Assessing the vulnerability of a habitat forming macroalga to climate warming: Roles of physiology, ecology and evolutionary processes in determining resilience

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Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Science,

Climate Change Cluster (C_3), School of Life Sciences, University of Technology Sydney

September 2016

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student:

Date: September 9th 2016

ACKNOWLEDGMENTS

What a journey this has been! I am immensely grateful to the many people who have helped me along the way. I would first and foremost like to thank my supervisors, Martina Doblin, Alistair Poore, Melinda Coleman and Peter Ralph for their endless knowledge, guidance, support and encouragement throughout my candidature and for helping me develop into the scientist I am today. I have learnt so much from you individually about science, about being a researcher as well as about life. I would also like to thank Alecia Bellgrove and Aimee Hauser for showing me the ropes in the molecular world as well as Anthony Van Rooyan for helping me with the microsatellite work. I would also like to thank Chris Evenhuis for his help with data analysis.

I would like to thank the UTS Australian Postgraduate Award, for supporting me during my PhD candidature as well the School of Life Sciences and Climate Change Cluster (C_3) for their generosity in funding my project.

Thank you to the members of the Climate Change Cluster (C_3), for your support as well as the technical staff Paul Brooks and Gemma Armstrong for technical support, keeping me safe in the lab and in the field as well as for the many laughs during our mid-hallway conversations. Thank you so much to the members of the Coastal Oceanography and Algal reSearch Team (COAST) for their support and friendship over the years. I also want to thank Jessica Tout, Lauren Messer, Allison McInnes and Michaela Larsson for your friendship over the years. You have been more than just colleagues at UTS, but life-long friends. We have had a lot of laughs and memories together and I thank you for always being there for me, through the thick and the thin.

To all the volunteers I have had over the years, I am grateful for your help within the field. Thank you, Daniel Lewis, Caitlin Lawson, Margarita Albas, Charlotte Robinson (that time when it was storming…) Madeline Ross, Sanjana Saxena, Kirralee Baker and Lisa Roberts.

Finally, I would like to dedicate this thesis to my husband Dan and our families. I want to thank my husband Dan (and best field assistant ever) for his love, support and encouragement throughout this journey. You are my rock. You believed in me, when I didn't believe in myself. You saw me through the good, the bad and the ugly and you kicked my butt when I needed it. Thank you, for your patience, for looking after me and for being there with me every step of the way. To our families, thank you for all your love and support over the years. To my brothers, Jason and Jonathan, thank you for your support and thoughtful conversations over cyber space and for all your silly antics that brought a smile to my face, even oceans apart. To my parents, Jeff and Nina, thank you for teaching me to follow my heart -even if it means to the opposite end of the world, for teaching me to love the natural world around me and for all your love and encouragement. To my dad, the green warrior, thank you for inspiring me to love science, for our endless conversations about the natural world, and for teaching me to look after our best investment – our environment.

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ABSTRACT

Anthropogenically mediated climate change is having profound impacts on the distribution, abundance and functioning of species worldwide. Marine macroalgae are important foundation species due to their role in facilitating biodiversity through provision of resources and moderating stress. Accurate predictions of how macroalgae will respond to global warming require a better understanding of factors that lead to the vulnerability of species. This thesis aimed to examine the exposure and underlying biological traits that explain the sensitivity and resilience to warming in a dominant and endemic intertidal macroalga, *Hormosira banksii,* with the ultimate goal of assessing its vulnerability to changes in climate regime.

H. banksii populations inhabiting two spatial scales, regional (central (cooler) and marginal (warmer)) and local (between tidal heights) were sampled. At each spatial scale, the performance of *H. banksii* was assessed to determine whether morphology influences function (relative water content and photosynthetic efficiency of PSII) in adults while the traits (growth and photosynthetic efficiency) of early life history stages (< 5 days old) were assessed to determine thermal niche. Adults in marginal populations had smaller thallus and vesicle size, which affected the ability of *H. banksii* to recover photosynthetically from thermal and desiccation stress. Distinct thermal performance curves of growth and photosynthetic efficiency of early life history stages revealed the marginal population had lower thermal safety margins and lower thermal optima compared to the central population that has broader thermal safety margins and higher thermal optima. The genetic structure was characterised among regions, locations and tidal heights to test the hypothesis that genetic diversity would decrease towards distribution limits and differ between tidal heights. Marginal populations had lower estimates of genetic diversity than central populations, and

there was evidence of isolation by distance – i.e., limited gene flow over long distances (~500 km). Genetic differentiation was not found between tidal heights, suggesting gene flow is not restricted by reproductive strategies of *H. banksii*. Furthermore, maternal provisioning of eggs did not indicate advantages in performance such as faster growth rate of early life stages, which would aid in recruitment. Physiological tolerances of adults and embryos, population genetic structure, inbreeding and limited gene flow all suggest that the warm marginal populations of *H. banksii* are vulnerable to changes in temperature regime. Local habitat effects such as topography and tidal cycles, however, are potentially more important in governing the physiology of *H. banksii* and can buffer the full extent of climate change occurring at the regional scale. In view of this, changes in the distribution and abundance of some populations of *H. banksii* with global warming, along with changes in the functioning of ecosystems which *H. banksii* support may be observed in the near future.

Chapter 1
General Introduction and Thesis Outline

1.1. INTRODUCTION

Growing anthropogenic pressures including increasing atmospheric CO_2 associated with global climate change are having profound and diverse consequences for marine ecosystems. In particular, the direct consequences of increased CO_2 are elevating ocean temperatures and acidity (Intergovernmental Panel on Climate Change, IPCC 2014). Since 1961, 80% of the heat added to the climate system has been absorbed by the ocean to a depth of 3000 m, indicating the ocean is a major heat sink (Lough 2009). Rising temperatures create additional changes to the climate system, such as intensified atmospheric pressure gradients causing an increase in frequency and intensity of storms, rising sea level, increased ocean stratification, altered patterns of ocean circulation and more frequent extreme climate events (Doney *et al.* 2012; IPCC 2014; Kerr 2011). These changes are already having significant effects on coastal marine systems including habitat fragmentation, shifts in the distribution of species, changes in ecological function and extinction of some species (Parmesan & Yohe 2003; Poloczanska *et al.* 2011; Root *et al.* 2003). These perturbations in climate are expected to continue far into the foreseeable future.

Much of the research regarding impacts of climate change has focussed on coral reefs and terrestrial forests, with considerably less attention devoted to other foundation species such as macroalgae (Harley *et al.* 2012; Hoegh-Guldberg & Bruno 2010; Wernberg *et al.* 2012). By forming large stands on emergent and submerged rocky reefs macroalgae support communities that are hotspots of diversity (Bolton 1994). These habitats are analogous to coral reefs and rainforests in that the foundation species facilitate biodiversity through providing resources such as habitat, food and shelter and by moderating environmental stress (Dayton 1972; Jones *et al.* 1994). Macroalgal communities also provide an estimated USD $3.8 trillion worth of ecosystem services per year including fixation of nutrients and carbon,

production of biomass and oxygen as well as storm protection, making them a valuable ecological and economical resource (Costanza *et al.* 1997).

Given their global importance, threats to macroalgal communities due to the potential impacts of climate change are of major concern. Macroalgae that inhabit rocky intertidal platforms are thought to be highly sensitive to changes in climate regime (Harley *et al.* 2012; Harley *et al.* 2006). Being exposed to extreme environmental gradients, such as diel changes in irradiance, elevated temperatures, desiccation, nutrient limitation and osmotic stress (Raffaelli & Hawkins 2012), intertidal organisms often exist close to their physiological limits (Somero 2010; Stillman & Somero 2000; Sunday *et al.* 2012). Macroalgae must simultaneously contend with anthropogenic stressors such as nutrient enrichment, pollution and contaminants (Bellgrove *et al.* 2010; Doblin & Clayton 1995; Seery *et al.* 2006), urban development, and recreational use, including trampling (Addison *et al.* 2008; Keough & Quinn 1998). The severity of these stressors can vary spatially (regionally at different latitudes and locally within habitat mosaics) as well as temporally (at different times of the year as well as between tidal cycles; Helmuth *et al.* 2006; Helmuth *et al.* 2002; Mislan *et al.* 2009), and the combinations of stressors can be additive, antagonistic or synergistic in their effects (Crain *et al.* 2008; Ferreira *et al.* 2014; Martínez *et al.* 2012). Furthermore, environmental change is occurring at a pace that is likely to exceed the ability of marine organisms to adapt (Quintero & Wiens 2013). Species can respond to these changes through adjustment in physiology, abundance, and distribution, including loss of localised populations and shifts in distribution towards cooler temperatures (Chen *et al.* 2011; Harley *et al.* 2012; Parmesan & Yohe 2003; Root *et al.* 2003; however, see Poloczanska *et al.* 2011). The extent to which macroalgae are resilient to environmental change depends on their biological and life history traits as well as their potential to evolutionarily adapt to these changes.

Of particular importance are organism responses to rising temperatures. Temperature plays a fundamental role in the physiology and distribution of macroalgae as it regulates photosynthesis through temperature sensitive enzymes (Allakhverdiev *et al.* 2008). On local scales, temperature increases with height on the shore, where temperature and desiccation often govern the upper limits of distribution (Harley & Helmuth 2003; Schonbeck & Norton 1978). On regional scales, temperature governs species boundaries with the distributional limits often coinciding with physiological and ecological limits (Hampe & Petit 2005). Previous studies have highlighted that the effects of temperature are not necessarily linearly related to latitude, and that local scale effects such as topography, aspect and tidal cycle may act to enhance or act as a buffer to warming temperatures and result in mosaics of 'hot spots and cold spots' (Helmuth *et al.* 2006). Variation in the physiological responses to warming may therefore occur among regions with varying climates and also locally within macroalgal communities.

In order to predict the impact of global warming on macroalgal communities, we require a better understanding of spatial variation in tolerance to thermal stress, and which species traits influence this tolerance. To address this, Williams *et al.* (2008) developed an integrative framework which proposed that the vulnerability of an organism depends on its *sensitivity* to environmental change, its *exposur*e to that change, its *resilience* or ability to recover from perturbations and its potential to adapt to change (Fig. 1.1). The degree of exposure to environmental change can vary regionally across a species distribution as well as on local scales among habitats that can enhance or buffer these effects (Williams *et al.* 2008). Species sensitivity is underpinned by physiological tolerance of individuals to stress, resilience of populations (i.e., degree to which populations can recover after disturbance) and, on longer

time scales, the likelihood of adaptation (dependent on levels of underlying genetic diversity (Williams *et al.* 2008; Fig. 1.1).

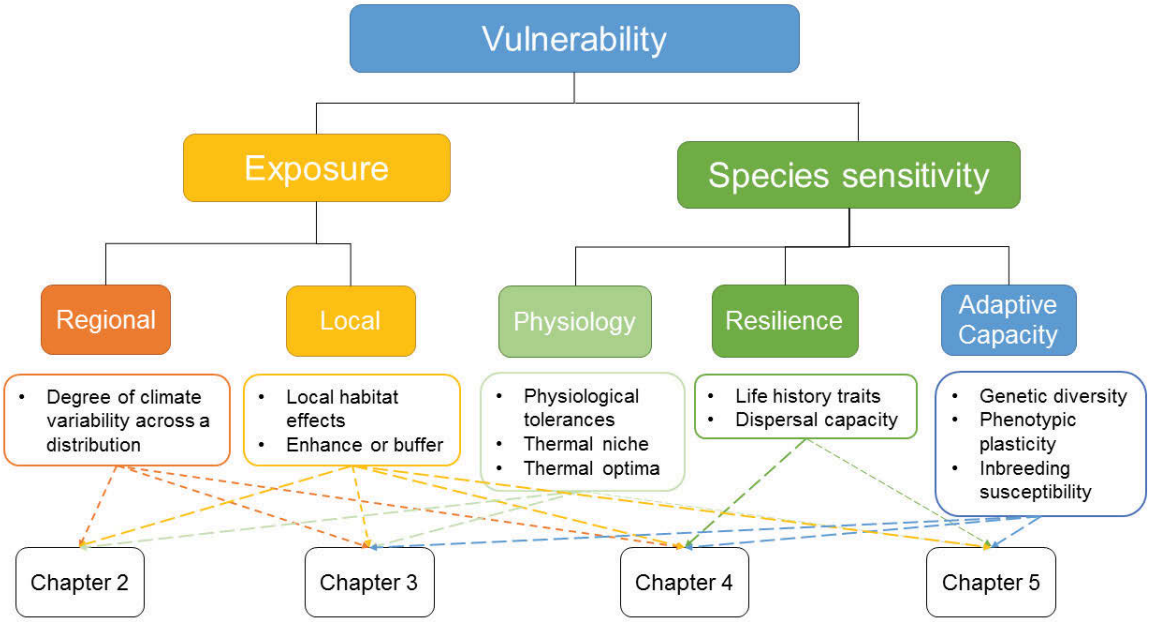

Figure 1.1: General framework to assess the vulnerability of species to global climate change. Framework adapted from Williams and others (2008). The parts of the framework addressed in each chapter are indicated by the dashed lines.

1.2. Variation in thermal tolerance of intertidal algae

While changes in physiology, ecology, and underlying genetic diversity to environmental change are well documented in terrestrial species, studies that have integrated these components of the vulnerability of macroalgal populations are relatively few (Nicastro *et al.* 2013; Pearson *et al.* 2009; Zardi *et al.* 2015). Physiological tolerances have mostly been examined in the context of determining lethal temperatures that set distributional limits on the vertical shore, (Davison & Pearson 1996; Hawkins & Hartnoll 1985; Schonbeck & Norton 1978) and setting marginal limits amongst species distributions (Bennett *et al.* 2015; Lima *et al.* 2007; Zardi *et al.* 2015). In order to determine the vulnerability of a species to warming, however, we must also understand how factors such as exposure and traits leading to species

sensitivity interact with each other in determining resilience in macroalgal populations. As such, **the overarching aim of this thesis is to determine the vulnerability of an ecologically important foundation species *Hormosira banksii* to ocean warming**, at different spatial scales: regionally between rear-edge (warm) and central (cooler) populations and locally (between different tidal heights on the shore). *H. banksii* is a dominant intertidal macroalga found within wave swept platforms of Australia and New Zealand (Lilley & Schiel 2006; Womersley 1987). Within this thesis, two different life stages are assessed to determine how temperature fluctuations shape physiological tolerances at different spatial scales. I also investigate how phenotypic plasticity in the morphology of adults influences their physiology. The underlying genetic diversity and gene flow among populations are also examined to determine the resilience of *H. banksii* to disturbance. Finally, transgenerational effects are investigated to test whether maternal provisioning (a non-genetic source of variation) can buffer embryos from thermal stress.

1.3. Physiological tolerances of individuals

Determining how global warming will affect macroalgal communities requires a better understanding of the environmental factors that govern physiological tolerances at the individual level and how they change spatially: among regions with different climate regimes and with local differences among habitats. Temperature is important in shaping physiological tolerances in many primary producers as it directly affects metabolic activity and is one of the main controllers of photosynthesis (Allakhverdiev *et al.* 2008). Physiological response to stress is translated into measures of fitness, such as growth (carbon assimilation through photosynthesis), survival and reproduction which is then translated to larger scales of distribution (Helmuth 2009).

The range of temperatures that an organism can tolerate is often developed through exposure to fluctuations in temperature throughout its thermal history, both seasonally at different latitudes as well as locally with direct interaction with the environment the organism perceives (Helmuth *et al.* 2010; Sunday *et al.* 2011). Thermal performance curves are used to visualise the relationship of temperature effects on fitness related traits (Eggert 2012). Typically, fitness related traits increase with temperature to a thermal optimum and then rapidly decline as temperatures surpass the optimum for metabolic function (Davison 1991; Eggert 2012; Gilchrist 1995; Huey *et al.* 2012). For photosynthesis, energy captured by the antenna of photosystem II (PSII) can be used in electron transfer and downstream carbon fixation. As temperatures surpass the optimum range for photosynthesis, energy is redirected to photoprotection such as non-photochemical quenching (NPQ) or the dissipation of heat (Allakhverdiev *et al.* 2008; Kramer *et al.* 2004). Reduced primary production through photodamage or repair of photosystems in temperatures outside of thermal optima can reduce growth or reproduction. With increased temperatures associated with global warming, an understanding of an organism's current physiological tolerances is needed both at small scales (e.g., across the vertical shore of a rock platform) and over large regional scales governing distribution boundaries.

1.3.1. Morphology and physiology

Plasticity in traits such as morphology can strongly modify the response of an individual to climate stressors (Miner *et al.* 2005; Sultan 2000). For intertidal organisms, the ability to modify their phenotype in response to changing environmental conditions is particularly important as they must be able to endure highly variable conditions. Species or genotypes within species with a high degree of plasticity may be favoured to persist in the changing

conditions due to ocean warming, than species or genotypes with little ability to alter their phenotype (Williams *et al.* 2008).

Phenotypic plasticity in morphology is one way that stress is ameliorated in the intertidal (Charrier *et al.* 2012). Some of the best examples of intraspecific variation in morphological plasticity are found in macroalgae that can adjust their phenotype (some within a manner of days) with interaction to its environment (Diaz-Pulido *et al.* 2007; Fowler-Walker *et al.* 2006; Koehl *et al.* 2008; Kübler & Dudgeon 1996). Although plasticity in morphology is well documented, less is known about the physiological consequences of this morphological plasticity (Demes *et al.* 2013; Dudgeon *et al.* 1995). Macroalgae have been found to decrease in size with height on the shore within a single platform (Sideman & Mathieson 1985; Wright *et al.* 2004) as well as towards species distributional limits as environmental conditions and habitats are less favourable (Araújo *et al.* 2011; Zardi *et al.* 2015). Reduced fitness in species can be exhibited as stunted morphologies, reduced growth or lowered reproduction (Sideman & Mathieson 1985; Stengel & Dring 1997b; Wright *et al.* 2004).

1.3.2. Regional scale variation

Marginal populations (i.e., populations at their distributional range edges) particularly those in lower latitudes (in tropical to subtropical climates) at the receding or rear-edge (Bridle & Vines 2007; Hampe & Petit 2005), have often been suggested to be the most susceptible to the effects of climate change (Bridle & Vines 2007; Provan 2013). They are often small, show spatial separation/isolation, and are limited by gene flow and connectivity to central populations that can reduce their potential to adapt as there are a lower range of genotypes available for functional responses (Eckert *et al.* 2008; Hampe & Petit 2005; Provan 2013). Individuals inhabiting marginal populations are also suggested to be at their physiological

threshold as environmental conditions and habitats are less favourable (Bridle & Vines 2007). Sunday *et al*. (2011, 2014) have shown that across a broad array of terrestrial and marine phyla, warm-adapted species inhabiting low latitudes and environments with higher average temperature have narrower thermal niches than cooler, high latitude species as they have adapted to lower seasonal temperature fluctuation throughout their life history. This is complicated further as warm-adapted species have been suggested to have a limited capacity to increase heat tolerance through acclimation compared to more cold adapted species living in temperate environments (Somero 2010; Stillman & Somero 2000). If warm-adapted populations of macroalgae are incapable of acclimating or adapting to higher average temperatures, then they may be susceptible to extirpation through extreme climate events (such as heat waves). Further, if these thermal sensitivities are a reflection of genetically fixed tolerances to increased temperatures then some macroalgal species will be in jeopardy from climate change in the next one to two centuries (Somero 2010). Investigation into physiological factors that govern distribution boundaries in macroalgal populations found that latitudinal gradients in air and sea surface temperatures often correlate with distributional patterns and range limits (Lima *et al.* 2007; Martínez *et al.* 2012; Nicastro *et al.* 2013; Pearson *et al.* 2009; Zardi *et al.* 2015). In Australia, however, studies that have assessed the vulnerability of macroalgal populations nearing their marginal limits have mainly focused on subtidal species (Smale & Wernberg 2013; Wernberg, Russell, Moore*, et al.* 2011). Given that south eastern Australia has been identified as a climate change hot-spot (Lough & Hobday 2011), advancing our understanding of how global warming and environmental change will affect the resilience of intertidal habitat-forming macroalgae is critical.

1.3.3. Small scale variation

Much of the research that has investigated the effects of climate change on species' distributions has focussed on using broad scale metrics of air temperature and sea surface temperatures (SST) to estimate habitat temperature. Research by Helmuth *et al.* (2002, 2006, 2010), however, has demonstrated that many broad scale observations do not capture the small-scale changes of climate and variation in local environmental factors which vary in space and time. The large heterogeneity and dynamic nature of the intertidal environment can cause highly variable temperature and environmental conditions which can vary within a few metres and within a few hours (Helmuth *et al.* 2006). These local scale variations include changes in topography, wave exposure, wind and tidal cycles that generally correlate with one or more abiotic factors such as temperature and desiccation (Davison & Pearson 1996; Harley & Helmuth 2003). Shore topography where cracks and crevices, aspect, wind and gradient of the shore, can create spatial heterogeneity that can enhance or buffer climatic effects (Fuller *et al.* 2010; Harley & Helmuth 2003). As such, the distribution of intertidal macroalgae with height on the shore is highly variable, with the position on the shore suggested to be linked to a species physiological tolerance to environmental factors with thermal and desiccation stress often limiting upper tidal limits (Hawkins & Hartnoll 1985; Schonbeck & Norton 1978). A better understanding is required in determining whether small scale variation in temperature such as within a single platform is more important in shaping physiological tolerances and morphology of macroalgae than larger regional effects.

1.4. The effects of genetic diversity and gene flow on determining vulnerability to climate change

1.4.1. Genetic variation within a population and the potential for adaptation

Phenotypic plasticity alone may not be sufficient as a mechanism to cope with warming (alongside other changes). On longer time scales, genetic adaptation may be required (Chen *et al.* 2011; Jump & Penuelas 2005; Parmesan & Yohe 2003). Macroalgae may therefore need to ultimately undergo evolutionary adaptation in order to be resilient to global warming (Hoffmann & Sgrò 2011). Evolutionary adaptation depends on the level of genetic diversity that currently exists in populations, dispersal capacity (gene flow) and connectivity of those populations (Hoffmann & Sgrò 2011). In naturally occurring populations, genetic variation in a trait suggests that there are genotypes within a population with a suite of functional responses to environmental stress for natural selection to act upon. This has been suggested to be linked to an increase in resilience or recovery from perturbations (Reusch *et al.* 2005). The standing genetic diversity and gene flow amongst macroalgal populations have recently become of interest in understanding the connectivity of populations (Assis *et al.* 2013; Coleman *et al.* 2011a; 2011b; Neiva *et al.* 2012; Teixeira *et al.* 2016; Zardi *et al.* 2015). A better understanding is required of the current genetic diversity and gene flow of macroalgal populations if we are to make accurate predictions of how other species will respond to future climate change.

Heritable genetic variation in traits in macroalgal populations can also be determined through quantitative breeding designs (Guntrip & Sibly 1998; Lynch & Walsh 1998). The presence of sire (male) x environment interactions indicate that there is heritable genetic variation in a trait. Sire (male) x environment interactions are generally used as they are considered to reflect the genotype and are free from maternal effects in which dams (females) can

contribute (Lynch & Walsh 1998). Mothers can often pass down not only the genotype but also the environmental history that a mother experiences onto the phenotype of her offspring. This can often be difficult to tease out variation contributed by the genotype and variation contributed by the phenotype. Quantitative breeding designs in marine species have been used in only a handful of studies: to examine the photosystems in scleractinian coral (Császár et al. 2010), defensive secondary metabolites in an intertidal alga (Sunday *et al.* 2011), tolerance to metal contamination in marine invertebrates, (Galletly *et al.* 2007; Pease *et al.* 2010), sea urchin larval development (Foo *et al.* 2014; Foo *et al.* 2012) and recently in two species of macroalgae (Al-Janabi *et al.* 2016; Clark *et al.* 2013).

1.4.2. Gene flow between populations

The capacity for populations to adapt to changed local conditions will also depend on gene flow between populations. Genetic diversity varies on different spatial scales: e.g., across a species distribution as well as within one population (Assis *et al.* 2013; Coleman *et al.* 2011a; Kelly & Palumbi 2010; Teixeira *et al.* 2016; Valero *et al.* 2011) and is primarily governed by gene flow and connectivity (the rate of exchange of genetic material and resources between different populations; Coleman *et al.* 2011a). For marine macroalgae, gene flow occurs through dispersal of genetic material via gametes/zygotes or by rafting of adult thalli (McKenzie & Bellgrove 2008). For short-distance dispersal and gene flow, gametes and zygotes of some macroalgae are often only dispersed within a few meters (Dudgeon *et al.* 2001; Muhlin *et al.* 2008). Species of *Fucus* are limited to gamete release during calm waters at high tide (Pearson 2006; Serrão *et al.* 1996) or low tide (Pearson & Brawley 1996) that ensure 100% fertilisation success (Serrão *et al.* 1996). Limited dispersal may have long-term impacts through limitation of gene flow (Muhlin *et al.* 2008) putting populations at risk of inbreeding and reduced potential for evolutionary rescue by tolerant genotypes (Muhlin *et al.*

2008; Willi *et al.* 2006). Consequently, species that have restricted dispersal will demonstrate greater genetic differentiation between populations (Valero *et al.* 2011).

At larger scales, genetic structure is governed by gene flow between populations through different dispersal capabilities of marine algae and transport vectors such as ocean currents, geographic barriers, and patterns in climate (Coleman *et al.* 2011a; Coleman & Kelaher 2009; Muhlin *et al.* 2008). Long-distance dispersal connects populations across large distances as well as across habitat discontinuities, however, this method occurs much rarer than short-distance dispersal (Coleman *et al.* 2011b). This type of dispersal occurs via reproductively viable rafting thalli that float with ocean currents with aid of air bladders or vesicles and can release viable gametes or propagules for a considerable time after detachment (McKenzie & Bellgrove 2008). Due to limited capacity in dispersal in some macroalgae, long distance dispersal via detached thalli may be important in genetic connectivity between distant populations (Palumbi 2004). If novel genetic material can only be introduced through long-distance dispersal, neighbouring populations may become less genetically diverse over time with potential consequences for fitness and survival.

1.5. Potential for transgenerational effects

1.5.1. Life history stages

Understanding how species of macroalgae will respond to climate change is also challenging, due to complex life cycles (i.e. alternation of generation) of some species. Relatively little is known about the effects of climate change on early life history stages (Coelho *et al.* 2000; Harley *et al.* 2012), and importantly, physiological tolerances can change depending on which stage of life is exposed (Wright *et al.* 2004). The life cycle of *H. banksii* is quite simple, in terms of gametes are produced from diploid individuals rather than from haploid

gametophytes and lack alternation of generation, such as in some kelp species (Fig 1.2). Environmental changes, however, can impact different stages and processes throughout its life history, which can have effects in later stages in life (Ladah & Zertuche-González 2007). Acquiring extensive information on biological traits, the longevity of some species (i.e. > 20 years in *Ascophyllum nodosum,* Åberg 1992) and time to reproductive maturity (Araújo *et al.* 2011; Araújo *et al.* 2015; Coleman & Brawley 2005c; Viejo *et al.* 2011) can also be quite time consuming. Due to this, the effects of climate change on different life histories and the role of life history traits in determining resilience in macroalgae remains generally unknown (Araújo *et al.* 2011; Coleman & Brawley 2005a; Kain 2015; Wright *et al.* 2004).

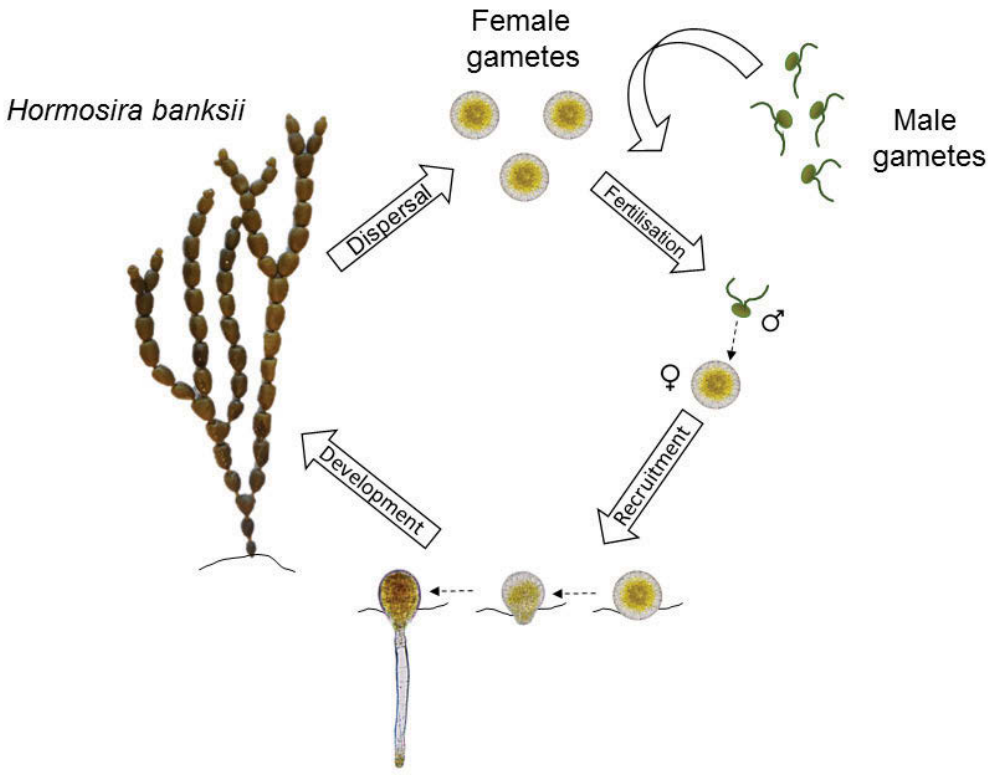

Figure 1.2: Conceptual diagram of *Hormosira banksii* life cycle of and processes which climate change may act upon.

1.5.2. Transgenerational effects

Climate change may also have an indirect effect on an individual's phenotype though transgenerational effects. Transgenerational effects in which the parental environment influences the fitness of their offspring can contribute to adaptive evolution (Mousseau & Fox 1998; Roach & Wulff 1987; Wolf & Wade 2009). The environment in which a parent experiences, particularly the maternal parent can have significant effects on the offspring through provisioning or enhancing fitness her offspring in order to give them an advantage in early life (Mousseau & Fox 1998). Resources gained by the maternal parent can be used to provision eggs or propagules and studies have indicated that larger offspring often have increased fitness and advantages in early life. This has been documented within terrestrial plants (Galloway 2005), vertebrates (Chambers & Leggett 1996) and invertebrates (Marshall 2008; Yanagi & Tuda 2010). The resources available to an individual for use in reproduction are acquired through the environment and therefore can be finite. Mothers may have to trade-off fewer larger offspring or many smaller sized offspring (Smith & Fretwell 1974). Although allocation of resources towards reproduction have been investigated in macroalgae (Araújo *et al.* 2015; Chu *et al.* 2011; Gillespie & Critchley 2001; Viejo *et al.* 2011), transgenerational effects, particularly maternal provisioning, lacks explicit testing.

1.6. Study organism

Hormosira banksii (Turner) Decaisne is a widely distributed brown macroalga found on temperate rocky shores of Australia and New Zealand, and is the focus of this thesis. It has been recorded from the west to the east coast of Australia (from Albany to Lennox Head), southern Australia, north-eastern Tasmania, New Zealand as well as Lord Howe Is., Norfolk Is., and the Kermadec and Chatham Islands (Miller *et al.* 2013; Osborn 1948; Womersley 1987). *H. banksii* is a dominant species on many intertidal platforms with morphological

ecotypes amongst rocky platforms and estuaries identified (Macinnis-Ng *et al.* 2005; Ralph *et al.* 1998). It is characterised by having branched chains of vesicles with each vesicle filled with gas or water (Coleman *et al*. 2011a; Macinnis-Ng *et al.* 2005; personal observation J.Clark) These ecotypes may reflect the plasticity of *H. banksii* to different environmental conditions, however, it remains to be investigated whether these differences in morphology confer advantages in stress resilience. This species is also ideal for population genetic research to answer questions about standing genetic diversity. As a member of the Fucaceae, it has been identified as having poor dispersal capacity due to negatively buoyant and phototactic eggs (Pearson & Brawley 1996; Serrão *et al.* 1996) and limited gene flow (Coleman *et al.* 2011a), despite showing evidence of long distance dispersal via floating thalli (McKenzie & Bellgrove 2008). Finally, with dioecious male and female plants, *H. banksii* is very easy to manipulate in culture making it ideal for manipulative experiments and breeding designs (Osborn 1948) and thereby amenable to answer questions regarding physiological tolerances, heritable genetic variation and transgenerational effects. As such, **the overarching aim of this thesis is to enhance our understanding of how a dominant intertidal macroalga in the southern hemisphere will respond to global warming and determine whether it possesses traits that lead to its vulnerability.**

1.7. Thesis outline

The goal of this thesis is to determine whether populations of *Hormosira banksii* possess traits that promote vulnerability to warming by investigating traits in physiology, ecology and underlying genetic diversity at two spatial scales: at the local scale, amongst different heights on the shore, as well as regional, at equatorward marginal edges and compare them to more centrally located populations. The following aims will be addressed within this thesis

:

1. **To determine whether there are physiological consequences of phenotypic plasticity in macroalgal populations;**
2. **To investigate how temperature variation experienced at marginal and central populations governs thermal niche;**
3. **To assess the adaptive capacity in terms of underlying genetic diversity and plasticity of *H. banksii* to future warming;**
4. **To explore the reproductive investment and maternal provisioning in *H. banksii.***

These specific aims will be addressed within the following chapters. In Chapter 2, the physiological consequences of *H. banksii* morphology at two spatial scales are investigated to determine whether form regulates function, and whether decreases in individual size and fitness occur at marginal populations and higher on the shore. In Chapter 3, thermal niche is estimated in early life history stages using thermal performance curves. I also assess whether heritable variation in thermal tolerances traits exist. Chapter 4 examines the underlying genetic diversity and gene flow at marginal and central populations as well as amongst different tidal heights. Chapter 5 explores transgenerational effects to determine whether maternal provisioning influences fitness of early life stages. Finally, Chapter 6 provides the general discussion, synthesis and conclusions.

Chapter 2

Shaping up for stress: Physiological consequences of variation in morphology of an intertidal alga

2. ABSTRACT

Variation in morphology of organisms has been recognised to have important functional and ecological implications, particularly in sessile marine organisms where morphology is often closely linked to the physical environment. Intertidal macroalgae have highly variable morphologies, with this plasticity suggested to increase fitness under stressful conditions. The functional consequences of morphological variation in macroalgae, however, have been rarely tested. This study quantified morphological variation in the habitat-forming, intertidal macroalga, *Hormosira banksii*, on two spatial scales: between tidal heights (local scale) and amongst locations (regional scale) to test the performance (relative water content and photosynthetic efficiency) of morphological variants during heat and desiccation stress under laboratory conditions It was predicted that algae inhabiting the warm end of their distribution, and high on the shore would have stunted morphology but be more heat and desiccation tolerant than those low on the shore and within the centre of their distributional range. Thallus and vesicle morphology varied within and among populations, with thalli at the distributional edge having a reduced biomass and length, with vesicles of greater surface area to volume ratio (SA:VOL) than those from central populations. Thalli from high on the shore were generally shorter than those low on the shore, with vesicles having a smaller SA:VOL. Vesicle morphology was found to predict relative water content and photosynthesis during desiccation and rehydration. As photosynthesis relies on functional thylakoid membranes, the differences in SA:VOL of vesicles between heights on the shore, may reflect water requirements needed to maintain tissue hydration during low tide. Photosynthetic efficiency during desiccation did not vary among *H. banksii* phenotypes, however, the ability to recover from high temperatures and desiccation stress did. Thalli from marginal populations showed greater thermal sensitivity as indicated through reductions in photosynthetic yield and delays in recovery after desiccation. It suggests that *H. banksii* possess a high degree of morphological plasticity reflecting local

climate, topography and environmental conditions, with this morphological variation having functional consequences. Phenotypic variation in morphology across local and regional scales will be important for resilience of this species to future climate warming.

2.1. INTRODUCTION

Morphological variation of organisms has often been closely linked with the physical environment as variation can often moderate stress and have functional and ecological importance. For sessile organisms, moderating stress through changes to the morphology of individuals can increase fitness and survivorship and translate to larger-scale patterns in distribution (Helmuth *et al.* 2006). Morphological variation within a species can be due to phenotypic plasticity, where a given genotype varies in morphology as a response to their environment (e.g. grazer-mediated wounding, Van Alstyne 1989) or through evolutionary processes where variation in selection among populations leads to morphological variation among populations (Roberson & Coyer 2004). Some of the best examples of intraspecific variation in morphology can be found in species of benthic macroalgae (Bell 1995; for a review Charrier *et al.* 2012; Kübler & Dudgeon 1996; Littler & Littler 1980; Wernberg & Thomsen 2005). Effects of the environment on the morphology of macroalgae have been documented for changes in water motion (Blanchette 1997; Fowler-Walker *et al.* 2006), temperature (Kübler & Dudgeon 1996), salinity (Falace & Bressan 2006), and depth (Roberson & Coyer 2004)). For example, in the subtidal kelp, *Ecklonia radiata,* wave exposure can have an effect on the thickness of blades, which in turn affects drag resistance (Fowler-Walker *et al.* 2006; Wernberg *et al.* 2003; Wernberg & Thomsen 2005). Indeed, highly dynamic environments can have profound effects on the morphology of macroalgae that may determine whether an individual or species can occupy certain habitats.

Although morphological plasticity of macroalgae is widespread in a range of environments, morphological variation may be particularly important in determining the fitness and survivorship of algae within the wave-swept platforms of the rocky intertidal (Blanchette 1997; Dudgeon *et al.* 1995; Roberson & Coyer 2004). Here, macroalgae are exposed to extreme environmental gradients and limited habitable space, coinciding with daily tidal regimes. The capacity for morphological plasticity may be important in determining whether a species of macroalgae inhabit a particular environment within the shore, or are able to occupy a range of environments across the intertidal region (Brown 1987; Gómez & Huovinen 2011; Skene 2004)

Emersion, temperature and tidal height have all been demonstrated to affect a wide range of morphological characters (Littler & Littler 1980). For example, forms with thick-walls and low surface area to volume ratios reduce the rate of desiccation (Bell 1995; Bell 1993; Oates 1985). Dissected or branched thalli may be at lower risk of overheating because they are more effective at dissipating heat, but likely to have higher rates of desiccation (Kübler & Dudgeon 1996). Many of these observations have been linked to vertical position on the shore, suggesting that physiological function is related to desiccation and temperature tolerance during tidal cycles (Dudgeon *et al.* 1995; Skene 2004; Smith & Berry 1986; Williams & Dethier 2005). Intertidal macroalgae have been reported to fix between 30 and 86 % of their daily carbon while emersed (Madsen & Maberly 1990), thus the effectiveness of carbon fixation depends on the ability of thallus tissues to remain hydrated. As macroalgae do not possess an active mechanism to control water loss such as stomata, or roots to replenish evaporative water loss, morphology is fundamentally important in moderating tissue temperature and desiccation rate (Bell 1995; Bell 1993).

The intrinsic trade-off for macroalgae on wave-exposed shores is to be resilient to intertidal stress (temperature, light and desiccation stress) while being able photosynthesise to grow and reproduce (Bell 1995; Bell 1993; Kübler & Dudgeon 1996; Littler & Littler 1980; Viejo *et al.* 2011). Intraspecific variation in morphology should be evident along environmental gradients as the physical environment has strong impacts on growth processes such as the temperature sensitive enzymes related to photosynthesis (Allakhverdiev *et al.* 2008). Individual macroalgae inhabiting areas of greater temperature and desiccation stress, such as those high on the shore, are suggested to have a higher tolerance for these stresses, but exhibit retarded growth and stunted morphologies in contrast to individuals living lower on the shore (Hawkins & Hartnoll 1985; Stengel & Dring 1997a). This may be related to trade-offs in resources for photoprotection as more energy is required to divert energy away from photosystems (Allakhverdiev *et al.* 2008).

Similarly, along latitudinal gradients, fitness of individuals commonly diminishes towards the distributional limits as environmental conditions and habitat become sub-optimal for growth and reproduction (Brown 1984). Species of macroalgae nearing their distributional limits have been shown to have reduced population and individual sizes (Araújo *et al.* 2011; Zardi *et al.* 2015) and a higher tolerance to increased temperatures (Ferreira *et al.* 2014). In some species, however, a reduced tolerance to heat and desiccation stress have also been recorded (Pearson *et al.* 2009). Variation in morphology can also occur through complex interactions of life history traits, local selection pressures and local environmental conditions as not all species follow these regional scale patterns (Sagarin & Gaines 2002). Furthermore, a growing body of work suggests that stress experienced at the scale of the individual organism, which varies with local scale conditions and topography, may be more important in shaping an organism's physiology than large regional scale effects (Helmuth *et al.* 2006). Quantifying intraspecific variation in

morphology at different spatial scales, and the relationship between morphology and function, is thus needed to understand how macroalgae inhabit highly variable environments while maintaining physiological function.

In this study, I investigate the physiology of different morphological phenotypes using an ecologically important species of intertidal macroalga, *Hormosira banksii* (Turner) Descaisne. This fucoid is the most broadly distributed brown macroalga in Australasia, with the edge of its northern distribution range located at a cold-warm transition zone. It often forms dominant, monospecific stands on wave-swept shores, occupying the entire mid to high intertidal area, playing a similar ecological role as species of *Fucus* in North America and Europe. *H. banksii* plays a key role in the ecological functioning of intertidal ecosystems, providing habitat, resources and modifying local environmental conditions for other organisms (Lilley & Schiel 2006; Schiel 2006). *H. banksii* ecotypes with different morphology inhabit different ecosystems such as exposed rocky intertidal platforms and estuaries (Macinnis-Ng *et al.* 2005; Mueller *et al.* 2015; Ralph *et al.* 1998) but there is little mechanistic understanding of how this variation in morphology influences physiology. The widespread distribution and limited gamete dispersal of *H. banksii* suggests that locally adapted phenotypes are likely, and that the species has broad tolerance to thermal and desiccation stress. Quantifying how differences in morphology translate to physiological function on both local (on the vertical shore) and regional (latitudinal) scales will enable an understanding of how this species can persist across a wide range of environmental conditions. Under experimental conditions, mimicking a low tide in the laboratory, thalli from different tidal heights were exposed to thermal and desiccation stress to investigate whether different morphologies are better suited to these stressors. I hypothesize that morphological variation will be greatest on the local scale, with high shore thalli having a greater thermal and desiccation tolerance in contrast to low shore thalli. On the regional scale, I

hypothesize that thalli from populations nearing distributional limits will have decreased photosynthetic capacity and stunted morphologies in contrast to more temperate populations.

2.2. METHODS

2.2.1. Sample collection and study sites

H. banksii was sampled at four locations: two in the subtropical marginal edge of its range, Angourie (29º28'41.51" S, 153º 21' 49.53" E) and Minnie Water (29°46'34.23" S, 153°18'07.43" E) and two in the temperate centre, Pearl Beach (33°32'57.70" S, 151°18'32.36" E) and Bilgola Beach (33°38'54.48" S, 151°19'39.59" E). These locations were on exposed headlands comprising rocky reef substrates where *H. banksii* was the dominant intertidal macroalga with percent cover of approximately 20%, 60%, 50%, and 60% respectively. Rockpool habitats were not sampled, and consequently individuals at the northern-most location (warm edge) of the current *H. banksii* distribution (Flat Rock, NSW; 28º 50' 32.79" S, 153º 36' 29.47" E) were not used, as they were only found in rockpools at this location. Instead, the northern-most location sampled was Angourie, NSW. Thalli were haphazardly collected from both high and low on the shore across the platform, two hours before maximum low tide and kept overnight at 4 ºC before analysis within laboratory conditions.

2.2.2. Thallus and vesicle morphology

Each thallus was photographed for detailed measurements using the imaging software Image J (ver. 1.48v, National Institutes of Health, USA) (n = 10). The morphological traits measured from each thallus were length of the longest frond (excluding holdfast), number of branches (dissection) and number of vesicles. From the mid-branch region of each thallus, six vesicles were chosen randomly and their length, diameter and wall thickness measured using digital callipers (Mitutoyo Corp., Model No. CD-6"C, Japan). Total vesicle surface area, total volume,

cavity volume and surface area to volume ratio (SA:VOL) were then calculated based on methods described by Ralph *et al.* (1998) and (Macinnis-Ng *et al.* 2005). Entire thalli were then dried at 60 ºC for 48 hours and weighed on a digital balance (NewClassic MF ML3002/01, Mettler Toledo, Switzerland).

2.2.3. Relative water content and photosynthetic efficiency during desiccation and re-immersion

Given the morphological variation among thalli collected from different locations and from different heights on the shore (see Results), morphological variants were then tested for differences in their tolerance to desiccation and temperature stress. Six thalli from each height on the shore at each location were exposed to one of three treatments. The first two treatments consisted of thalli desiccated at one of two temperatures, 20 ºC or 30 ºC for 4 hours, followed by a recovery period where thalli were placed in filtered 20 ºC seawater in low light (~10 µmol photons m^{-2} s^{-1}) for 2 hours. The third treatment was a control where thalli were submerged in filtered (0.7 µm Whatman GFF) 20 ºC seawater under the same light exposure for the duration of the experiment. All thalli were allowed to recover a further 12 hours in the dark after the 2-hour initial recovery in low light. Prior to experimentation, thalli of equal length were allowed to fully hydrate in filtered 20 ºC seawater for 30 min, then blotted with tissue and weighed. After a 20 min period of dark adaptation, initial maximum quantum yield (F_V/F_M) was determined by applying a saturating pulse of blue light (2000 µmol photons m^{-2} s^{-1}) using a pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Walz, Germany).

Desiccation treatments commenced when lights were turned on and thalli were placed on desiccation trays which consisted of an aluminium tray placed over a heat plate, with each tray having metal grates to aid in maintaining air flow beneath each frond and a mesh to ensure thalli

were protected from burning. Thalli were illuminated using LED lights (custom-made LED light with a cool white spectral output) at 400 μmol photons m^{-2} s^{-1} until steady state was reached (approx. 20 min). Experimental light intensity was designed to induce photoinhibition, as determined in a pilot study using PI Curves (Fig. S2.1). To understand the photosynthetic function of *H. banksii* under simulated tidal emersion, a steady state analysis was used which allowed determination of photosynthetic efficiency of photosystem II (PSII) (Kramer *et al.*). Effective quantum yield of PSII ($\Delta F/F_M'$) and thallus mass was measured every 30 minutes during the desiccation period. After 4 hours of desiccation, recovery of the photosynthetic health of re-immersed thalli was assessed by measuring the maximum quantum yield (F_V/F_M) every 30 minutes for two hours and then again 12 hours later. Wet weights were recorded to the nearest 0.01 g every 30 min, and at the end of the experiment, thalli were dried for 48 hours at 60°C and reweighed. Relative water content (RWC) was calculated at each time using the formula:

$$\text{Relative water content} = \frac{Desiccated\ weight - dry\ weight}{fresh\ weight - dry\ weight} \times 100\ \%$$

2.2.4. Statistical analyses

All morphological traits were contrasted among locations and heights on the shore with analysis of variance (ANOVA). Thallus traits were analysed using a two-factor mixed model design with location (random, 4 levels) and height on the shore (fixed, 2 levels). Vesicle traits were analysed using a three-factor mixed model with location (random, 4 levels), height on the shore (fixed, 2 levels) and individual (random, nested within location and height 10 levels) as factors. For both sets of analyses, planned comparisons among the four locations were used to contrast the two locations at the range edge with the two locations in the range centre.

The similarity in morphology of thalli among locations and heights on the shore was visualised using principle components ordination (PCO). Given that the parameters were measured on different scales, morphological traits were normalised before the similarity matrix using Euclidean distance was constructed. Draftsman plots were used to visualise correlations among traits and showed that vesicle volume and surface area were highly correlated with SA:VOL and thus only SA:VOL was used in the PCO.

Photosynthetic yields and relative water content were contrasted between 20 °C and 30 °C using ANOVAs with location (random, four levels), height on the shore (fixed, two levels) and temperature (fixed, two levels) as factors. Separate analyses were conducted for these traits at each of six separate time points: initial (before the treatments started), after 1 h of desiccation, at the end of the desiccation period at 4 h, initial recovery after 30 min submerged at the end of 2 hours of recovery and final maximum quantum yield (F_V/F_M) 12 hours later. Univariate ANOVAs were conducted in the PERMANOVA+ routine of PRIMER-E (v. 6), with probabilities calculated by permutation (999) using Monte Carlo *p*-values.

2.2.5. Predicting function from morphology

Distance-based linear models (DISTLM) were used to identify which morphological traits best predicted physiological function. Vesicle-level morphometrics were used as predictor variables to determine whether they regulated each of the response variables, photosynthetic efficiency of PSII and relative water content, at two critical time points: at the end of the desiccation treatment (4 h) and after thallus re-immersion (30 mins). Vesicle morphometrics from each thallus were averaged to ensure data matrices matched for the DISTLM. The DISTLM was performed separately for each response variable at each of the two time points, to determine the model which best fit the variables and identify what variable or combination of variables best

characterised the functional traits. The routine followed an information criterion model (AIC) with 'Best' as a selection criterion to best explain the largest variation in functional traits attributed by morphology. This was performed on morphometric and functional traits for the 20 °C treatment only, as most vesicles in the 30 °C treatment were not functional at the end of the desiccation treatment.

2.3. RESULTS

2.3.1. Thallus and vesicle morphology

The morphology of *H. banksii* was highly variable among locations, between regions and with height on the shore, based on analysis of nine morphometric parameters of vesicles and thalli. *H. banksii* thalli varied in form from having several long fronds with little branching and a few large vesicles, to having many short, branched fronds with small vesicles (Fig. 2.1).

Principle co-ordinate analysis revealed differences in morphology between tidal heights were correlated with vesicle morphometrics, primarily SA:VOL (Fig. 2.2 a). The thallus traits of length, biomass and number of branches, did not significantly differ between heights on the shore, however, total length varied amongst locations (Fig. S2.2, Table 2.1). Vesicles found high on the shore had larger volumes and surface areas, and smaller SA:VOL ratios than those found low on the shore (Fig. S2.2, Table 2.2), however, cavity volume and wall thickness differed between heights on the shore for the central population only (Fig. S2.3, height x region interaction, Table 2.2).

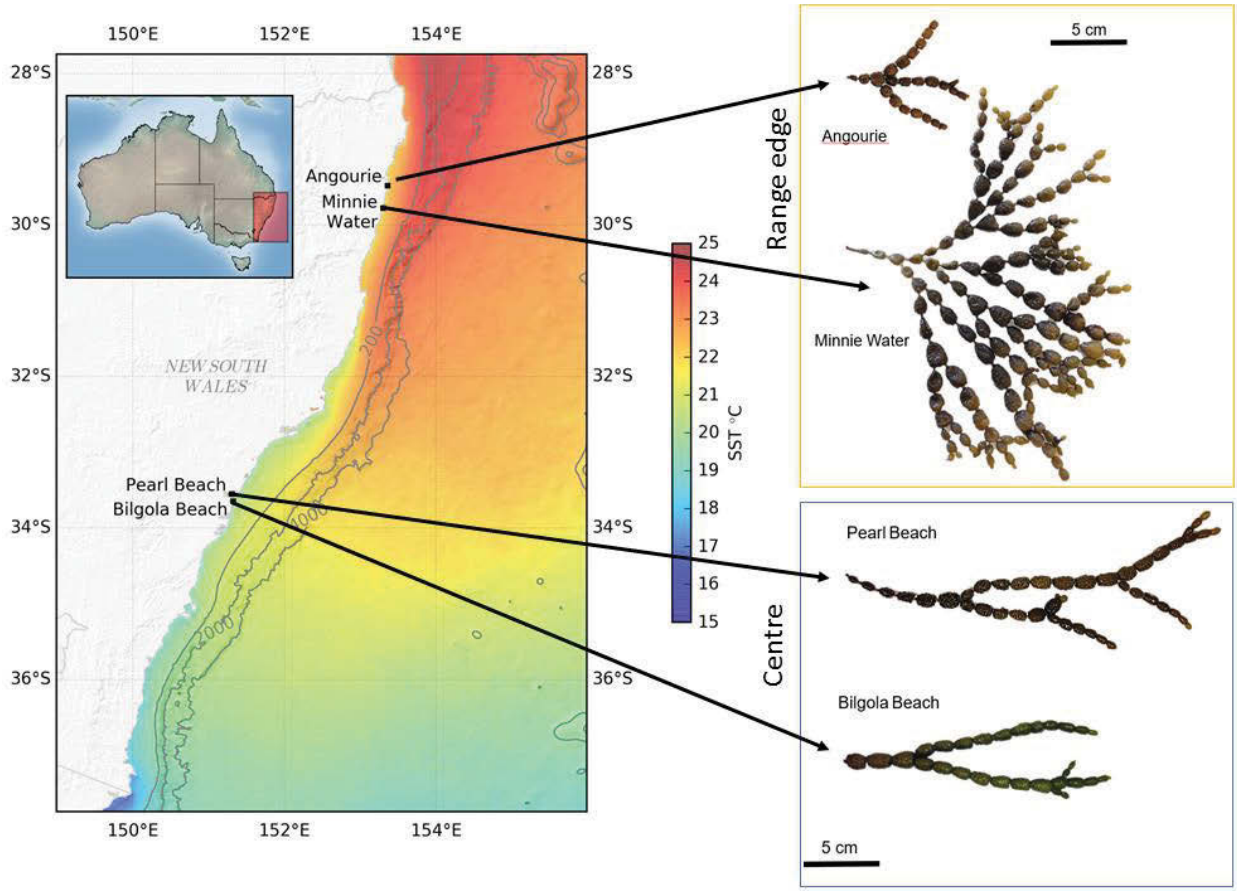

Figure 2.1: Example of *H. banksii* phenotypes found across different locations, Angourie, Minnie Water, Pearl Beach and Bilgola Beach. Orange box (top) shows phenotypes from edge populations, while blue box (bottom) shows central populations.

Differences in morphology among locations were largely attributed to thallus level morphometrics (length, biomass, branching and number of vesicles) as revealed by principle co-ordinates analysis (Fig. 2.2b). Significant differences among locations and regions were found for thallus level morphometrics (Table 2.1); however, there was no consistent pattern of decreasing individual size with decreasing latitude (Fig S2.2, Table S2.1). The northern-most population sampled, Angourie, exhibited the smallest thallus morphology (Fig. S2.2, Table S2.1), while thalli from Minnie Water, a marginal population, were the largest among all locations (Table S2.1). The magnitude of the differences between high and low on shore varied among regions for all vesicle traits, but the only trait with a region by height interaction was vesicle surface area (Fig. S2.2, Table 2.2).

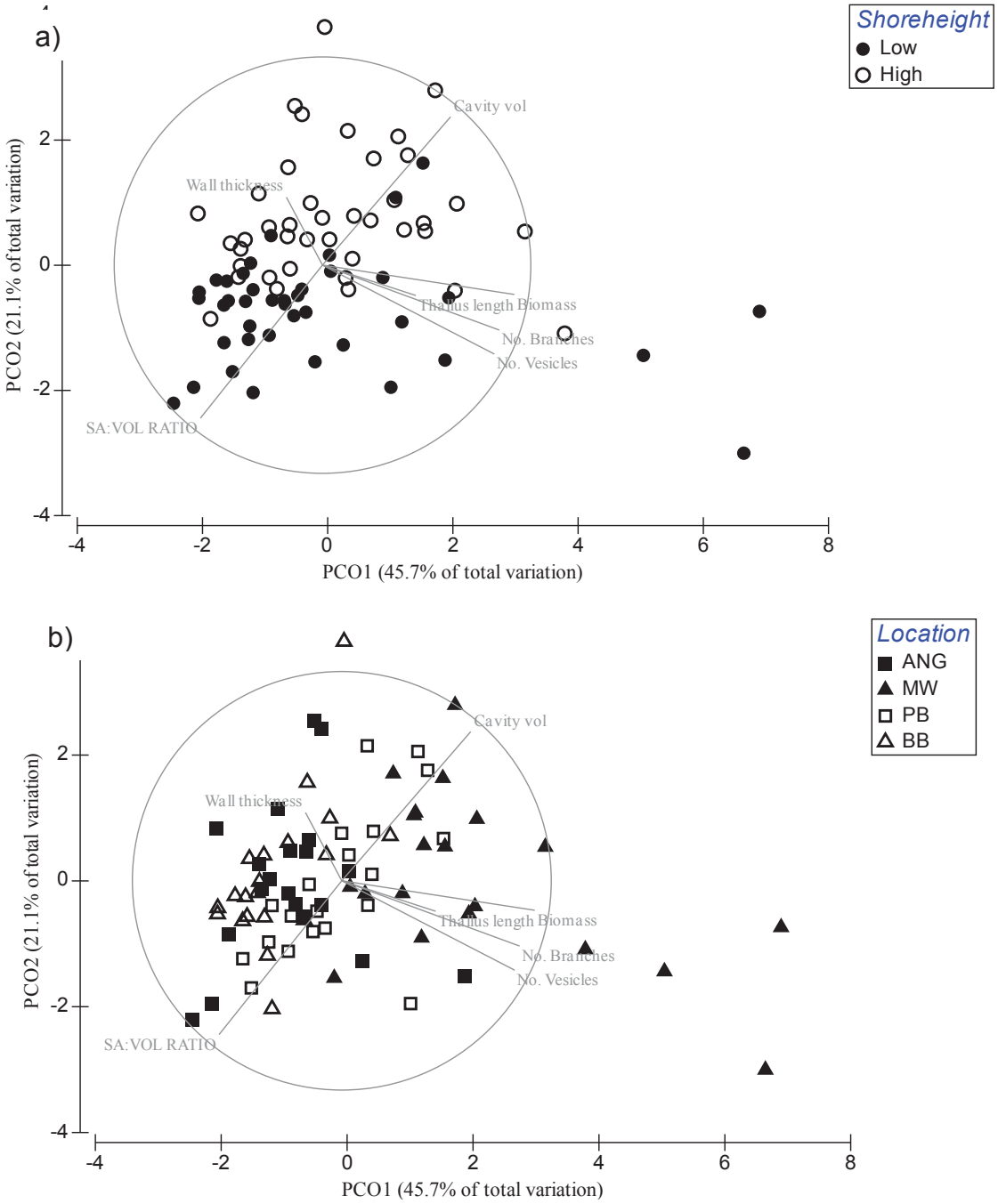

Figure 2.2: Principle coordinates ordination of thalli from 4 locations (Angourie ANG, Minnie Water MW, Pearl Beach PB and Bilgola Beach BB) and across two tidal heights (low and high on the shore) based on all morphometric traits. In a) thalli are labelled by height on the shore and in b) thalli are labelled by location and region (filled symbols = marginal, open symbols= centre). The percentage of variation is represented by the first two principal coordinate axes which attributes 66.8% of variation amongst all morphometrics.

Table 2.1: Results of mixed model ANOVA of *H. banksii* thallus morphometrics with planned regional contrasts. Location is random and has 4 levels (ANG, MW, PB, BB). Height on the shore is fixed and has two levels (low and high shore; n = 10)

ANOVA Source	Thallus											
	Biomass				Total Length			No. Branches			No. Vesicles		
	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Lo	1	66.04	10.97	**0.001**	15014.00	5.65	**0.019**	980.00	18.84	**0.001**	9790.30	10.91	**0.002**
Re	3	79.84	20.80	**0.001**	29677.00	18.40	**<0.001**	676.33	16.88	**0.001**	8474.70	11.72	**0.001**
He	1	4.86	0.46	0.514	9320.80	1.44	0.322	20.00	0.60	0.490	6142.50	4.41	0.129
Lo x He	3	10.55	2.75	0.051	6486.00	4.02	**0.010**	33.33	0.83	0.463	1394.30	1.93	0.129
Re x He	1	24.00	3.99	**0.048**	7547.20	2.84	0.097	80.00	1.54	0.205	3712.80	4.14	**0.040**
Res	72	3.84			1613.30			40.07			723.41		

Bold denotes significance P < 0.05

Table 2.2: Results of mixed model ANOVA of *H. banksii* vesicle morphometrics. Location is random and represents 4 locations sampled (ANG, MW, PB and BB) with regional contrasts, shore height is fixed (low shore and high shore) and individual which is nested within location and shore height is random (n = 10).

ANOVA Source	Volume				Surface Area			SA:VOL Ratio			Wall Thickness			Cavity Volume		
	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Lo	3	2.92E+06	11.14	**0.001**	4.73E+05	11.79	**0.001**	0.40	15.81	**0.001**	0.79	8.10	**0.001**	1.10E+06	12.96	**0.001**
Re	1	9.78E+04	0.37	0.550	3.58E+03	0.09	0.786	0.05	1.93	0.175	0.06	0.75	0.397	1.58E+06	19.30	**0.001**
Height	1	1.51E+07	27.56	**0.016**	2.34E+06	21.39	**0.016**	1.82	47.38	**0.044**	0.13	0.72	0.438	2.94E+06	15.54	0.067
Lo x He	3	5.50E+05	2.09	0.107	1.09E+05	2.73	**0.045**	0.04	1.51	0.229	0.17	1.77	0.168	1.89E+05	2.22	0.105
Re x He	1	1.51E+06	5.79	**0.017**	3.26E+05	7.76	**0.009**	0.11	4.37	**0.048**	0.44	5.80	**0.016**	3.59E+05	4.39	**0.041**
Ind(Lo x He)	72	2.62E+05	7.65	**0.001**	4.01E+04	7.56	**0.001**	0.03	8.21	**0.001**	0.10	4.18	**0.001**	8.52E+04	5.03	**0.001**
Ind(Re x He)	36	2.61E+05	3.59	**0.001**	4.20E+04	3.76	**0.001**	0.03	3.47	**0.001**	0.08	2.07	**0.002**	8.17E+04	3.02	**0.001**
Res	400	3.43E+04			5.30E+03			0.00			0.02			1.69E+04		

Bold denotes significance P < 0.05

2.3.2. Relative water content and photosynthetic efficiency during desiccation and re-immersion

Temperature had an effect on the capacity of different morphologies of *H. banksii* to retain water during desiccation (Table 2.3). After an hour of desiccation in the 20 °C treatment relative water content (RWC) of thalli varied among locations and was greater in high shore thalli than those low on the shore (Fig. 2.3). In contrast, thalli exposed to 30 °C responded similarly between heights on the shore (Fig. 2.3, Table 2.3). At the end of the desiccation period (4 h), the effects of temperature on RWC varied among locations (location x temperature interaction, Fig. 2.4b, Table 2.3). All thalli exposed to 30 °C displayed less than 20% of their initial water content, while thalli exposed to 20 °C had RWC ranging from 20–40% with variation among locations (Fig. 2.4b). The effects of temperature on RWC did not vary between thalli collected at different heights on the shore (Table 2.4a).

Increased temperature reduced the ability of thalli to rehydrate after desiccation, with the magnitude of this effect varying among locations and between heights on the shore (Fig. 2.4a, Table 2.3). Once re-immersed (within the first 30 minutes of recovery, 4.5 h) the recovery of RWC varied between low and high shore thalli, and among locations, with all thalli recovering ~40% of their RWC in the first 30 minutes (Fig. 2.3, 2.4b). Significant interactions between temperature and location were present for the remainder of the recovery period, suggesting that different morphologies varied in their response to temperature (Fig 2.3, Table 2.3). At the end of the recovery period (18 h), all thalli desiccated at 20 °C were able to recover ~30 % more initial water content than thalli desiccated at 30 °C (Fig. 2.3). In addition, marginal populations showed greatest recovery at 20 °C than central populations. Conversely, thalli from central populations desiccated at 30 °C were able to recover more RWC than marginal population thalli (Fig. 2.3). Thalli found high on the shore exposed to 20 °C were able to recover 20 % more RWC than thalli found low on the shore (height x

temperature interaction Table 2.3) while thalli exposed to 30 °C showed no difference in RWC.

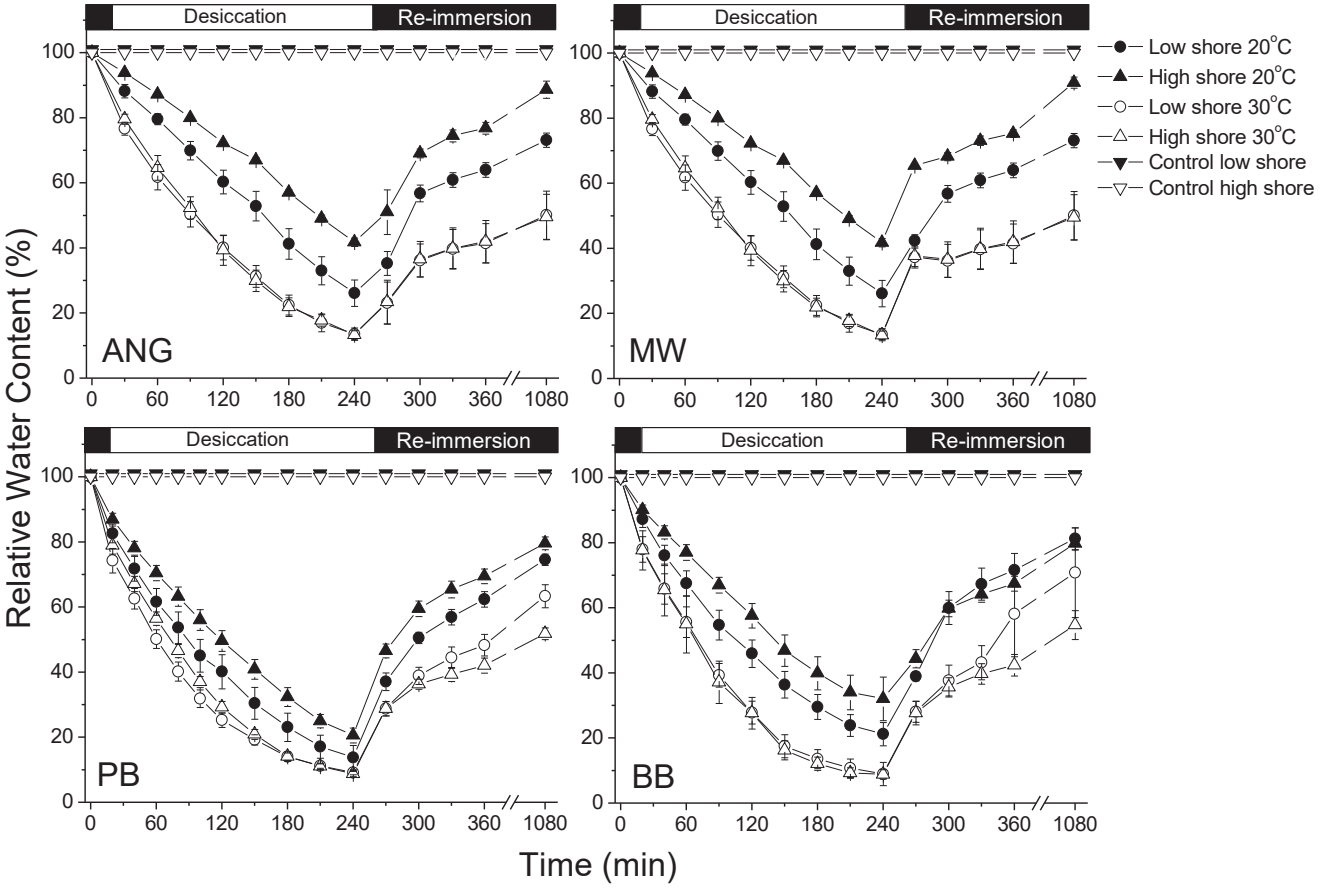

Figure 2.3: Changes in mean (± SE) relative water content (%) with time for high and low shore thalli under two temperature treatments 20 °C (filled) and 30 °C (unfilled) at 4 populations. Edge populations: Angourie (ANG), Minnie Waters (MW); centre populations: Pearl Beach (PB), and Bilgola Beach (BB). Bars above graph represent desiccation periods (white bars) where lights (400 μmol photons m^{-2} s^{-1}) and heat treatments were on and re-immersion periods (black bars) where thalli were re-immersed, lights and heat turned off. Control treatments: low shore control (filled inverted triangle) and high shore control (open inverted triangle).

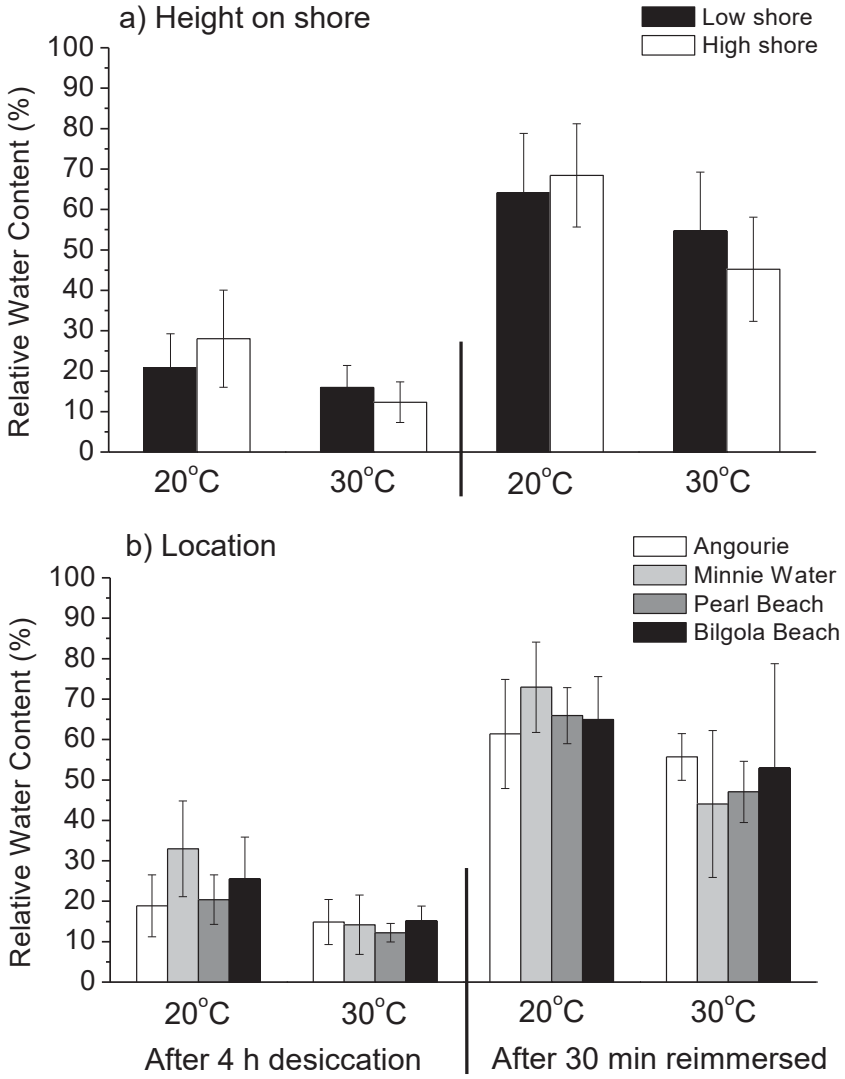

Figure 2.4: Mean (± SD) relative water content (%) of thalli collected from a) two heights on the shore and b) four locations at two time points: the end of the desiccation (4 h) and the initial measurement of recovery 30 minutes after thalli were resubmerged. Edge populations: Angourie and Minnie Water; centre populations: Pearl Beach and Bilgola Beach.

Table 2.3: Results of analysis of variance (ANOVA) for percent relative water content of *H. banksii* for three treatments, 20 °C, 30 °C at 5 times points: after 1 hour of desiccation, at the end of the desiccation period (4 h), initial recovery after thalli were submersed (30 min), recovery after 2 h, and at the end of the recovery period (after 14 h submersed). Location factor had planned regional contrasts between marginal (ANG, MW) and central populations (PB, BB). *P*-values were calculated from 999 permutations under a reduced model with a significance level of 0.05. (n = 6).

ANOVA Source		Desiccation (1 h)			Desiccation (4 h)		
	df	MS	*F*	*P*	MS	*F*	*P*
Lo	3	398.07	3.13	**0.025**	366.15	8.74	**0.001**
Re	1	262.12	1.93	0.167	142.06	2.32	0.132
He	1	928.26	59.78	**0.032**	395.40	11.67	0.065
Te	1	5245.80	21.20	**0.032**	3496.20	12.56	0.060
Lo x He	3	15.53	0.12	0.945	33.89	0.81	0.494
Re x He	1	1.22	0.00	0.928	2.26	0.04	0.860
Lo x Te	3	247.44	1.95	0.123	278.39	6.65	**0.002**
Re x Te	1	0.10	0.00	0.983	10.75	0.18	0.699
He x Te	1	83.09	1.78	0.269	400.00	7.40	0.065
Lo x He x Te	3	46.76	0.37	0.787	54.06	1.29	0.320
Re x He x Te	1	42.02	0.31	0.593	0.83	0.01	0.909
Res	80	127.07			41.88		

ANOVA Source		Recovery (30 min)			Recovery (2 h)			End of Recovery (14 h)		
	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Lo	3	137.36	2.69	**0.049**	119.78	0.90	0.455	261.54	1.79	0.139
Re	1	408.68	7.01	**0.006**	0.00	0.00	0.998	13.45	0.09	0.775
He	1	631.83	24.84	**0.029**	0.06	0.00	0.914	8.61	0.03	0.809
Te	1	5683.20	22.91	**0.035**	8904.00	21.11	**0.025**	9675.60	25.15	**0.047**
Lo x He	3	25.43	0.50	0.676	307.89	2.32	0.085	293.93	2.01	0.117
Re x He	1	43.94	0.75	0.380	559.47	3.90	**0.045**	703.10	4.48	**0.031**
Lo x Te	3	248.08	4.86	**0.004**	421.76	3.18	**0.031**	384.65	2.63	0.062
Re x Te	1	5.56	0.10	0.735	13.60	0.10	0.767	52.22	0.34	0.601
He x Te	1	319.35	3.21	0.180	755.79	80.62	**0.006**	1144.30	48.77	**0.016**
Lo x He x Te	3	99.60	1.95	0.141	9.37	0.07	0.982	23.46	0.16	0.942
Re x He x Te	1	20.10	0.35	0.569	9.50	0.07	0.786	18.62	0.12	0.742
Res	80	51.01			132.84			146.48		

Bold denotes significance P < 0.05

Thalli from different regions, locations and heights on the shore varied in their photosynthetic efficiency of PSII in response to desiccation (Table 2.4). Fully hydrated thalli showed significant differences in maximum quantum yield (F_V/F_M) prior to desiccation exposure amongst different heights on the shore and locations (Fig. 2.5). Initial F_V/F_M was lower in the marginal populations and amongst low shore thalli. Once lights were turned on and desiccation commenced, effective quantum yield was significantly reduced by 60-70 % of initial F_V/F_M within 1 h across all thalli, compared to the submerged controls which were reduced by 50% (Fig. 2.5 Table 2.4). There were no interactions between temperature and locations or height on the shore. By the end of the desiccation period, initial F_V/F_M had declined by 95%, with the differences among locations varying for the two heights on the shore (location x height interaction; Fig. 2.5, 2.6a, Table 2.4).

During rehydration, the effect of temperature on the ability of thalli to recover PSII photosynthetic efficiency varied between marginal and central populations (region x temperature interaction, Table 2.4). Within the first 30 min of thalli being re-immersed, central populations (Bilgola Beach and Pearl Beach) regained a greater percentage of photosynthetic efficiency (~50%) than marginal populations (Angourie and Minnie Water (Fig. 2.5, 2.6b)). Angourie, the northern-most population sampled, lost 60 and 70% of initial F_V/F_M at 20 and 30 °C, respectively, while the other locations thalli regained much of their photosynthetic function at 20 °C but not 30 °C (location x height interaction, Fig. 2.5, 2.6b, Table 2.4). In the 30 °C treatment, less than 10 % of initial F_V/F_M was recovered, with high shore thalli generally unable to recover under increased temperature (Fig. 2.6a, Table 2.4).

Across all locations, thalli collected from high on the shore desiccated at 20 °C recovered a greater proportion of photosynthetic performance (~20% more F_V/F_M) than low shore thalli

after 2 hours of re-immersion (Fig. 2.7). At the end of the recovery period (14 h), desiccated thalli showed irreversible damage to photosystems across all locations, heights on the shore and temperatures, with an additional 10% reduction of F_V/F_M 12 hours later, whereas controls did not show photodamage (Fig. 2.6, 2.7). All thalli showed an 80-95% loss of initial F_V/F_M when desiccated at 30 °C (Fig. 2.7). The controls that remained immersed at both 20 and 30 °C showed similar photosynthetic efficiency among locations or heights on the shore during the desiccation and recovery periods (Fig. 2.5).

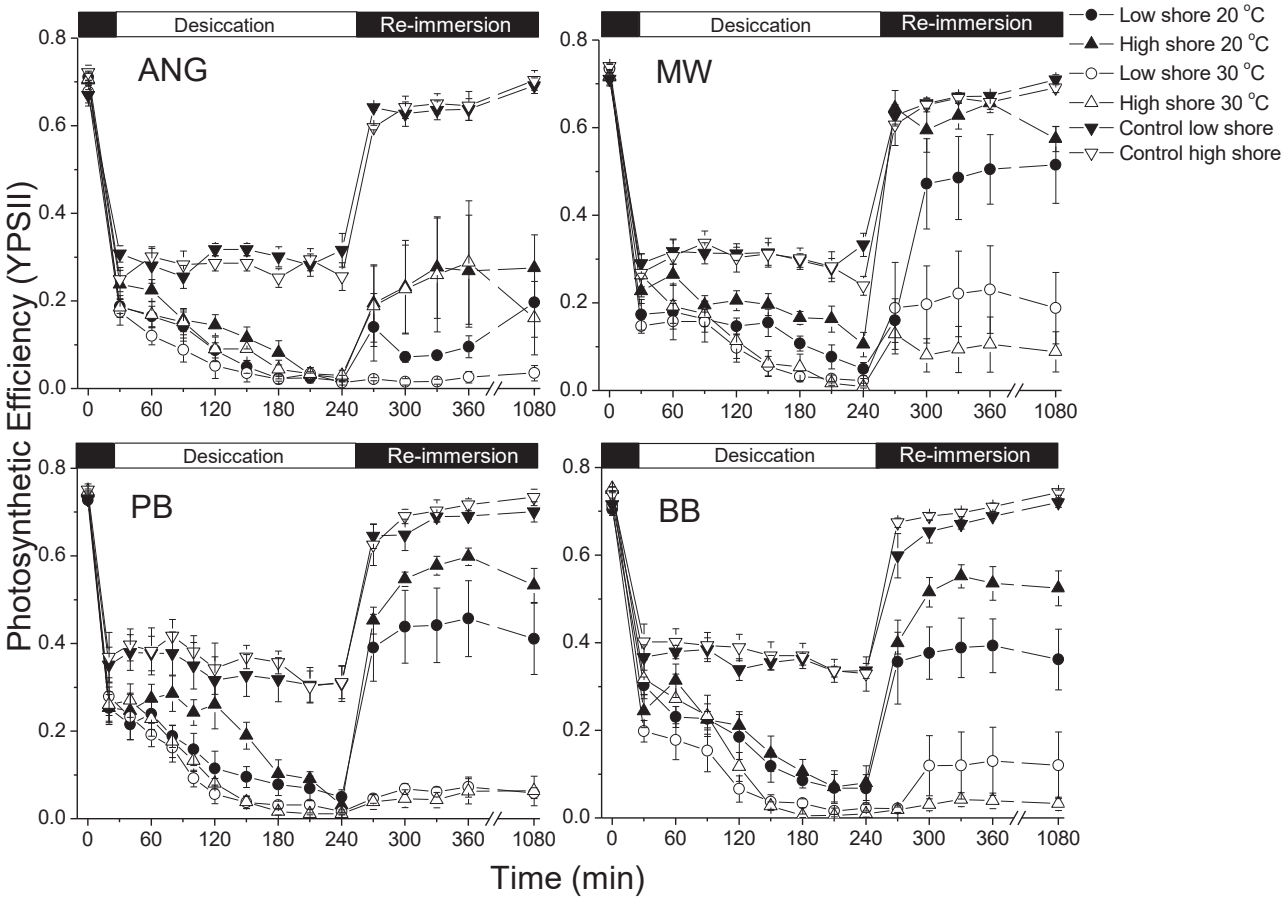

Figure 2.5: Mean (± SE) of photosynthetic efficiency of PSII (%) over time of thalli from high and low on the shore under two temperature treatments 20 °C (filled) and 30 °C (unfilled) at 4 populations. Edge populations: Angourie (ANG) and Minnie Waters (MW); centre populations: Pearl Beach (PB), and Bilgola Beach (BB). Bars above graph represent desiccation periods (white bars) where lights (400 µmol photons m^{-2} s^{-1}) and heat treatments were on and re-immersion periods (black bars) where thalli were re-immersed, lights and heat turned off. Control treatments: low shore control (filled inverted triangle) and high shore control (open inverted triangle).

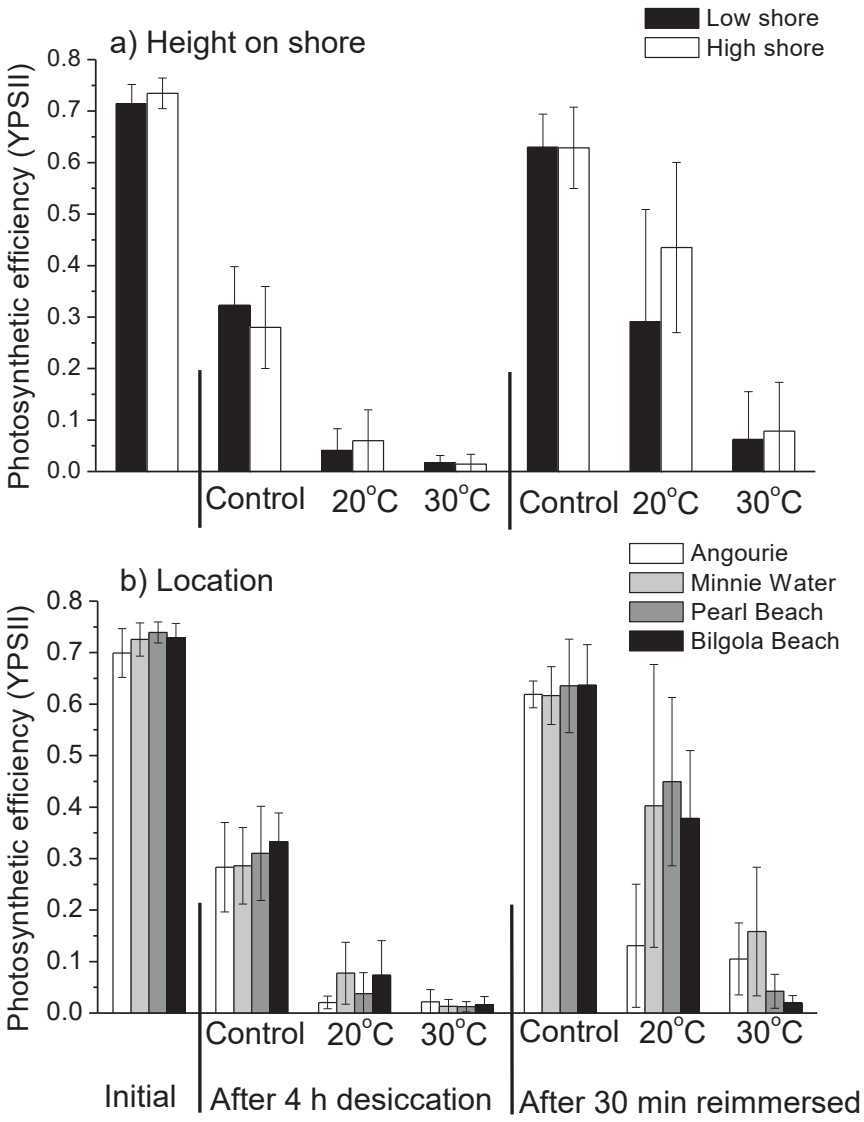

Figure 2.6: Mean (± SD) of photosynthetic yield of thalli from a) two heights on the shore and b) four locations. Photosynthetic yield was measured at three time points: 1) initial, 2) the end of the desiccation period (4 h), and 3) 30 minutes after thalli were resubmerged. The controls remained submerged while the 20 °C and 30 °C temperature treatments were emergent during the desiccation period.

Table 2.4: Results of analysis of variance (ANOVA) for photosynthetic yield of *H. banksii* for three treatments, 20 °C, 30 °C and control at 6 times points: before the treatment commenced, after 1 hour of desiccation, at the end of the desiccation period (4 h), initial recovery after thalli were submersed (30 min), recovery after 2 h, and at the end of the recovery period (after 14 h submersed). Location factor had planned regional contrasts between marginal (ANG, MW) and central populations (PB, BB). *P*-values were calculated from 999 permutations under a reduced model with a significance level of 0.05. (n = 6).

ANOVA Source	Desiccation (1 h)				Desiccation (4 h)		
	df	MS	*F*	*P*	MS	*F*	*P*
Lo	3	0.03	4.04	**0.010**	0.00	2.16	0.096
Re	1	0.08	10.98	**0.001**	0.00	0.48	0.477
He	1	0.08	20.16	**0.033**	0.00	1.65	0.266
Te	1	0.06	4693.30	**0.025**	0.02	5.80	**0.109**
Lo x He	3	0.01	0.52	0.678	0.00	0.58	0.628
Re x He	1	0.00	0.00	0.975	0.00	1.33	0.265
Lo x Te	3	0.00	0.00	1.000	0.00	3.16	**0.033**
Re x Te	1	0.00	0.00	0.961	0.00	0.02	0.885
He x Te	1	0.00	0.24	0.657	0.00	1.23	0.348
Lo x He xTe	3	0.00	0.20	0.910	0.00	1.44	0.250
Re x He xTe	1	0.00	0.45	0.491	0.00	0.42	0.551
Res	80	0.00			0.00		

ANOVA Source	Recovery (30 min)				Recovery (2 h)			End of Recovery (14 h)		
	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Lo	3	0.14	6.13	**0.003**	0.36	16.86	**0.001**	0.12	5.70	**0.003**
Re	1	0.19	7.46	**0.005**	0.08	2.02	0.165	0.00	0.07	0.791
He	1	0.07	5.90	0.093	0.07	6.07	0.109	0.06	3.46	0.175
Te	1	0.91	5.35	0.106	2.40	9.51	0.063	2.62	25.81	**0.028**
Lo x He	3	0.01	0.50	0.666	0.01	0.52	0.672	0.02	0.84	0.489
Re x He	1	0.03	1.22	0.279	0.00	0.04	0.835	0.00	0.09	0.755
Lo x Te	3	0.17	7.38	**0.001**	0.25	11.73	**0.001**	0.10	4.72	**0.005**
Re x Te	1	0.39	15.15	**0.001**	0.26	6.78	**0.009**	0.08	2.99	0.098
He x Te	1	0.04	1.75	0.298	0.14	4.51	0.123	0.10	4.16	0.130
Lo x He xTe	3	0.03	1.07	0.389	0.03	1.41	0.256	0.02	1.10	0.353
Re x He xTe	1	0.01	0.39	0.509	0.01	0.20	0.670	0.03	1.13	0.295
Res	80	0.02			0.02			0.02		

Bold denotes significance P < 0.05

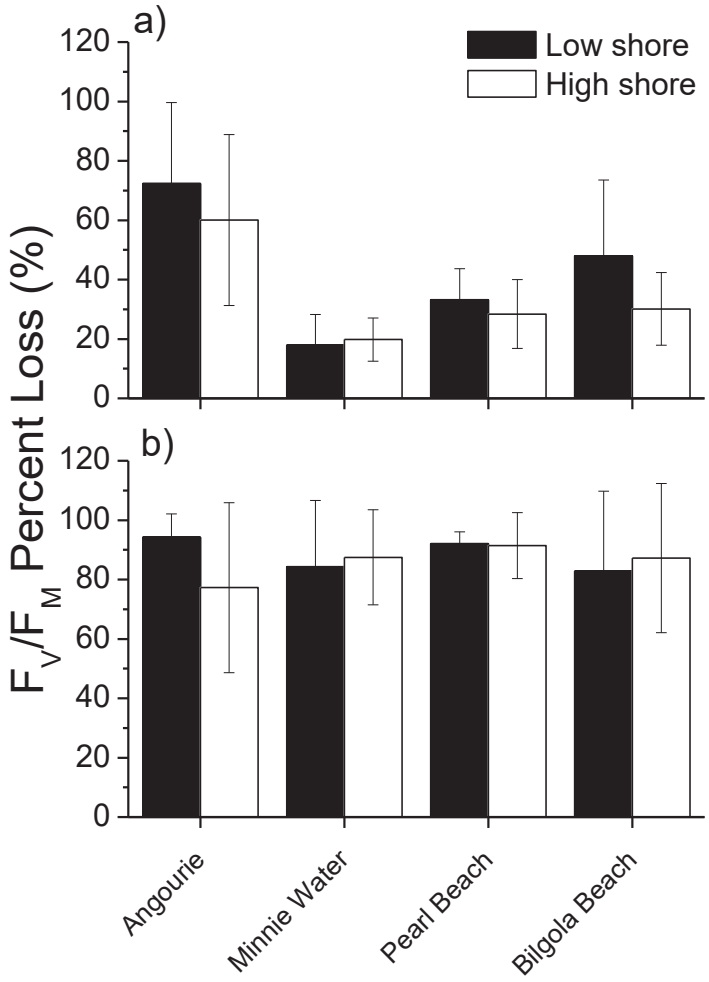

Figure 2.7: Mean (± SD) of percent loss of maximum quantum yield (F_V/F_M) between initial and final time point at a) 20 °C and b) 30 °C for thalli collected from two heights on the shore and four locations. Edge populations: Angourie and Minnie Water; centre populations: Pearl Beach and Bilgola Beach.

2.3.3. Predicting function from morphology

Vesicle morphometrics were found to be significant predictors for RWC and photosynthetic efficiency of PSII at two functionally important time points in the DISTLM analysis: the end of the desiccation period (4 h) and during initial recovery (30 min). Surface area to volume ratio was the most significant predictor variable for both time points. For relative water content, SA:VOL ratio alone fitted ~74% of the overall variation (Desiccation: $F_6 = 16.33$, $p = 0.006$, $R^2 = 0.731$; Recovery: $F_6 = 17.67$, $p = 0.003$, $R^2 = 0.747$), whereas SA:VOL fitted 70% of the overall variation in photosynthetic efficiency during desiccation ($F_6 = 13.81$, $p = 0.021$, $R^2 = 0.697$) but not for recovery ($F_6 = 5.21$, $p = 0.057$, $R^2 = 0.465$). Overall best solutions of the DISTLM indicated that SA:VOL ratio and cavity volume were the best predictors for recovery of RWC, with the lowest AIC values of 23.67 and greatest proportion of the variation explained ($R^2 = 0.748$) indicating that vesicle morphology, and particularly SA:VOL ratio, are important predictors underlying RWC in the *H. banksii* populations sampled.

2.4. DISCUSSION

In this study, significant intraspecific variation in morphology was found for the intertidal macroalga, *Hormosira banksii*, amongst different spatial scales with consequences for the physiological function of these macroalgae. Importantly, vesicle morphology, particularly SA:VOL ratio, fitted 70-74% of the overall variation in relative water content and photosynthetic efficiency during desiccation and recovery, indicating that function is dependent on morphology and links to the ability of vesicles to retain water content during low tide. Importantly, the capacity to withstand greater thermal and desiccation stress was lower amongst marginal populations compared to central populations which agrees with previous research (however, see Ferreira *et al.* 2014; Pearson *et al.* 2009). At the local scale, however, there were variable responses to thermal and desiccation stress between tidal heights

suggesting that local environmental conditions play an important role in morphological variation and consequently physiology and distribution (Ferreira *et al.* 2014; Zardi *et al.* 2013).

2.4.1. Local scale differences in morphology and the effects on relative water content and photosynthetic efficiency of PSII

Morphological variation found between heights on the shore within each population of *H. banksii* sampled suggests that local environmental exposure may influence morphology and function more than regional scale effects (Helmuth *et al.* 2006). As organisms experience the direct environment around them, small scale differences in topography, local weather, tidal regimes and slope of the intertidal may impose stress differently to macroalgae inhabiting different heights on the shore (Helmuth *et al.* 2010). Macroalgae inhabiting higher tidal heights are generally exposed to desiccation and thermal stress longer than lower on the shore therefore, physiology and morphology are adjusted to compensate for those differences. The effect of morphological variation between tidal heights was demonstrated during desiccation and recovery in this study as distinct differences in relative water content for thalli from different heights on the shore. Retaining water while emersed through the duration of the low tide is not only important for macroalgae to stay hydrated and endure desiccating conditions but is also important for photosynthesis to occur and in the long-term important for growth, reproduction and survivorship (Oates 1985).

The SA:VOL ratio of vesicles was the best predictor of relative water content and subsequently photophysiology, which has been confirmed in other macroalgae (Dromgoole 1980). Thalli high on the shore were smaller than thalli from low on the shore but had larger vesicles (smaller SA:VOL ratio). Saccate macroalgae such as *H. banksii* have internal

reservoirs of water within each vesicle which can replenish water loss within the tissue during desiccation. (Oates 1985; Osborn 1948). The ability of macroalgae to tolerate desiccation has been found to be related to retaining moisture within the molecular environment around the photosynthetic apparatus, which prevents membrane breakage (Kawamitsu *et al.* 2000), therefore photosynthesis can still occur as long as tissues remain moist. As thalli from high on the shore are emersed for longer and experience higher temperatures than low shore thalli (Chapter 3, Fig 3.1), vesicles require a larger water capacity in order to maintain physiological function during low tide. In studies with other macroalgae, the rate of desiccation is dependent on the thickness of the thalli blades (Bell 1995). As there were no differences found for wall thickness between heights on the shore, water retention in *H. banksii* is therefore dependent on the morphological properties of the vesicles. Physiological differences found between heights on the shore may therefore reflect morphological differences in vesicle water holding capacity rather than differences in the rate of water transfer through vesicle walls.

The 'critical water content' threshold describes the desiccation tolerance of a species, where algae can photosynthetically recover once re-immersed in water (Lüning *et al* 1990), with desiccation beyond this point causing irreversible damage (Dring & Brown 1982). Within this study, thalli in the 30 °C treatment that reached < 20% RWC had a reduced photosynthetic efficiency of PSII and a reduced capacity to recover, while thalli exposed to the 20 °C treatment maintained RWC above 20%. Previous studies have found that different macroalgae have different critical water content which may be a reason for why macroalgae are found at different heights on the shore. High shore species, such as *Fucus spiralis,* have a high tolerance to desiccation and can recover after desiccation of 80-90% of their original RWC (Dring & Brown 1982). *Mastocarpus papillatus,* found in the subtidal, has moderate

desiccation tolerance (> 20% RWC) needed for recovery; (Bell 1993), whereas *Durvillaea willana* is highly sensitive to desiccation, failing to recover after only a 10% loss of initial RWC (Brown 1987). The critical water content for *H. banksii* (20%) would suggest that it has a moderate tolerance to desiccation stress, however, the ability to recover also depends on the temperature it is subjected to. Increased temperatures have been found to cause a delay in the time it takes for full recovery in other macroalgae (Bell 1993). Temperatures that exceed upper thresholds of thermal tolerance in macroalgae can cause breakdown of proteins and temperature sensitive enzymes needed for photosynthesis, and consequently requires reallocation of resources for repair instead of use in carbon assimilation and growth (Allakhverdiev *et al.* 2008). This may explain the slow recovery of photosynthetic efficiency and RWC after desiccation at 20 °C and the lack of recovery in the 30 °C. While there was a difference in RWC of thalli from different tidal heights, there was no interaction between tidal height and temperature for photosynthetic efficiency, suggesting that the morphological phenotypes of *H. banksii* analysed in this study may be highly adapted to their environment, otherwise they would not be present if they are unable to mitigate stress. The morphological differences that enable *H. banksii* to retain RWC to maintain photophysiology during low tide are likely an important determinant for survival and distribution at different tidal heights.

2.4.2. Regional scale differences in morphology and the effects on relative water content and photosynthetic efficiency of PSII

Latitudinal clines in temperature are often predicted to result in a decrease in abundance and individual size towards the peripheral edges of species distributions (Viejo *et al.* 2011; Zardi *et al.* 2015). Empirical evidence, however, often does not reflect this trend with local effects of abiotic factors, disturbance and gene flow overriding large scale effects (Zardi *et al.* 2015). For example, in *H. banksii*, high temperature summer periods have been known to cause

tissue damage or 'sunburn' in thalli which can make them vulnerable to breakage, and potentially prune them back to create smaller individual size (Schoenwaelder 2002). In this study there was no clear difference in the size of *H. banksii* thalli sampled from the marginal to the central populations. Instead, population size (> 60% percent cover) and individual thallus biomass were greatest in one of the marginal populations, Minnie Water, 33 km south of the northern-most population sampled, Angourie. The findings of this study are contrary to studies where individual size in macroalgae declined towards distributional edges, attributed to both unfavourable abiotic conditions and life history traits (Araújo *et al.* 2011; Pearson *et al.* 2009).

The timing of daily tidal regime and local topography may also contribute to differences in morphology amongst locations and regions (Helmuth *et al.* 2002; Mislan *et al.* 2009; Mueller *et al.* 2015). For example, in studies with an intertidal mussel, tidal regime was found to differ among latitudes where low tides occurred in the middle of the day in equatorward locations compared to central populations where low tides occurred at night, potentially reducing the risk of high temperature and desiccation stress (Helmuth *et al.* 2002, 2006). If daily tidal lows occur in the middle of the day, it may create 'risky days' in which thermal and desiccation stress are intensified and cause concomitant effects in photosynthesis and growth which could create morphological variation (Helmuth *et al.* 2011; Mislan *et al.* 2009). Although tidal regime was not investigated here, it has previously been found to correlate with morphological differences amongst populations of *H. banksii* in Tasmania and New Zealand, with differences in desiccation stress suggested in both studies to contribute to the morphological variation (Bergquist 1959; Mueller *et al.* 2015).

Local environmental conditions and topography will also play an important role in in shaping morphology and physiology (Helmuth *et al.* 2010). For instance, Minnie Water is characterised by large boulders where *H. banksii* occurs in patches where water is trapped. Stress otherwise experienced by thalli on flat topography could be reduced, and thalli at this location can potentially photosynthesise for much longer than thalli sampled at other locations. This may be why thalli at this marginal population had the greatest population size (> 60 % cover) and biomass among all locations sampled. Furthermore, thalli had the greatest dissections of branching at Minnie Water, and branching occurred more often in marginal populations and amongst low shore heights compared to central populations and high shore heights. Overlapping branches are important in retaining moisture between thalli, and previous studies have found that thalli found in aggregates prolonged hydration (Bell 1992). Therefore, populations with greater percent coverage and have longer more branched thalli will be able to retain more moisture between thalli and thus photosynthesise for longer, compared to those with stunted morphologies or lower percent cover (Bell 1993).

The results of this study suggest that thalli would be at serious risk to increases in temperature, however, fatalities are not generally observed. As the intertidal is highly dynamic, local weather such as cloud cover and wind speed can modify air temperatures while humidity, wave splash, and irradiance can alter rate of thermal and desiccation stress (Helmuth *et al.* 2006, Martínez *et al.* 2012, Viejo *et al.* 2014). Furthermore, thalli were desiccated on metal grills, which have different thermal properties than basalt or sandstone which populations of *H. banksii* are found on. Finally, thalli were desiccated individually rather than in aggregates and as mentioned previously when desiccated in aggregates, thalli retain moisture for longer. These reasons may contribute to why *H. banksii* can tolerate lethal temperatures and desiccation in natural populations.

Despite thalli inhabiting warmer climates towards distributional limits, marginal populations of *H. banksii* were shown to be sensitive to increased temperatures even under favourable temperatures for photosynthesis (20 °C). This was demonstrated by a reduced capacity to recover photosynthetically from emersion stress amongst thalli from Angourie, and delayed recovery amongst thalli from Minnie Water. The small vesicle size of Angourie thalli (large SA:VOL ratio) suggests that there is less water available to replace during desiccation and thus a risk of exceeding the critical water content (Kawamitsu *et al.* 2000; Williams & Dethier 2005). The inability of thalli from Angourie to recover photosynthetically once re-immersed, suggests severe damage to photosystems. Thalli in the 20 °C treatment from marginal populations had RWC >20% suggesting that RWC recovers irrespective of desiccation and temperature. Other factors apart from emersion stress may have accounted for the delay in recovery of photosynthetic efficiency of PSII in marginal populations. Differences in chlorophyll-*a* pigment have been found to decrease with decreasing latitude which may cause sensitivity to light (Staehr & Wernberg 2009; Wernberg *et al.* 2016). Additive effects of high light intensities and high temperature have also been found to strongly correlate with distribution of species *Fucus* with heights on the shore (Ferreira *et al.* 2014; Gómez *et al.* 2004; Martínez *et al.* 2012).

2.4.3. Conclusions

The effects of variation in morphology on the functional traits of *H. banksii* at different spatial scales will not only have important implications on the biology and distribution of this species, but it will also be important in providing resilience of intertidal communities to climate warming. The results of this study indicated that marginal populations particularly the marginal edge population, Angourie, was more thermally sensitive to increases in temperature than other populations. The global increase in average ocean temperatures of 2-

3 °C predicted to occur by 2030 with global warming, may not necessarily affect intertidal macroalgae as they experience larger fluctuations in temperatures annually. Extreme climate events such as heat waves, however, may have detrimental and continuous effects on the future of *H. banksii* populations. These effects may be enhanced or buffered depending on local climate and topography, with these findings adding to the growing body of work that shows that stress experienced at the level of the organism can have more impact that large scale regional effects of climate (Helmuth *et al.* 2006).

As found in previous studies, morphological variation in *H. banksii* is not continuous (Macinnis-Ng *et al.* 2005; Ralph *et al.* 1998) despite potential high connectivity of eastern Australian populations due to the southward flowing Eastern Australian Current (Coleman *et al.* 2011a). Species of the *Fucales* are well known for their limited dispersal capacity with gametes often only being dispersed within 10 m of parental plants on the same platform (Bellgrove *et al.* 1997, 2004). It is uncertain whether the morphological variation observed in this study results from genotypic differences among populations, or phenotypic plasticity only. If these morphological differences reflect the underlying genotype and lead to increased fitness and survivorship, then local adaptation to environmental conditions may play an important role in the persistence of macroalgal populations in the face of global climate change. In conclusion, this study found regional and local differences morphology that relate to the physiological function of *H. banksii* to retain relative water content for photosynthesis during desiccation and recovery and provides a mechanistic understanding of how morphological variation contributes to differences in function at different spatial scales.

2.5. SUPPLEMENTARY FIGURES AND TABLES

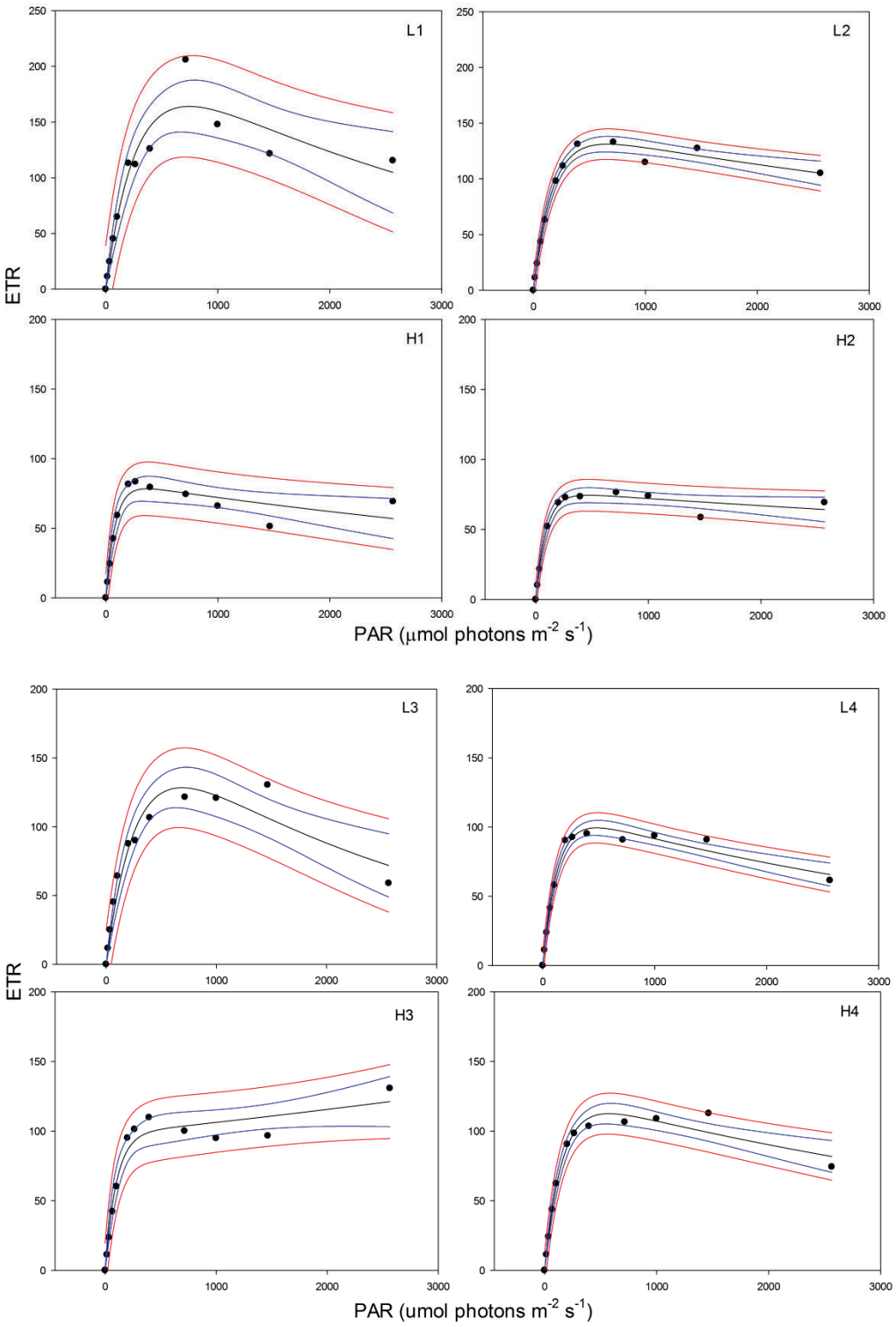

Figure S2.1: Pilot study that determined stressful light intensities for desiccation treatments. PI curves showed that 400 µmol photons m^{-2} s^{-1} was close to the saturation region and therefore an effective light intensity to use for examining stress responses. L1-4 are low shore thalli and H1-4 are high shore thalli.

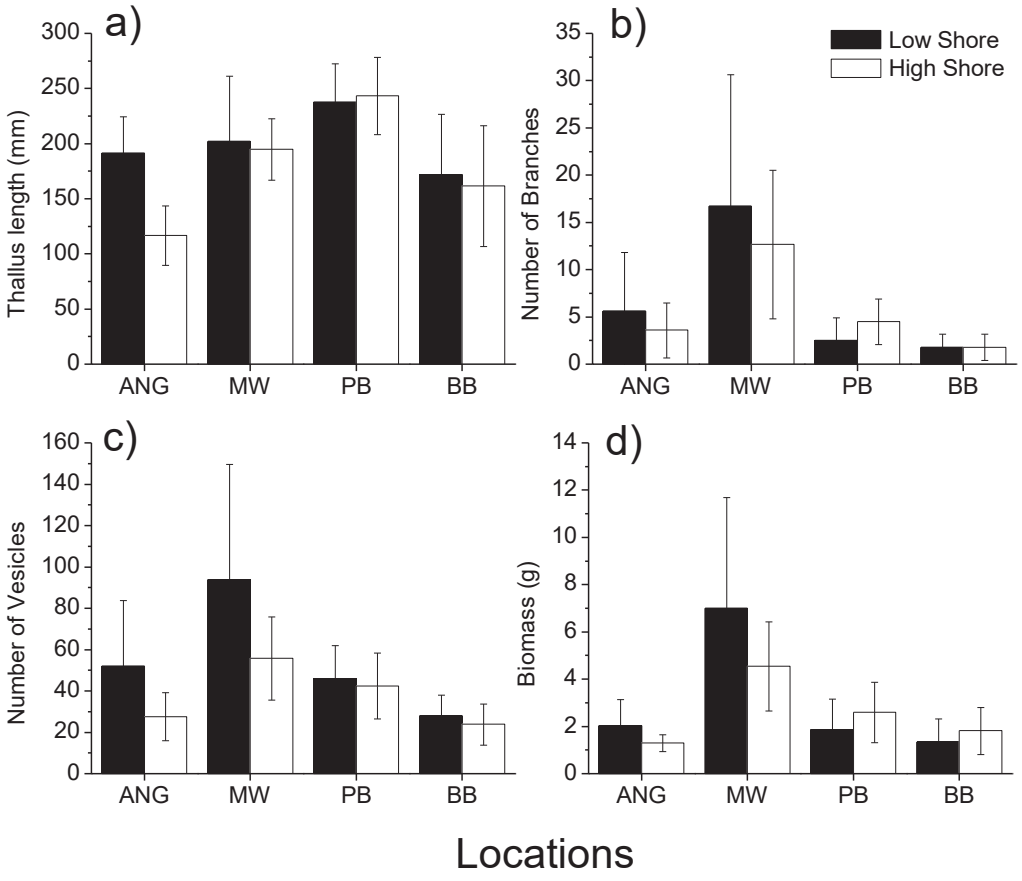

Figure S2.2: Mean ± SE of thallus morphometric traits collected from 4 locations (ANG = Angourie, MW = Minnie Waters, PB = Pearl Beach, and BB = Bilgola Beach) and two heights on the shore (n = 10).

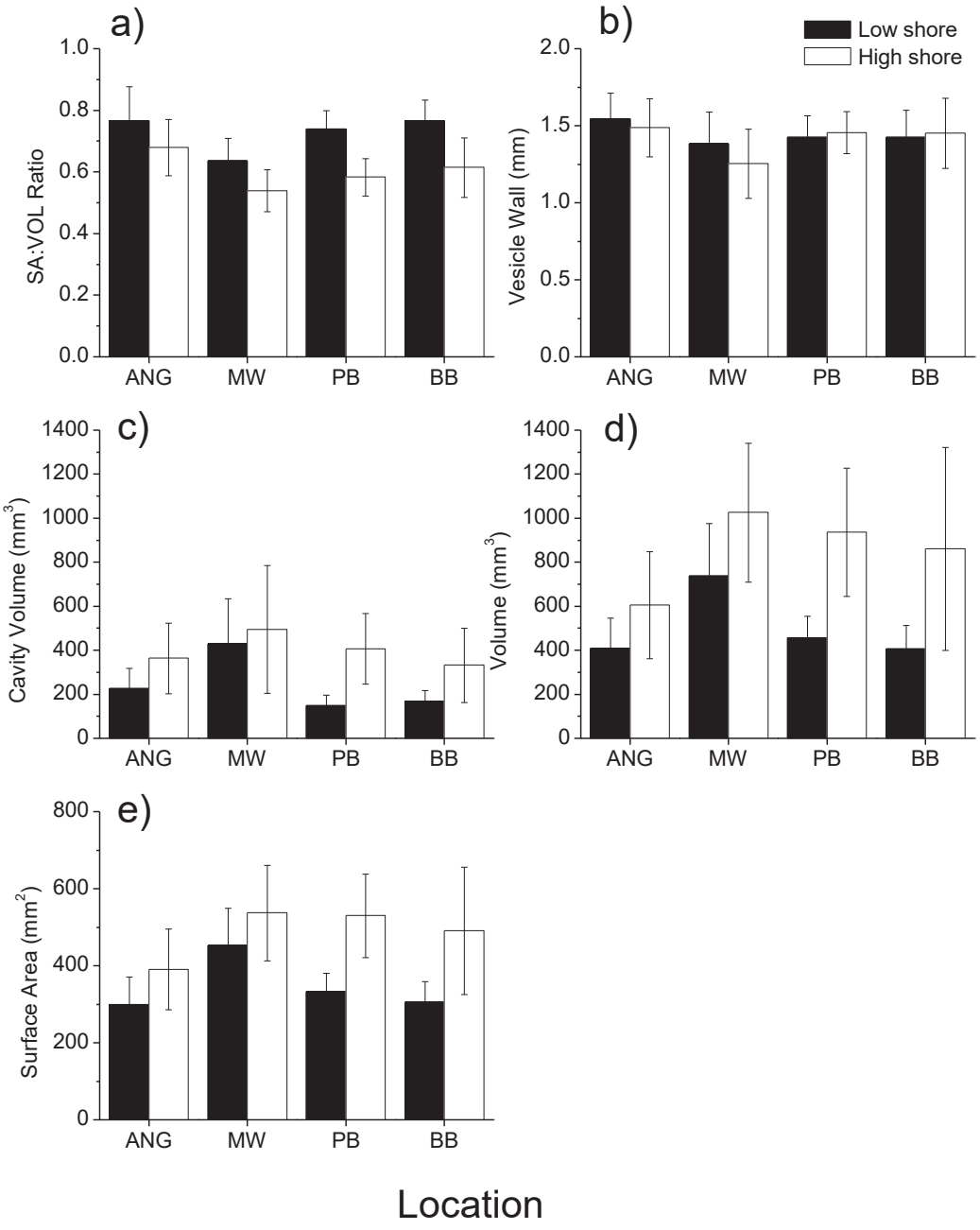

Figure S2.3: Mean ± SE of vesicle morphometric traits collected from 4 locations (ANG, MW, PB, and BB) and two shore heights (black= low shore, grey= high shore; n = 40).

Table S2.1: Mean (± SE) of thallus and vesicle morphometrics of *H. banksii* collected from low and high on the shore from four locations on Australia's east coast: Angourie, Minnie Waters, Pearl Beach and Bilgola Beach.

LOCATION	**Angourie**		**Minnie Waters**		**Pearl Beach**		**Bilgola Beach**	
SHORE HEIGHT	Low	High	Low	High	Low	High	Low	High
Thallus								
Total Length (mm)	191.38 ± 10.45	116.78 ± 8.53	202.25 ± 18.67	193.29 ± 9.08	237.66 ± 10.62	243.41 ± 11.03	171.92 ± 12.20	161.84 ±17.32
Branching (number)	5.60 ± 1.97	3.60 ± 0.92	16.70 ± 4.41	12.70 ± 2.49	2.50 ± 0.75	4.50 ± 0.76	1.80 ± 0.55	1.80 ± 0.44
Vesicles (number)	51.90 ± 10.06	27.60 ± 3.67	95.70 ± 17.44	55.80 ± 6.40	46.10 ± 7.67	42.50 ± 5.05	28.10 ± 4.00	23.90 ± 3.16
Biomass (g)	2.03 ± 0.35	1.30 ± 0.11	7.01 ± 1.48	4.55 ± 0.60	1.87 ± 0.300	2.60 ± 0.41	1.33 ± 0.15	1.81 ± 0.31
Vesicle								
Volume (mm^3)	408.55 ± 17.75	605.58 ± 31.42	737.65 ± 30.83	1026.63 ± 40.70	457.15 12.60	937.65 ± 37.59	407.50 ± 13.81	861.94 ± 59.53
Surface Area (mm^2)	299.78 ± 9.16	390.59 ± 13.52	452.93 ± 12.53	537.24 ± 15.95	332.52 ± 6.23	530.08 ± 14.04	305.09 ± 6.92	491.00 ± 21.27
SA:VOL	0.767 ± 0.014	0.679 ± 0.012	0.636 ± 0.010	0.539 ± 0.01	0.739 ± 0.01	0.583 ± 0.01	0.766 ± 0.02	0.615 ± 0.01
Wall Thickness (mm)	1.544 ± 0.022	1.488 ± 0.024	1.38 ± 0.026	1.254 ± 0.03	1.426 ± 0.02	1.45 ± 0.02	1.42 ± 0.02	1.45 ± 0.03
Cavity Volume (mm^3)	225.65 ± 11.93	364.22 ± 20.65	430.07 ± 26.35	495.26 ± 37.45	149.36 ± 6.03	406.80 ± 20.68	167.74 ± 6.53	332.77 ± 21.80

Chapter 3

Thermal variation experienced at different spatial scales reveals sensitivity to warming in a habitat-forming macroalga at the warm-edge of its distribution

3. ABSTRACT

Understanding the spatial variation of individual responses to increased temperatures is needed to predict and manage how future populations will respond to a changing climate. Physiological processes that occur at the level of the organism can have cascading effects on the distribution, abundance and fitness of organisms at significantly greater spatial scales. Here, I examined how tolerance to increased temperatures by the early life stages of an intertidal macroalga, *Hormosira banksii* varied at three scales: regional, local (with height on the shore) and among genotypes within a shore. Embryos produced from replicated males and females from high and low on the shore, and from a marginal and central population, were incubated at six seawater temperatures: 22, 24, 26, 28, 30, 32 °C (± 5 °C diel variation). Morphological (embryo length), ontogenic and photophysiological traits were measured after 120 h post fertilisation. Embryos from the marginal population (warm rear-edge) were sensitive to higher temperatures, with optimum growth occurring at a lower temperature than the central population (cool), and growth occurring in a narrower range (24–26 °C) than the central embryos (22–28 °C). High shore embryos from both populations grew faster across a wider range of temperatures than low shore embryos. The lower fluctuation in temperature in the subtropics, and at lower heights on the shore within both populations, suggests that algae from these locations may be thermal specialists, while embryos from the central population and higher on shore are more likely thermal generalists, tolerant to the greater fluctuation in temperatures experienced in those locations. Variation in thermal tolerance among genotypes within a shore, indicated by genotype-by-environment (G x E) interactions, was present but low in magnitude compared to variation in tolerance at the larger spatial scales (region, height on the shore). Projected warming will favour thermal generalists within the central population as they are tolerant of greater fluctuations in temperature, whereas thermal specialists in the marginal population may be more vulnerable to a warming climate.

3.1. INTRODUCTION

Organisms are exposed to a wide range of environmental stressors in nature, due to environments being spatially heterogeneous and temporally dynamic. While natural variation in environmental conditions occurs over various time scales (e.g., diel, seasonal), organisms are now faced with fluctuation caused by anthropogenically mediated climate change. By the end of this century, anthropogenic increases in atmospheric greenhouse gases will have increased ocean and air temperatures by 2–3 °C (IPCC 2014). Increases in the frequency, intensity and duration of extreme climate events such as storms, tropical cyclones, droughts, floods, cold spells and heatwaves are also predicted (IPCC 2014). Predicting how organisms will respond to changes in their environment depends on understanding the variation in tolerance to stressors which is often a function of previous stressor exposure, either within an organism's lifespan (acclimatisation) or in previous generations (adaptation), with each of these processes varying throughout an organism's distribution on several spatial scales (Hoffmann & Sgrò 2011; Hoffmann *et al.* 2005)

Determining the spatial scale at which variation in temperatures will most strongly influence fitness is needed to estimate the impact of warming on biological systems (Helmuth *et al.* 2014). Species' distributions can often span thousands of kilometres and individuals can be exposed to wide variation in temperature regimes at different spatial scales; regionally among latitudes, and locally, among habitats within a single location. The climate variability hypothesis proposes that seasonal fluctuation in temperature diminishes closer to the equator and generally increases with latitude (Stevens 1989; Sunday *et al.* 2011). This suggests that tropical species may be acclimatised or adapted to a narrower range of temperatures associated with lower variation in temperature seasonally, compared to central species (Stevens 1989; Sunday *et al.* 2011).

The range of temperatures physiologically tolerated by a species occupying different latitudes is thought to have developed through evolutionary processes of seasonal temperature variation, with the highest temperature of the season setting the hot temperature threshold and the coldest temperature in winter setting the cold temperature threshold (Stevens 1989; Sunday *et al.* 2012). Thermal generalists are predicted to evolve in environments that are heterogeneous in space or time whereas specialists are predicted to evolve in environments that are homogenous in either dimension (Gilchrist 1995; Kassen 2002). If organisms experience a greater fluctuating environment, it may be favourable to tolerate a wide range of conditions at the cost of having lower fitness at their optimum temperature. In contrast, if organisms experience a narrow range of temperatures, a thermal specialist strategy may be favourable where performance is maximised under an optimal temperature while the breadth of performance is reduced (Berger *et al.* 2014; Gilchrist 1995; Huey & Kingsolver 1989).

There is increasing recognition that environmental conditions experienced at local scales are also important in shaping organismal responses, with modifying factors such as local climate and topography within a habitat translating to mosaics of "hotspots" and "coldspots" across a distribution (Helmuth 2009; Helmuth *et al.* 2006; Helmuth *et al.* 2002). Morphological differences among individuals and orientation towards the sun add to local habitat topography and daily fluctuations in exposure, all of which can shape differing physiological thresholds (Harley 2008; Helmuth & Hofmann 2001). This growing body of work has highlighted that variation at the scale of individuals can have a greater effect on physiology than broad scale differences observed over kilometres (Helmuth 2009; Helmuth *et al.* 2002). Consequently, species declines in response to increasing thermal stress may not occur evenly across their range (Helmuth *et al.* 2006; Pearson *et al.* 2009) and understanding intraspecific variation in tolerance is required to predict changes across a species' range.

Intraspecific variation in thermal tolerances can arise from acclimatisation, which are changes to an organism's phenotype developed within life time through previous exposure to diel or seasonal changes in temperature, or local adaptation, where past selection and limited gene flow across generations has led to populations that vary in thermal tolerance. Local adaptation may occur on several spatial scales. At large scales, populations at the margins of biogeographic ranges are suggested to have limited gene flow from central populations suggesting that genetic diversity erodes and genetic differentiation increases towards range limits (Eckert *et al.* 2008). At small scales, adaptation can occur over a few meters if local environmental pressures create a selective gradient in which natural selection selects for tolerant genotypes (Schmidt & Bertness 2000). The likelihood of local adaption to spatial variation in thermal regimes, and for adaptation to future changes in climate, is also dependent on the levels of heritable genetic variation in thermal tolerance (Clark *et al.* 2013; Foo *et al.* 2014; Foo *et al.* 2012).

The rocky intertidal has been suggested to be a sentinel habitat for global warming, primarily due to resident organisms being already close to their thermal limits (Somero 2010; Stillman & Somero 2000). Habitat-forming macroalgae are particularly important primary producers in this habitat due to their role as ecosystem engineers through modifying local environmental conditions and providing resources (Dayton 1972; Jones *et al.* 1994) that can strongly facilitate associated biodiversity (Schiel 2006). Studies on the effects of temperature in governing species distribution and marginal ranges are relatively few amongst macroalgal dominated communities (but see Bennett *et al.* 2015; Ferreira *et al.* 2014; Martínez *et al.* 2012; Pearson *et al.* 2009). Temperature is a fundamental determinant of algal fitness as it regulates photosynthesis as well as enzymes that govern metabolic activity (Allakhverdiev *et al.* 2008; Falkowski & Raven 2013). Variation in thermal tolerance among marine macroalgae

has been mostly studied in the context of determining how lethal temperatures set distributional limits both across species ranges and vertically on the shore (Davison & Pearson 1996; Hawkins & Hartnoll 1985; Schonbeck & Norton 1978). On regional scales, individuals in marginal populations have been shown to have decreased resilience to heat and desiccation stress (Pearson *et al.* 2009) and reduced size (Zardi *et al.* 2015), suggesting that populations are physiologically stressed towards range limits (Araújo *et al.* 2011). Variation in thermal tolerances on the local scale, among heights on the shore, is well known and is related to daily tidal regimes and topography, and the ability to effectively photosynthesise during periods of increased desiccation and thermal stress (Brown 1987; Chapman 1995; Dring & Brown 1982; Schonbeck & Norton 1978; Williams & Dethier 2005). Less well understood is the contribution of heritable genetic variation in thermal tolerance, required if there is to be any local adaptation to a given thermal regime or evolution of increased tolerance with increasing temperatures. Genetic variance in thermal tolerance has been documented in the early life stages of only two species of macroalgae, the subtidal macroalga, *Fucus vesiculosus,* (Al-Janabi *et al.* 2016) and the intertidal, habitat-forming macroalga, *Hormosira banksii* (Clark *et al.* 2013).

In this study, I quantify multiple performance traits of an ecologically and functionally important intertidal macroalga, *Hormosira banksii,* to assess variation in thermal tolerance at three nested spatial scales: among regions, among heights on the shore and among individual genotypes within a shore. *H. banksii* is a dominant macroalga on wave swept rocky shores in Australia and New Zealand. Its gamete dispersal is typically less than 10 m (Bellgrove *et al.* 1997, 2004), suggesting that local adaptation to spatial variation in thermal regimes could occur due to the strong selection gradients in the intertidal and limited gene flow among populations (Coleman *et al.* 2011a). I focus on microscopic embryos as they are potentially

more susceptible than adults to anthropogenic and natural stressors and are a critical early life history stage leading to recruitment (Brawley & Johnson 1991).

3.2. METHODS

3.2.1. Study sites and collection of samples

H. banksii populations were sampled during the austral autumn (April - May) at two locations in eastern Australia: a warm marginal location, Minnie Water, at the northern rear-edge of its distribution, (29º46'34.23" S, 153º18'07.43" E) and a central location, Pearl Beach, 460 km further south (S 33º32'57.70", E 151º18'32.36") towards the cooler climates within the centre of its range. *H. banksii* was the dominant, intertidal macroalgal species (60% percent cover) at both locations. *H. banksii* is found at a single location further north than Minnie Water but hosts rockpool ecotypes only. Annual variation in thermal exposure at the regional scale was estimated using water temperature as detected by satellite (MODIS-Aqua), obtained via GIOVANNI (NASA GES DISC, http://giovanni.gsfc.gov.au). Local weather stations were used to estimate regional scale air temperature (Bureau of Meteorology, Australian Government, http://www.bom.gov.au). To document the temperature variability within *H. banksii* beds at both locations, single HOBO® pendant loggers (Onset®, USA) were drilled to the substrate at high and low tidal heights and temperatures were recorded between April and July. Loggers only overlapped for 7 days in June, therefore were only used to view the temperature variability within each shore height at each location.

Adult thalli were collected two hours before absolute low tide to prevent desiccation-induced spawning (Gunthorpe *et al.* 1995). Thalli were collected from the low shore, directly adjacent to the seaward edge of the rock platform, and the high shore, in the upper intertidal, 5–10 m

distant from low shore region. Thalli were transported on ice and gametes extracted within 48 hours.

3.2.2. Effects of temperature on embryo growth and ontogenesis

Embryos of *H. banksii* were grown under six temperatures to quantify the relative importance of variation in thermal tolerance among regions, among heights on the shore and among genotypes. To induce spawning, thalli were gently agitated in tap water (room temperature), blotted dry, placed into individual containers and allowed to desiccate at room temperature. After 20 min, gametes were released from conceptacles and the sex of the thallus identified by the colour of its gametes: olive green for females and orange for males (Osborn 1948). Three males and three females from each shore height within each location were used in a North Carolina breeding design (Lynch & Walsh 1998), where each male was cross fertilised with each corresponding female in a fully factorial design, yielding nine unique embryo crosses. Each egg and sperm solution was filtered through nylon mesh (100 μm for egg solution and 40 μm for sperm solution) to filter out debris and larger algal material before being mixed to initiate fertilisation. Aliquots of each egg and sperm solution were distributed amongst multiple petri dishes filled with 0.7 μm (Whatman GFF) filtered seawater containing coverslips for zygotes to attach to.

Petri dishes with settled zygotes were then randomly allocated to each of six temperatures; 22, 24, 26, 28, 30 and 32 °C, with the latter two temperatures representative of high temperatures for these populations at the warmer edge of their distribution. To examine thermal responses under more realistic fluctuating environments (and hence estimate realized rather than fundamental thermal reaction curves; Paaijmans *et al.* 2013), a 5 °C diel cycle was implemented. This temperature regime was determined from examining field data from the

HOBO® pendant loggers. Embryos were incubated in a 12:12 hour light cycle at 30 ± 5 µmol photons $m^{-2}s^{-1}$ to simulate the light environment under an adult canopy.

At 120 h after fertilisation, embryos growing on coverslips were removed from treatments, wet mounted on a microscope slide and photographed using a light microscope (Olympus BX50, Japan) with AnalySIS imaging software (v 5.0, Japan). Total embryo length (defined as extension along the primary rhizoid axis) was calculated from digital images using Image J (National Institutes of Health, USA V1.6.0_24). To examine stages of development, embryos were scored into five ontogenetic stages: 0 = fertilisation through condensation of the chloroplasts; 1 = protrusion of embryo cell wall to create a pear shape which later develops into the rhizoid; 2 = division of the embryo germinating cell and elongation of a single rhizoid; 3 = elongation of the rhizoid coupled with secondary and tertiary rhizoid development; and 4 = paraphysis development on top of the germinating cell. These equate to stages 1, 2, 3-5 and 6 (a-d), respectively, in Clarke and Womersley (1981).

3.2.3. Effects of temperature on photophysiological traits

A pulse modulated fluorometer (Microscopy-PAM, Walz GmbH, Germany) was used to examine photophysiological traits of individual embryos. The system comprises a modified epi-fluorescence microscope (AxioScope.A1, Zeiss) equipped with a modulated LED light source and a photomultiplier for detection of modulated chlorophyll-a fluorescence. Embryos attached to cover slips (120 h post fertilisation) were wet mounted and scanned under green light (non-stimulatory for photosynthesis). Selection of embryos for measurement involved maximising the number of embryos in a field of view, as coverslips were discarded after each fluorescence assay. Gain settings were adjusted so that base fluorescence (F_t) was between 0.1 and 0.3 for each embryo, ensuring fluorescence signals were detectable but not too high to

cause saturation during measurements. To use F_t as a proxy for pigment content, values collected at different gain settings were standardised to a single gain setting to make them comparable amongst all embryos.

The measurement protocol involved dark adapting embryos for 5 min before a single saturating pulse of blue light (blue Zeiss LED-Module 470 nm; pulse duration = 0.6 s; pulse intensity > 3000 µmol photons m^{-2} s^{-1}; F_M determination), followed by a two-step steady-state light curve. The steady state analysis included consecutive 5 min exposures to actinic blue light of 32 and 113 µmol photons $m^{-2}s^{-1}$ (sub-saturating and saturating irradiance, respectively, previously determined in a steady state P vs I fluorescence curve). Saturating pulses of blue light were spaced 30 s apart to monitor F_M, and F_t. Due to the length of time needed for measurements, photosynthetic measures were only taken for 24, 28 and 32 °C.

Maximum quantum yield of photosystem II (PSII), F_V/F_M, was calculated according to the equation $(F_M-F_O)/F_M$ (Schreiber 2004). Effective quantum yield of PSII, $\Delta F/F_M$', was calculated using $(F_M'-F_t)/F_M'$. The proportions of energy being used in photochemistry Y(PSII), regulated non-photochemical quenching Y(NPQ), i.e. energy dissipation through the rapid conversion of xanthophyll pigments) and unregulated non-photochemical quenching of excitation energy (Y(NO; i.e., heat dissipation) were calculated for each actinic light level assuming Y(PSII) + Y(NPQ) + Y(NO) = 1 according to (Kramer *et al.*). To estimate the capacity of embryos to deal with high light, the relative NPQ between high light (HL) and low light (LL) steps was calculated: (HLY(NPQ)/LLY(NPQ). A value less than one means there is less NPQ under high light and the xanthophyll cycle has exceeded its capacity to deal with excess energy.

3.2.4. Statistical analyses

Variation in temperature data obtained by HOBO® pendant sensors within low and high shore *H. banksii* beds were analysed using Levene's test of variance. Variation in embryo length and ontogenic development was analysed with permutational ANOVA, with location (Minnie Water and Pearl Beach), height on the shore (low and high) and temperature (six levels) as fixed factors, and male and female identity as a random factor nested within each combination of height on the shore and region. Photophysiological traits were analysed using ANOVA with location, height on the shore and temperature as fixed factors (with the low fecundity of some combinations requiring that males and females were pooled). Univariate ANOVAs were conducted in the PERMANOVA routine of Primer (v6) and the proportion of variance explained by each factor calculated by least square estimates of variance components (Graham & Edwards 2001).

3.3. RESULTS

3.3.1. Temperature regimes

Annual air temperatures ranged from 9.7 to 26.7 °C at Minnie Water (marginal), and 4.8 to 27.6 °C at Pearl Beach (cooler; Fig. 3.1a, b). On an annual basis, the range of air temperature was lower at Minnie Water compared to Pearl Beach (17.0 °C vs 22.8 °C respectively; Fig. 3.1a, b). Minnie Water experiences fewer days per year where air temperature exceeds 35 °C than Pearl Beach (on average ~1 vs ~8 d y^{-1}, respectively; Fig. 3.1a). Sea surface temperature was also greatest at Minnie Water compared to Pearl Beach, ranging from 20.9 to 25.3 °C vs 18.8 to 23.4 °C in 2013 (the year of sampling), respectively.

At the local scale, HOBO® sensors recorded similar mean temperatures for low and high on the shore within each location at Minnie Water (21.9 ± 2.8 °C vs 21.5 ± 3.6 °C respectively,

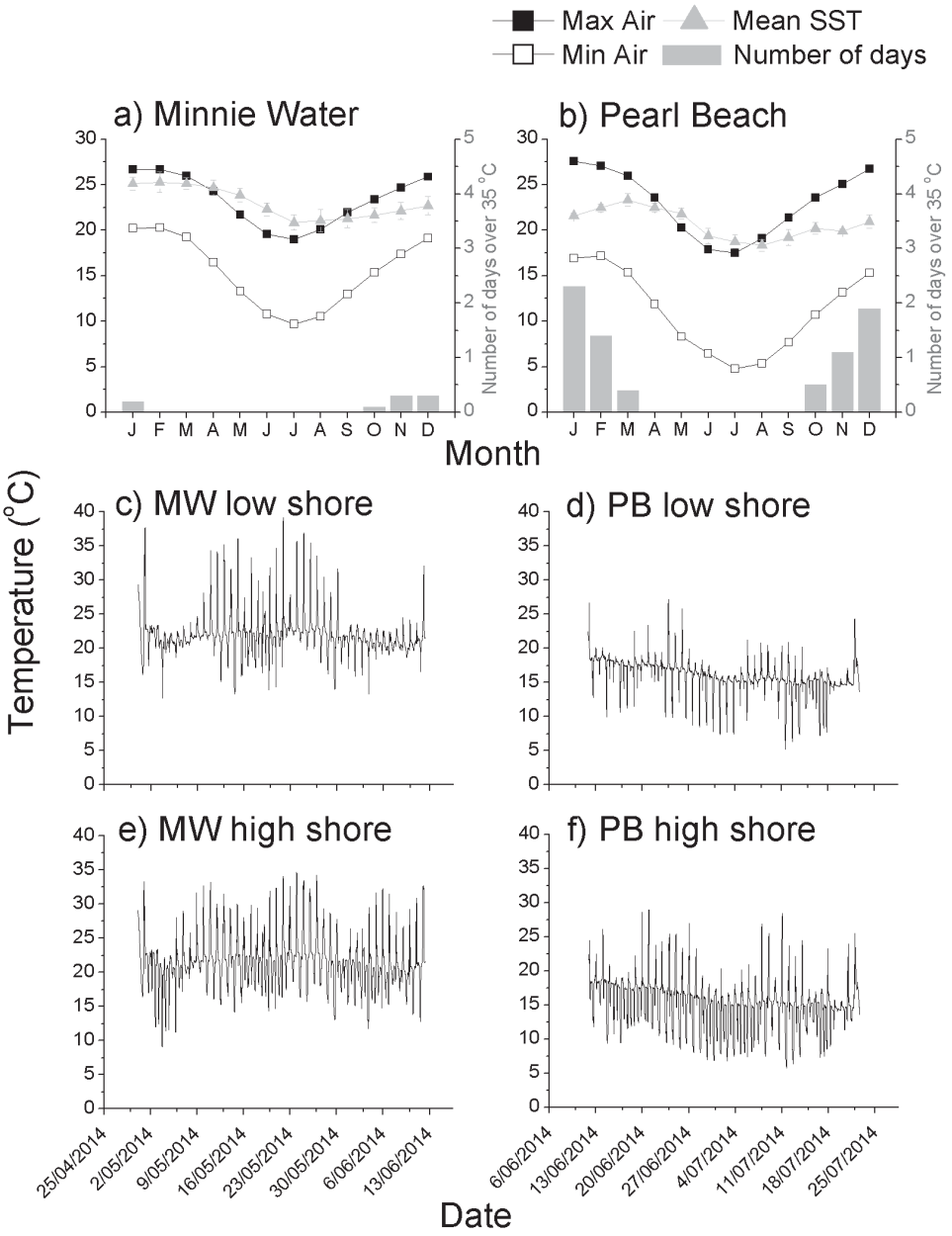

Figure 3.1: Mean maximum (■) and minimum (□) air temperature recorded by the Bureau of Meteorology between 1877 and 2013, and mean (± SD) offshore sea surface temperature (as detected by MODIS-Aqua satellite in 2013; ▲) for Minnie Water (a) and Pearl Beach (b). Grey columns represent the number of days over 35 °C. Lower plots show local intertidal temperature within the adult canopy (recorded using HOBO® pendent sensors that logged temperature every 10 min during a deployment in austral autumn) at low on the shore (c, d) and high shore height (e, f) at Minnie Water (marginal) and Pearl Beach (central), respectively.

Fig. 3.1c, e) as well as Pearl Beach (16.0 ± 2.4 °C vs 15.3 ± 3.5 °C respectively, Fig. 3.1d, f). Dispersion of temperature data, however, showed high shore temperatures were more variable than low shore temperatures at each location (Minnie Water: $F_{1,2074}$= 55.89, P = 0.001; Pearl Beach: $F_{1,1960}$, P = 0.001).

3.3.2. Effects of temperature on embryo growth and ontogenesis

Temperature had a strong effect on embryo length with the effect of temperature varying with location, and height on the shore (Fig. 3.2, Table 3.1). Temperature interacted most strongly with location, resulting in distinct thermal performance curves at each location (Fig. 3.2, significant location by temperature interaction, 26% of the total variation in embryo length, Table 3.1). The thermal performance curves of the marginal population (Minnie Water) were narrower and showed a 2 °C shift towards cooler temperatures in the thermal optimum compared to the central population (Pearl Beach, Fig. 3.2). Embryos from Minnie Water exhibited reduced growth at temperatures beyond 26 °C, whereas those from Pearl Beach grew slower beyond 28 °C. Embryos from Minnie Water reached maximum lengths across a narrower range of temperatures (24–26 °C; Fig. 3.2) than those from Pearl Beach (22–28 °C; Fig. 3.2). At the most extreme temperature, 32 °C (outside the temperature range for both populations; Fig. 3.1, 3.2), embryos from Pearl Beach grew 3-4 fold slower than their thermal optima (~2.5 µm day^{-1}) and those from Minnie Water 4-5 fold slower. Growth at 32 °C was characterised by enlargement of the germinating cell rather than through rhizoid development.

At some of the temperatures tested, embryos from high on the shore reached a greater maximum length than those from the low shore (Fig 3.2, significant interaction between height on the shore and temperature, Table 3.1). At Pearl Beach, embryos from high on the shore were longer than those on low on the shore when grown at lower temperatures (22–26

°C), but were of similar length at higher temperatures (28–32 °C) (Fig. 3.2b). At Minnie Water, there was little difference between embryos from the high and low shore (Fig. 3.2a).

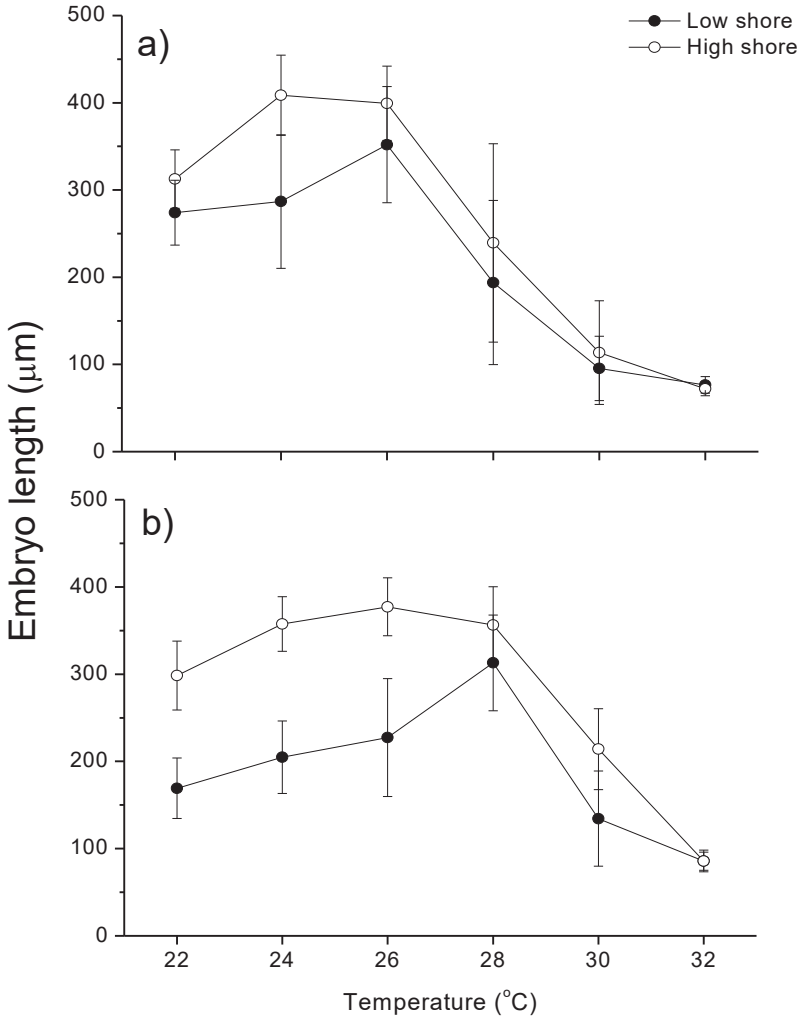

Figure 3.2: Mean (± SD) embryo length after 5 days at 6 different temperatures (22, 24, 26, 28, 30 and 32 ° C) for Minnie Water (a) and Pearl Beach (b) population. Closed circles (●) represent embryos from low shore adults whereas open circles (○) represent embryos from high shore adults. Data from all crosses are pooled. (n = 54).

Table 3.1: Results of analysis of variance of *Hormosira banksii* embryo length 120 h post fertilisation (defined as embryo growth in this study). Embryos from Pearl Beach and Minnie Waters at low and high shore heights in 9 different crosses were grown at 6 temperatures (22, 24, 26, 28, 30 and 32 ºC). Location (Lo), height on the shore (He), and temperature (Te) are fixed factors, with male (Ma) and female (Fe) nested in shore height and location as a random factor. Results were achieved using 999 permutations and tested at a significance level of 0.05.

ANOVA Source	**Embryo Length**			
	df	*F*	*P*	Effect Size (%)
Lo	1	0.75	0.568	0.00
He	1	22.48	**<0.001**	11.38
Te	5	62.60	**<0.001**	36.48
Lo x He	1	1.79	0.155	0.84
Lo x Te	5	23.67	**<0.001**	26.86
He x Te	5	3.99	**<0.001**	3.55
Ma(Lo x He)	8	3.55	**0.014**	1.74
Fe(Lo x He)	8	1.12	0.405	0.08
Lo x He x Te	5	1.45	0.119	1.08
Te x Ma(Lo x He)	40	1.03	0.454	0.09
Te x Fe(Lo x He)	40	1.47	0.070	1.34
Ma(Lo x He) x Fe(Lo x He)	16	9.66	**<0.001**	1.84
Te x Ma(Lo x He) x Fe(Lo x He)	80	6.70	**<0.001**	7.25
Res	1080			7.63

Bold denotes significance at P < 0.05

The effect of temperature did not vary significantly with male identity for embryo length (temperature x male interaction; Table 3.1), which would provide evidence of heritable genetic variation in thermal tolerance. The effect of temperature, however, did vary with parental identity (a significant temperature x male x female interaction, Fig. 3.3, Table 3.1) with ~7% of the variance in the entire data set attributed to the variation in temperature effects among male/female combinations.

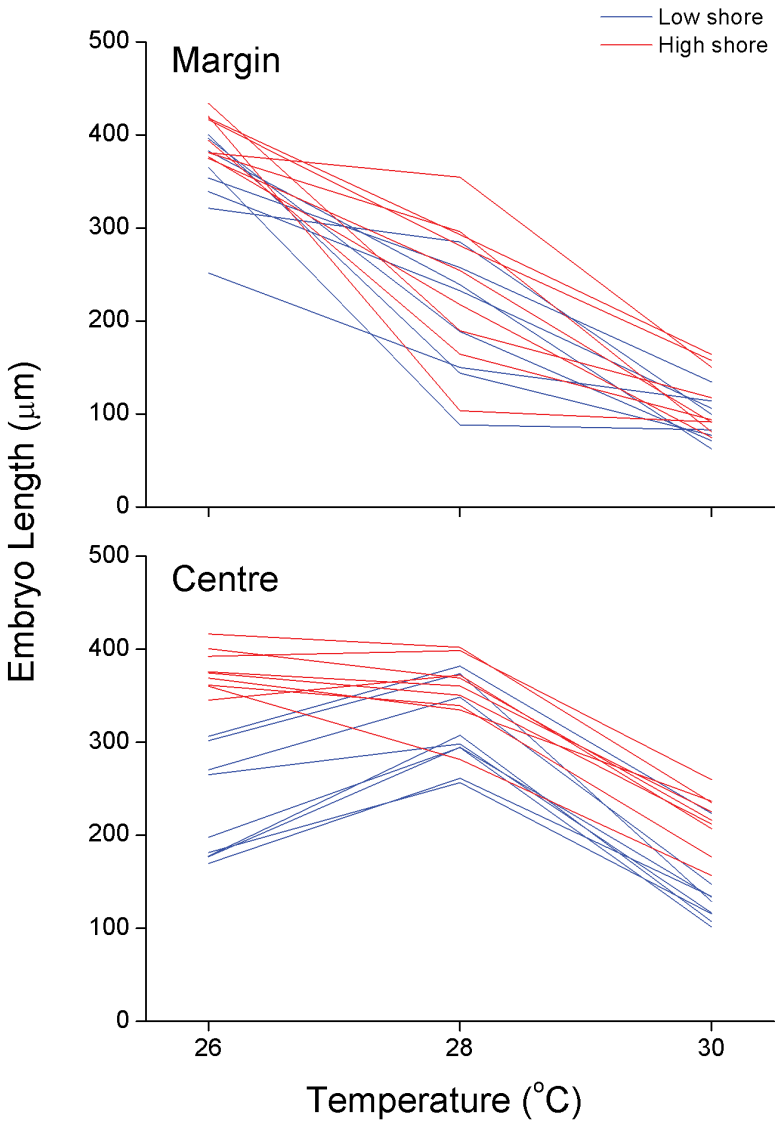

Figure 3.3: Thermal reaction norms showing *H. banksii* embryo length at 120 h across 26, 28 and 30°C at Minnie Water (a) and Pearl Beach (b). Lines represent mean embryo length of each male and female cross (n=6 embryos per cross). Blue lines represent embryos from low on the shore and red lines are embryos from high on the shore.

The effects of temperature on the ontogeny of embryos varied among locations, heights on the shore and among genotypes (Fig. 3.4). Overall, embryos from Minnie Water developed more rapidly than those from Pearl Beach (significant location by temperature interaction, Table 3.2), with up to 60% of Minnie Water embryos reaching stage 3 or 4 after 5 days in contrast to only 20% from Pearl Beach (Fig. 3.4 a, b). The proportion of Minnie Water embryos with delayed development (i.e. stage 0) increased steadily in temperatures that surpassed optimal

temperatures for growth (24–26 °C) reaching ~85% at 32 °C. At Pearl Beach, between 5 and 15% of embryos had delayed development across all temperatures except for embryos at 32 °C which ~40% of embryos were delayed in development (Fig. 3.4c and d). The proportion of embryos in stage 3 and 4, or with delayed development in stage 0, did not vary with height on the shore in either population (non-significant temperature x height on shore interactions, Table 3.2). The effect of temperature, however, varied significantly with male identity for the proportion of embryos in stage 3 or 4 (Table 3.2) indicating that there is genetic variation in the effects of temperature on rates of development.

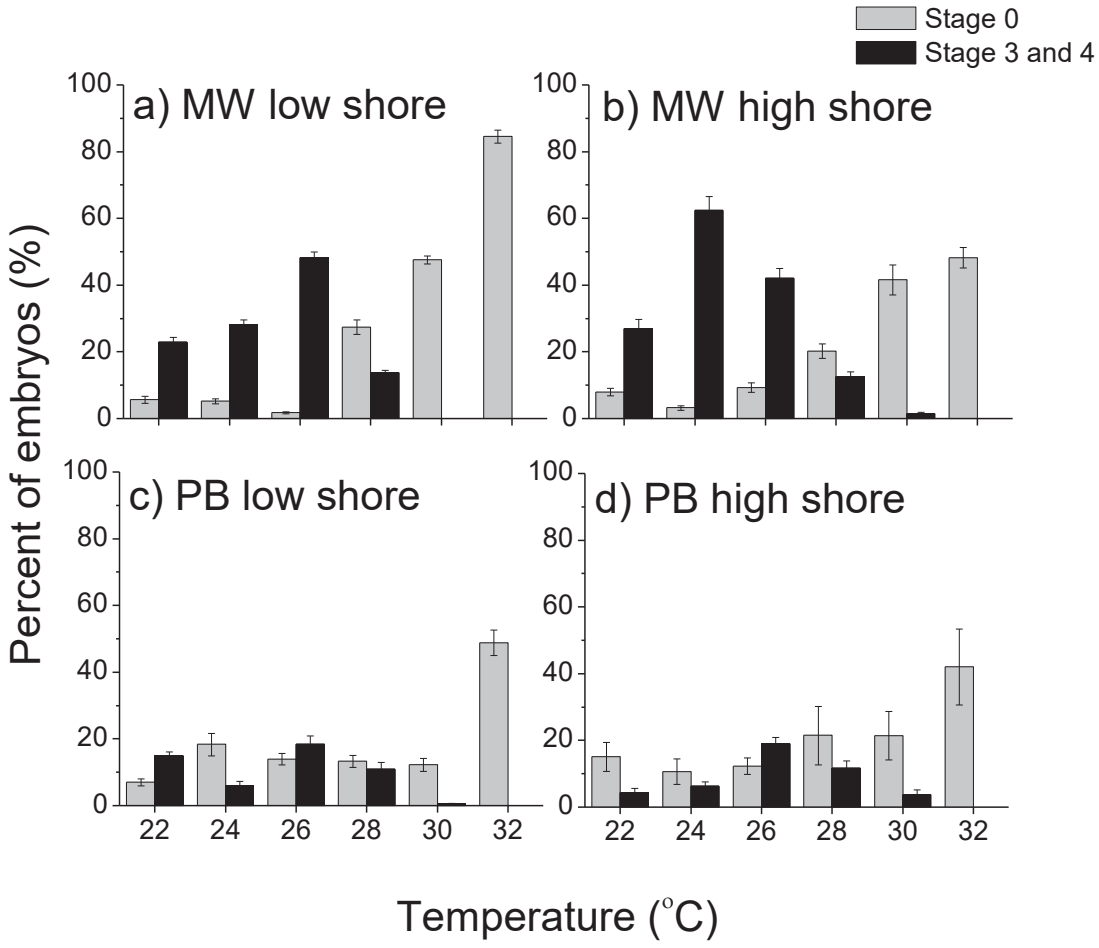

Figure 3.4: Percentage (± SD) of *H. banksii* embryos reaching stage 3 and 4 or remaining in stage 0 after incubation at 6 different temperatures (22, 24, 26, 28, 30 and 32 ° C) for 5 days following fertilisation. Embryos are from marginal (warm) Minnie Water (a, b) and central (cool) Pearl Beach (c, d). Grey columns represent ontogenic stage 0 (fertilisation to condensation of the chloroplasts) amongst low shore (a, c) and high shore (b, d). Black columns represent stages 3 and 4 (elongation of the rhizoid coupled with secondary and tertiary rhizoid development and paraphysis development on top of the germinating cell) amongst low shore (a, c) and high shore (b, d). Data represent pooled crosses (n= 40).

Table 3.2: Results of analysis of variance of the percent of *Hormosira banksii* embryos in each developmental stages, stage 0 and stage 3 & 4 at 120 h after fertilisation. Embryos from Pearl Beach and Minnie Waters from low and high on the shore in 9 different crosses were grown at 6 temperatures (22, 24, 26, 28, 30 and 32 ºC). Location (Lo), height on the shore (He), and temperature (Te) are fixed factors, with male (Ma) and female (Fe) nested in shore height and location as a random factor. Probabilities were calculated using 999 permutations and tested at a significance level of 0.05.

	Developmental stage				
Source	Stage 0			Stage 3 & 4	
	df	*F*	*P*	*F*	*P*
Lo	1	0.71	0.596	8.08	**0.002**
He	1	0.32	0.950	0.38	0.895
Te	5	10.60	**0.001**	9.30	**0.001**
Lo x He	1	0.50	0.784	0.61	0.730
Lo x Te	5	2.23	**0.009**	4.06	**0.001**
H x Te	5	1.09	0.341	1.11	0.338
Ma (Lo x He)	8	2.80	**0.042**	4.51	**0.004**
Fe (Lo x He)	8	2.30	0.060	1.54	0.218
Lo x He x Te	5	0.77	0.720	1.12	0.337
Te x Ma (Lo x He)	40	0.85	0.718	1.95	**0.006**
Te x Fe (Lo x He)	40	1.84	**0.013**	1.28	0.162
Ma (Lo x He) x Fe (Lo x He)	16	1.38	0.148	1.02	0.439
Res	80				

Bold denotes significance P < 0.05

3.3.3. Effects of temperature on photophysiological traits

Temperature had a direct effect on the photochemical efficiency of PSII at a sub-saturating and saturating light intensities (LY(II), HY(II)) amongst all embryos, but there was no variation among location or heights on the shore (Table 3.3; Fig. S3.1). Embryos all showed a similar response to increasing temperature, with maximum quantum yield and photosynthetic efficiency being relatively constant between 24 °C and 28 °C and decreasing at 32 °C (Fig. S3.1 and S3.2). Maximum quantum yield (F_V/F_M) was generally greater in embryos from Minnie Water compared to Pearl Beach embryos but did not differ among heights on the shore (Fig. S3.1, Table 3.3).

Embryos from different heights on the shore used regulated nonphotochemical quenching as a means of photoprotection, with Y(NPQ) remaining similar under ambient and high light intensity (Fig 3.5, Table 3.3). Embryos from Minnie Water, however, diverted more energy proportionally to Y(NPQ) under high light intensities than those from Pearl Beach (Fig. 3.5). This was also evident with the ratio of regulated nonphotochemical quenching under ambient and high light (HL: LL Y(NPQ), Fig. 3.6, Table 3.3) where the embryos from Minnie Water had ratios above 1 (indicating increased regulated quenching of energy at saturating light intensity) compared to embryos from Pearl Beach which had ratios below 1 (indicating increased unregulated quenching, or potential photodamage under high light). Baseline fluorescence (F_t), a proxy for photosynthetic pigment content, was significantly greater in the embryos from Pearl Beach and those from high on the shore (Fig. S3.3, Table 3.3). For all photophysiological traits, there were no significant interactions between temperature and location or height on the shore, indicating that embryo responses to temperature did not vary at regional and local scales (Table 3.3).

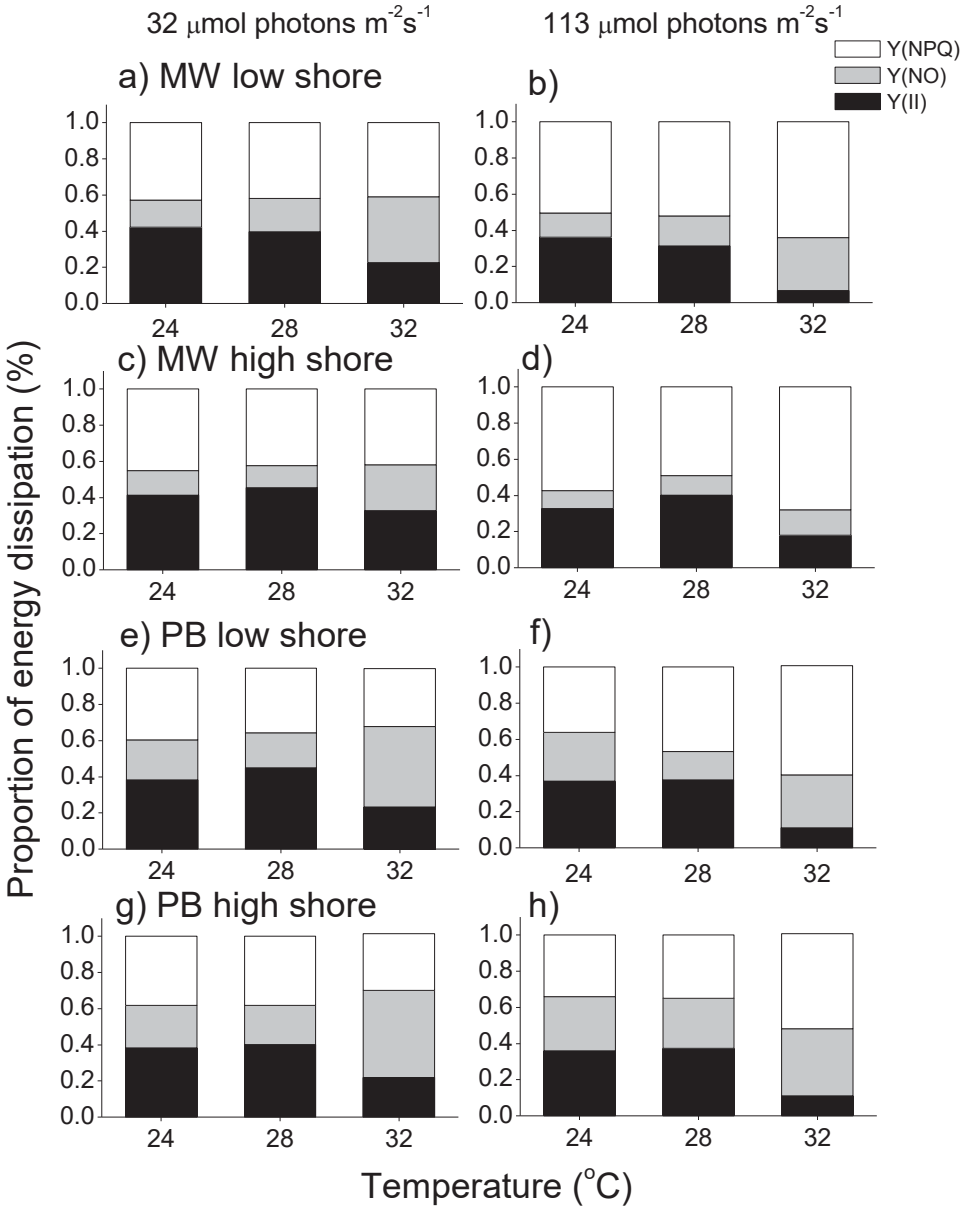

Figure 3.5: Mean proportion of energy dissipated amongst three complementary pathways photochemistry Y(II), unregulated nonphotochemical quenching Y(NO) and regulated nonphotochemical quenching Y(NPQ) in embryos from Minnie Water (a-d) and Pearl Beach (e-h) from low on the shore (a, b, e, f) and high on the shore (c, d, g, h) after incubation for 5 days at three temperatures: 24, 28 and 32 °C. Photophysiological measurements were made under two light irradiances: 32 μmol photons m-2 s-1 (a, c, e, g) and 113μmol photons m^{-2} s^{-1} (b, d, f, h).

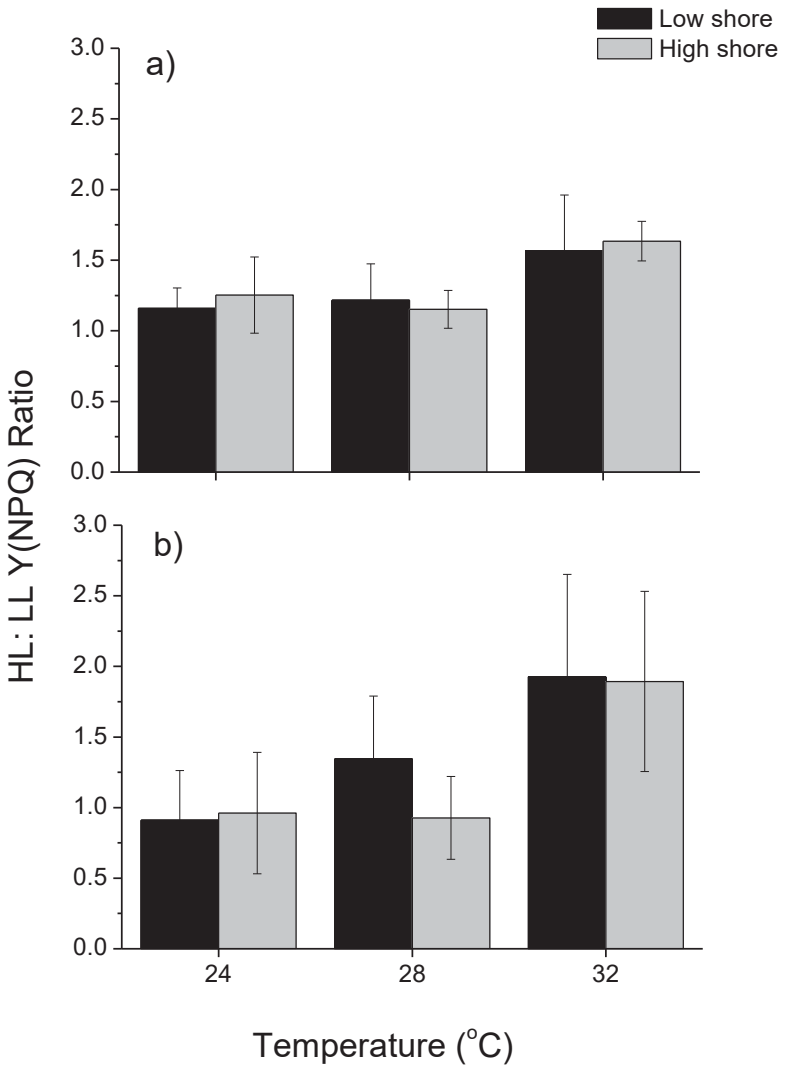

Figure 3.6: Mean (±SD) ratio of regulated nonphotochemical quenching (Y(NPQ)) amongst high and ambient light intensities (113 and 32 µmol photons m^{-2} s^{-1}, respectively) for embryos from low and high on the shore from two locations a) Minnie Water and b) Pearl Beach.

Table 3.3: Results of analysis of variance of the temperature effects on F, maximum quantum yield (F_V/F_M), complementary photosynthetic pathways of photosynthesis Y(II), nonregulated nonphotochemical quenching Y(NO) and regulated nonphotochemical quenching Y(NPQ). Location (Lo) height on the shore (He) and temperature (Te) are fixed factors. Probabilities were calculated using 9999 permutations and tested at a significance level of 0.05.

Source		**F**		**F_V/F_M**		**HL:LL YNPQ**	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Lo	1	12.17	**0.003**	4.39	**0.050**	0.09	0.790
He	1	4.83	**0.038**	1.91	0.178	0.74	0.430
Te	2	1.61	0.222	5.40	**0.011**	9.35	**< 0.001**
Lo x He	1	0.69	0.417	0.09	0.771	0.84	0.397
Lo x Te	2	0.82	0.446	0.89	0.433	2.18	0.123
He x Te	2	0.06	0.938	0.02	0.981	0.46	0.671
Lo x He x Te	2	0.10	0.902	0.86	0.4373	0.18	0.872
Res	23						
Source		**LY(II)**		**LY(NO)**		**LY(NPQ)**	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Lo	1	0.46	0.500	3.20	0.091	5.44	**0.028**
He	1	0.56	0.468	0.20	0.666	0.01	0.922
Te	2	8.76	**0.001**	6.50	**0.006**	0.89	0.433
Lo x He	1	1.37	0.255	0.73	0.405	0.01	0.927
Lo x Te	2	0.62	0.562	0.40	0.681	0.09	0.918
He x Te	2	0.26	0.783	0.05	0.947	0.04	0.964
Lo x He x Te	2	0.55	0.578	0.10	0.913	0.29	0.755
Res	23						
Source		**HY(II)**		**HY(NO)**		**HY(NPQ)**	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Lo	1	0.52	0.477	6.66	**0.016**	8.03	**0.009**
He	1	1.47	0.252	0.001	0.982	0.58	0.444
Te	2	25.29	**< 0.001**	1.70	0.207	3.68	**0.044**
Lo x He	1	1.81	0.190	2.68	0.112	0.54	0.467
Lo x Te	2	0.44	0.659	0.24	0.783	0.28	0.757
He x Te	2	1.18	0.327	0.19	0.828	0.74	0.482
Lo x He x Te	2	1.11	0.346	0.21	0.809	0.19	0.831
Res	23						

Bold denotes significance at P < 0.05

3.4. DISCUSSION

Determining the sensitivity of organisms to their changing environment requires an understanding of variation in their responses to climate change stressors at multiple spatial and temporal scales. The results of this study suggest embryos from adults exposed to greater temperature variation (i.e., at the central location and from high on the shore), are physiologically more tolerant to a broader range of temperatures compared to embryos exposed to lower fluctuations (i.e., marginal location and from low on the shore). Furthermore, embryos from the central location have a higher thermal optimum for growth and rates of development compared to embryos from the marginal location, which is somewhat counterintuitive. Importantly, significant genotype-by-environment interactions (male x temperature interaction) were found for embryo ontogenetic development which suggests that there is genetic variation in thermal tolerance traits. These results have important implications as they suggest that marginal populations may be sensitive to increases in temperature that are forecast for the future. The loss of rear-edge populations will have cascading effects on the genetic diversity of the species, but also on other organisms that depend on *H. banksii*.

3.4.1. Regional scale variation in thermal tolerance

The significant temperature by location interactions for growth and rates of development suggests that the regional scale differences in thermal regimes at the study locations influenced embryo growth and development (Table 3.1, 3.2). Embryos from the central location were tolerant of a broader temperature range compared to those from the marginal location that had a 2 °C shift towards cooler temperatures in their thermal optimum. The increased thermal sensitivity to high temperatures and lower thermal optimum in embryos from the marginal location is contrary to previous research where populations in lower

latitudes have been found to be more tolerant of higher temperatures, as they generally experience higher temperatures throughout their life history (Gerard & Bois 1988; Kelly *et al.* 2012; Stillman & Somero 2000; Sunday *et al.* 2012). For example, in congeneric species of porcelain crabs in the genus *Petrolisthes,* species found in lower latitudes consistently had greater thermal tolerances than species found in higher latitudes (Stillman & Somero 2000). The measurements among *H. banksii* populations in different regions, however, showed that while average air temperatures may have been higher at Minnie Water than Pearl Beach, the marginal location experiences lower seasonal variation in temperature and fewer days over 35 °C compared to the central location (Fig. 3.1). This thermal regime is associated with embryos having a narrower thermal optimum for growth and development (24–26 °C) and is consistent with the climate variability hypothesis (Stevens 1989). Bennett *et al.* (2015) recently showed that a non-edge population of a subtidal macroalgal species had similar thermal safety margins to marginal populations but different absolute temperature tolerances, and cautioned that not all species at their distributional limits have a narrower thermal safety margin. Our data reflect similar patterns with embryos showing different absolute temperature tolerances, however, exhibited narrower safety margins in marginal populations. One explanation for this pattern is that intertidal species are exposed to environmental stress imposed by the terrestrial and marine environment, therefore differences such as emersion and air temperature variation may be more important in shaping thermal safety margins, particularly for the central populations sampled.

Populations at range margins have been previously reported to be physiologically stressed due to proximity of physiological limits to temperature thresholds (Hairston *et al.* 2005; Pearson *et al.* 2009; Zardi *et al.* 2015). In this study, embryos from the marginal (rear-edge) and central locations were found to have similar effective quantum yields at both sub-saturating and saturating irradiances (Staehr & Wernberg 2009). Photosynthesis may be

uncoupled from growth during early life stage development of macroalgae, with reserves of mannitol being utilised as a source of energy (Major & Davison 1998). While no measurements of energy reserves in *H. banksii* embryos were made, this could provide a reason why differences were not evident in photosynthetic efficiency during this study. In addition, the faster growth and development of embryos from the marginal location at non-stressful temperatures may be indicative of a higher metabolic rate with long-term exposure to warmer temperatures, which in turn increases the acquisition of carbon (Davison & Pearson 1996). Conversely, slower rates of development among embryos from the central location at similar temperatures may be indicative of a trade-off in order to grow across a broader range of temperatures (Gilchrist 1995).

The lack of any interactions between temperature and location for photophysiological parameters in *H. banksii* suggests that embryos have a high degree of plasticity, and can adjust their photosystems to tolerate differences in light and temperature regimes. Despite reductions of growth at 28 °C for marginal embryos and 30 °C for central embryos, *H. banksii* was still able to maintain a high level of PSII efficiency in ambient and stressful light intensities across 24 and 28 °C, suggesting acclimation of photosystems (Major and Davison 1998). The adjustment of photosystems to different temperatures and light intensities to optimise photosynthesis may be an important trait for intertidal macroalgae as temperature and light gradients can change rapidly. In sporophytes of the subtidal kelp *Ecklonia radiata,* physiological performance was maintained in higher temperatures through an increase in critical light demand (E_C) (Staehr and Wernberg 2009). This reduction allowed for similar levels of light limited photosynthesis to be achieved in warm and cool adapted populations found at different latitudes, consistent with this study. While these results provide evidence for variation in thermal tolerance between two populations that differed in their thermal

regimes, further spatial sampling is required for formal tests of latitudinal variation in thermal tolerance, or differences between range-edge and central populations.

3.4.2. Local scale variation in thermal tolerance

Within a location, embryos of *H. banksii* from high on the shore differed in their response to temperature compared to those low on the shore. Low shore embryos from the central location grew fastest at 28 °C while high shore embryos showed greatest rhizoid extension from 24 to 28 °C. Embryos from high on shore at Pearl Beach were almost double the length of low shore embryos between 22 to 26 °C, but showed similar growth from 28 to 32 °C. Temperature loggers placed amongst *H. banksii* on the lower and upper shore confirmed that the individuals high on the shore experienced greater temperature variation than those lower on the shore at both locations.

These results are consistent with a growing body of research that suggests that local scale topography and environmental conditions may be more important in driving physiology and species' distributions than larger regional effects of climate (Helmuth 2009; Helmuth *et al.* 2006; Helmuth *et al.* 2002). For example, local scale topography and environmental conditions experienced by individuals of the intertidal mussel *Mytilus californianus* can result in body temperatures varying between 6 to 13° C within a population at a given time (Harley 2008; Helmuth & Hofmann 2001). Consequently, temperatures experienced by individuals may not be easily predicted by larger scale variation in temperatures (e.g., among latitudes), but instead be a mosaic of smaller scale hotspots and coldspots. In this study, the marginal location, Minnie Water, is characterised by large boulders (Fig. S3.4) that can shade *H. banksii* and trap small pools of water, potentially reducing the stress experienced by individual thalli in contrast with temperatures experienced on flatter rock platforms such as

Pearl Beach (Fig. S3.4). The shore morphology at Minnie Water could thus modify the thermal exposure of individuals at this location and lead to similar growth rates of embryos from low and high on the shore in this marginal population.

Maintaining thermal tolerance across broader temperatures can be physiologically costly, therefore high shore embryos may not grow optimally across all temperatures (Huey *et al.* 2002). In this study, high shore embryos had a reduced thermal optimum at 24 °C (Minnie Water) and 26 °C (Pearl Beach) relative to embryos from low on the shore (Fig. 3.2). The lower thermal optima amongst high shore embryos compared at both locations, however, may reflect increased energy dissipation (i.e., regulated non-photochemical quenching; YNPQ) and decreased photochemistry (YII) with increased temperatures and light intensities (Table 3.3) likely experienced for longer periods during low tide high on the shore (Davison & Pearson 1996). In addition, the reduced growth and narrow thermal optima amongst low shore embryos in both populations may reflect light limited photosynthesis of adults as they experience longer periods spent submerged, while optimising growth within a narrow range of temperature that they most commonly experience (Huey *et al* 2002). There were no interactions between temperature and height on the shore for photosynthetic parameters, suggesting phenotypic plasticity for these photophysiological traits. Given that intertidal macroalgae at different heights on the shore must contend with greater variation in light and temperature during daily tidal cycles, it suggests that photosystems need to be able to acclimate to different light and thermal regimes within short time periods (Hanelt *et al.* 1997). Over longer time scales, adaptation of the population at the local scale involving genotypes tolerant to the prevailing thermal and light regime may also be important (Al-Janabi *et al.* 2016; Hanelt *et al.* 1997).

3.4.3. Organismal scale variation in thermal tolerance

Within locations and heights on the shore, there was further variation in thermal responses among individuals and genotypes of *H. banksii*. The presence of heritable genetic variation for rates of ontogenetic development (indicated by significant male by temperature (G x E) interaction) indicates the potential for selection by temperature to alter early life history traits. This is consistent with earlier investigations of this species (Clark *et al*. 2013) and suggests that as mean temperatures increase, genotypes that are better able to tolerate higher temperatures will be favoured (Deutsch *et al.* 2015; Fusi *et al.* 2015; Sunday *et al.* 2012). A significant interaction between female identity and temperature was also found for the proportion of embryos that did not develop (stage 0), suggesting a role for either female genotype or non-genetic maternal effects in thermal responses. Maternal effects have been identified previously in different organisms (e.g. fish, Chambers & Leggett 1996; bryozoans, Marshall 2008; terrestrial plants, Galloway *et al.* 2009; sea urchins, Foo *et al*. 2014) and are potentially relevant in *H. banksii* where egg size differs amongst different females (J.Clark unpublished and Chapter 5). As maternal effects can influence the genotypic and phenotypic traits of the offspring, the maternal environmental history may impact the resources available for reproduction which can affect egg size and growth trajectory of offspring (Wolf & Wade 2009). For growth, there was no significant male by temperature interaction, but an interaction between temperature and parental identity (i.e., male x female x temperature; Table 3.1). This suggests that different genotypes are more susceptible to different temperatures. There were no interactions between temperature and male or female identity for any of the photosynthetic parameters, suggesting that photosynthesis is highly regulated amongst individuals. This agrees with previous studies in which no heritable genetic variation was found in *H. banksii* photosynthetic traits (Clark *et al.* 2013).

The likelihood of adaptation to a changing thermal regime is dependent on levels of standing genetic variation. Similar to a previous study on two temperate populations of *H. banksii* (Clark *et al.* 2013), the current study provides evidence for genetic variation in growth responses to temperature in natural populations. Contrasting the relative magnitude of within-population variation to variation in thermal responses on larger spatial scales, this study shows that the interaction between temperature and location comprised an effect size of 27% of the total variation in growth, the interaction between temperature and heights on the shore with an effect size of 3.6%, and the interaction between temperature and effect size of male-female combination as 7.3%. This within-population variation in thermal tolerance will be particularly important under a changing climate as populations with greater diversity will have a broader suite of tolerant genotypes for selection to act upon (Reusch *et al.* 2005). Al-Janabi *et al.* (2016) found that *Fucus vesiculosus* early life stages were more resilient to environmental stress when they were from high genetic diversity populations, compared to embryos from low genetic diversity populations. An understanding of the genetic diversity of *H. banksii* across its distribution will therefore be useful to examine the potential for genotypic sorting, and hence improve predictions of how this species will respond to future warming.

3.4.4. Further considerations for predicting Hormosira banksii response to warming

Here I used embryos of *H. banksii* to investigate thermal responses because they were more amenable to manipulation and are potentially more vulnerable than their adult counterparts (Brawley & Johnson 1991; Nielsen *et al.* 2014a). For example, early life stages may have lower thermal plasticity compared to adults, because adults can adjust metabolic and photosynthetic rates in order to maximise survival at the expense of growing under stressful conditions (Alestra & Schiel 2015). Temperature could also impact other life history processes such as fertilisation, germination and recruitment (see Harley *et al.* 2012, Andrews

et al. 2015), and may therefore play a more complex role in the response of *H. banksii* to warming. Importantly, as early life stages are precursors to future populations, what occurs during the juvenile stage may have important repercussions and bottlenecks to future populations of macroalgae.

In the interest of understanding effects of global warming, this study only investigated temperature, however, it is recognised that other stressors are important in determining the vulnerability of macroalgae to environmental change. This includes abiotic factors (ocean acidification, altered salinity and nutrient regimes, sea-level rise), biotic interactions (competition, herbivory, disease) as well as other anthropogenic stressors (habitat destruction, pollution). Similarly, understanding the effects of low temperature stress on thermal performance is also important as cold tolerances may exert more influence on macroalgal traits (Zardi *et al.* 2015). As lethal temperatures in both summer and winter prune out intolerant genotypes through seasonal mortalities, a better understanding of responses to both ends of the thermal spectrum is required to increase the accuracy of predictions.

3.4.5. Conclusions

The findings of this study indicate important variation in thermal tolerance of *H. banksii* at several scales: among individuals, between heights on the shore and between location. Species with high phenotypic plasticity, broad physiological tolerances as well as potential to adapt given genetic variation in traits that relate to thermal tolerance, are likely to be best able to adjust to new temperature regimes (Bates *et al.* 2014; Gilchrist 1995; Huey *et al.* 2012). The range edge population of *H. banksii* had optimal performance in a narrow range of temperatures, consistent with historical exposure to lower variation in temperature. Consequently, this population may be more susceptible to future warming than the central population and potentially other temperate locations, as embryos were more sensitive to

higher temperatures. In contrast, the central population has experienced greater fluctuations in temperature, with its broader thermal response curve creating a 'thermal buffer' against increased temperatures (Ketola *et al.* 2013). The projected increase in mean temperatures of 2 to 3 °C predicted with global warming within the next decade (IPCC 2014), may not necessarily cause mortality to central populations as these species are exposed to a wide range of daily environmental gradients and extreme temperatures, particularly in summer months. Rather, the increase in average temperatures may cause physiological effects such as damage to the photosynthetic apparatus which may have downstream effects such as stunted growth, decreases in fecundity and declines in long-term recruitment success. The sensitivity to higher temperatures in marginal populations, however, may make them susceptible to discrete climate events such as heat waves (Bennett *et al.* 2015; Wernberg *et al.* 2010). If temperatures surpass lethal temperatures for prolonged periods during these thermal excursions, we may see declines and subsequent shifts in populations if species fail to recover from these events (Smale & Wernberg 2013).

3.5. SUPPLEMENTARY FIGURES

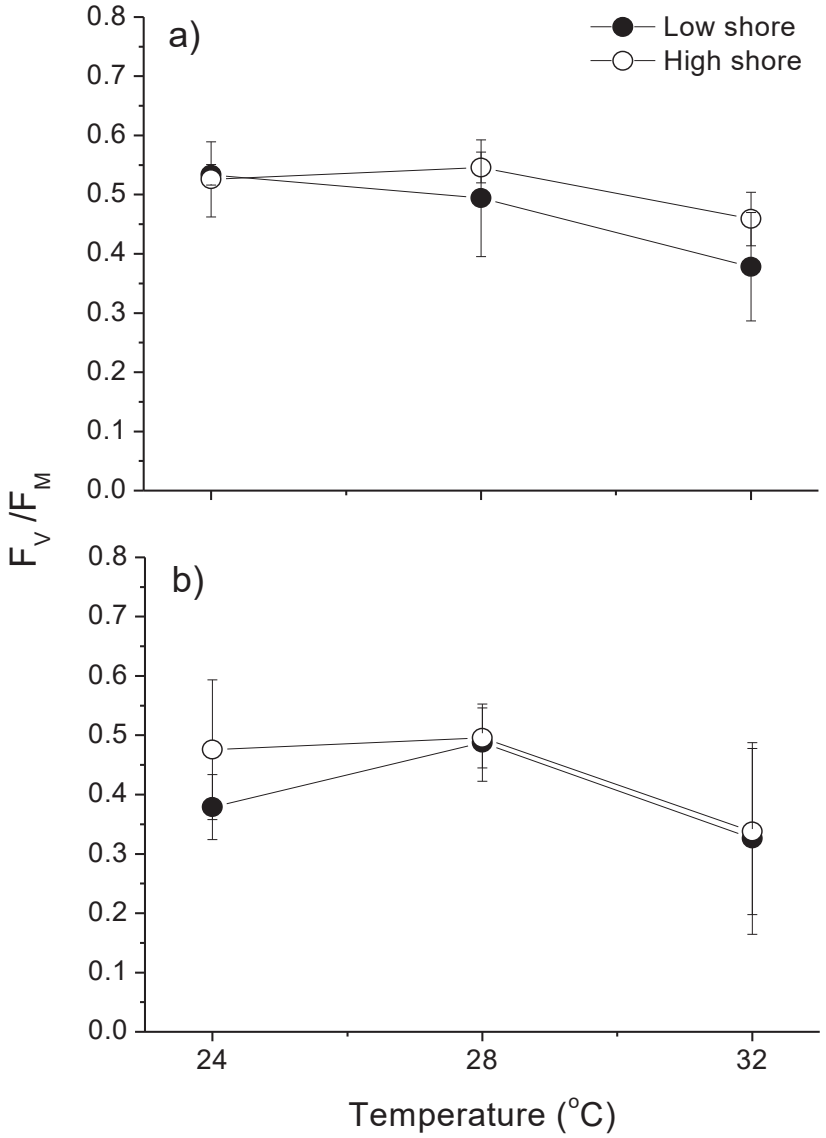

Figure S3.1: Mean (± SD) maximum quantum yield of PSII (F_V/F_M) of embryos from Minnie Water (a) and Pearl Beach (b) after incubation for 5 days at three temperatures: 24, 28, 32 °C. Closed circles (●) indicate F_V/F_M measured from embryos low on the shore and open circles (○) indicate F_V/F_M measured from embryos high on the shore.

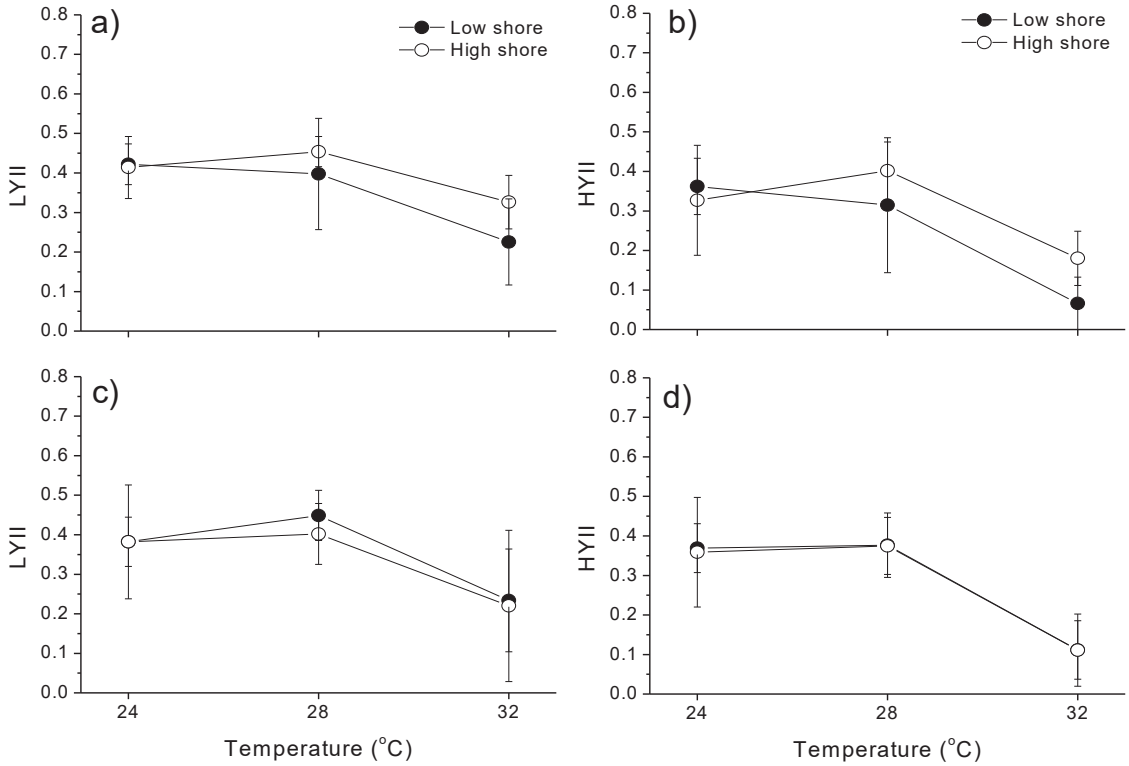

Figure S3.2: Mean (± SD) effective quantum yield of PSII (photosynthetic efficiency) of embryos measured under low light (LYII; 32 µmol photons m^{-2} s^{-1}) and high light (HYII; 113 µmol photons m^{-2} s^{-1}) from Minnie Water (a, b) and Pearl Beach (c, d) after 5 days incubation at three temperatures: 24, 28, 32 °C. Closed circles (●) indicate Y(II) of embryos low on the shore and open circles (○) indicate Y(II) of embryos high on the shore (n=6).

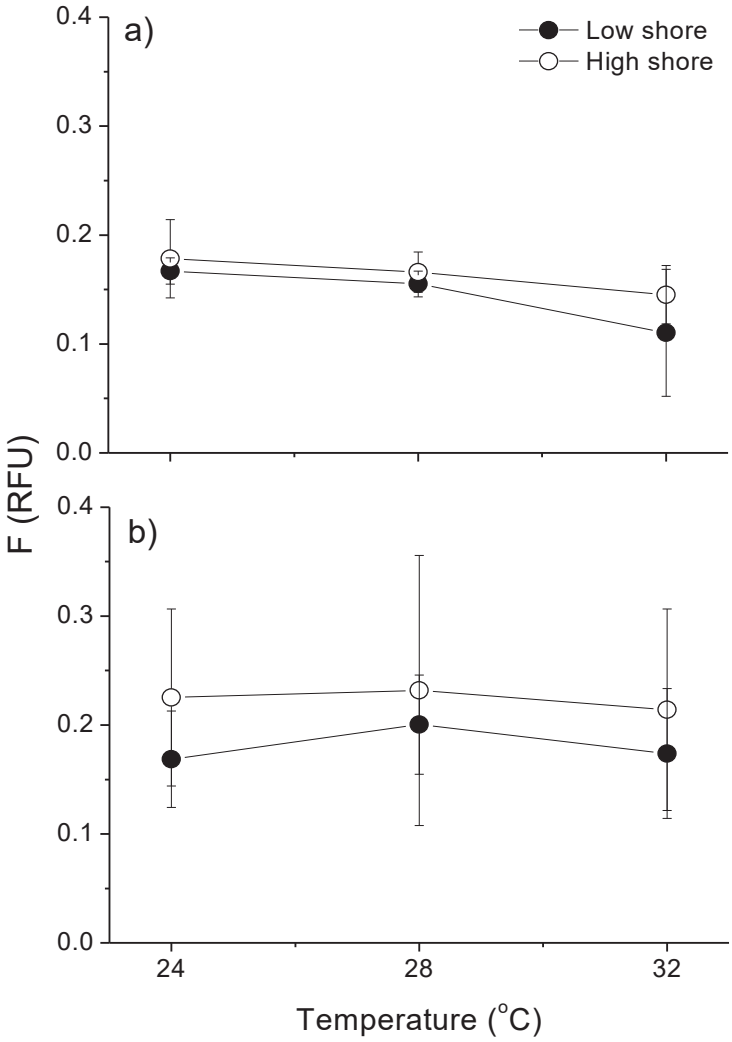

Figure S3.3: Mean (±SD) baseline chlorophyll-a fluorescence (F_t) of embryos from Minnie Water (a) and Pearl Beach (b) after incubation for 5 days at three temperatures: 24, 28, 32 °C. Closed circles (●) indicate F_t measured from embryos low on the shore and open circles (○) indicate F_t measured for embryos high on the shore.

Figure S3.4: The habitat of *H. banksii* at the marginal location, Minnie Water (top), and central location, Pearl Beach (bottom) indicating differences in local-scale topography.

Chapter 4

Genetic structure of a habitat-forming macroalga at multiple spatial scales

4. ABSTRACT

Predicting how organisms will respond to perturbations including global warming requires an understanding of underlying patterns of genetic diversity, structure and gene flow. Gene flow maintains connectivity between and genetic diversity within populations, which may confer resilience to perturbations. Populations that inhabit chronically warmer locations (e.g. rear-edge populations) are suggested to be physiologically stressed and have smaller effective population sizes due to limited gene flow and lower estimates of genetic diversity. Likewise, strong selection pressure across environmental gradients can create genetic structure at small scales (meters). In this study, patterns of genetic diversity and structure were characterised in the habitat-forming macroalga *Hormosira banksii* at three different spatial scales: between marginal and central regions (100s km apart), between locations (10s km apart) and locally between vertical tidal heights (10s m apart). Results revealed strong genetic structure between neighbouring populations (>10 km) with evidence of isolation by distance over long distances (~ 500 km). Genetic diversity (expected heterozygosity) appeared to be lower in marginal populations. In contrast, no genetic structure was found between tidal heights, suggesting that the reproductive strategy of *H. banksii* may limit gene flow over long distances, but not over small distances within a single location. As lower genetic diversity and limited gene flow are linked to reduced adaptive potential, *H. banksii* may be vulnerable to future warming at the equatorward (warm) limits of its distribution. This highlights the importance of conservation strategies, including protected areas, in maintaining connectivity, particularly for species with limited dispersal capacity.

4.1. INTRODUCTION

A challenging task in ocean change biology is determining the impact that global warming will have on biological systems and predicting the response of organisms to perturbations in climate regimes (Parmesan & Yohe 2003). Ultimately, for populations to be resilient to warming climates, organisms must undergo evolutionary adaptation to global warming and this requires adequate genetic diversity for natural selection to act upon (Hoffmann & Sgrò 2011; Reusch *et al.* 2005). The underlying genetic diversity of a species captures the variation of genotypes present and the extent of their phenotypic plasticity, which in turn provides a range of responses to stress that can lead to resilience from perturbations (Reusch *et al.* 2005). Prediction of how species will respond to global warming requires knowledge of how past evolutionary processes have shaped current genetic diversity within a species biogeographic range, extant connectivity among populations as well as whether genetic diversity confers resilience to warming and other anthropogenic mediated stressors.

Species living within the centre of their distribution are suggested to be living within optimal environmental conditions for growth, reproduction and survival with these conditions as well as organismal fitness diminishing towards distributional limits (Bridle & Vines 2007). Reduced fitness at distributional limits often coincides with reduced gene flow and connectivity, habitat fragmentation (local separation of populations) and reduced effective population sizes (Coleman *et al.* 2011a; Eckert *et al.* 2008). Many studies have found trends of decreasing genetic diversity and increases in genetic differentiation towards species range edges (for a review see Eckert *et al.* 2008; Provan 2013). Regionally, genetic structure among populations is largely governed by gene flow or connectivity which, for sessile marine species, is determined by species dispersal capacities and the strength, presence and

alternations in vectors of dispersal such as ocean currents, geographic barriers, and seasonal climate variability (Billot *et al.* 2003; Coleman *et al.* 2011b; Muhlin *et al.* 2008).

On local scales, strong environmental gradients can create selective pressure which can cause fine-scale genetic structure over scales as small as a few meters (Pardo & Johnson 2005; Schmidt & Bertness 2000). Intertidal environments are one such environment, where abiotic gradients are extreme and highly heterogeneous both spatially and temporally. Daily tidal regimes create strong gradients of temperature, light and desiccation, increasing from the ocean's edge to higher on vertical shores (Davison & Pearson 1996). As temperature and desiccation are well known factors that govern distribution of species along vertical tidal heights (Schonbeck & Norton 1978), genetic structure may be evident. This directional pressure selects for organisms that have high phenotypic plasticity or organisms that have adapted to a highly dynamic environment and can create distinct distributional patterns (Zardi *et al.* 2011). It is suggested that high tidal heights represent an extreme environment that may favour only a few well-adapted genotypes (Innes 1988).

Macroalgae are important foundation species (Dayton 1972), because of their autogenic role in facilitating biodiversity through moderation of environmental stressors as well as providing resources such as food, shelter and habitat to other organisms (Jones *et al.* 1994). Despite patterns of decreasing genetic diversity reported towards species range limits (Eckert *et al.* 2008; Provan 2013), at chronically warmer, low-latitude populations (rear-edge) of species distributions, estimates of genetic diversity in macroalgal populations have been reported to vary. For example, there was no centre-marginal gradient in genetic diversity reported for the intertidal macroalga, *Fucus guiryi* (Zardi *et al.* 2015). In contrast, the subtidal *Saccorhiza polyschides* showed increasing genetic diversity towards marginal populations, despite reports

of bottlenecks (Assis *et al.* 2013). The intertidal alga, *Fucus vesiculosus,* was found to follow a centre-marginal gradient with lower genetic diversity found in equatorward populations (Teixeira *et al.* 2016). These differences in genetic diversity patterns may reflect different evolutionary histories where current distribution boundaries may be the result of recolonization from refugia following the last glacial maximum (Assis *et al.* 2013; Buchanan & Zuccarello 2012; Nicastro *et al.* 2013; Provan 2013).

On small spatial scales, genetic structure in macroalgae has been related to reproductive traits such as dispersal capacity and reproductive mode as well as habitat characteristics (intertidal vs subtidal; Valero *et al.* 2011). In a review of 17 species of *Laminariales*, species that were located mainly in the intertidal were found to have significant patterns of genetic differentiation at the smallest scale (< 1 km), whereas species that had morphological structures such as air bladders that favoured long distance dispersal had the lowest levels of genetic structure (Valero *et al.* 2011). Furthermore, it has been suggested that genetic structure in marine species is more related to adult habitat depth than duration of the pelagic stage (Kelly & Palumbi 2010) and that the intertidal landscape limits migration between high and low shores (Valero *et al.* 2011).

There are limited studies that have analysed genetic structure across very small scales such as between intertidal heights, despite the potential for these strong environmental gradients to structure dispersal and gene flow. The few studies that have been undertaken show that intertidal habitats can strongly influence genetic structure. For example, in *F. distichus* significant genetic structure was found across spatial scales as small as 10 cm suggesting genetic isolation due to limited water exchange between rockpools (Coleman & Brawley

2005b). High genetic structure on small spatial scales (across tidal heights) has also been found in a red alga inhabiting rockpools (Engel *et al.* 2004).

Eastern Australia has been identified as a climate change hotspot (Lough & Hobday 2011) and is therefore an ideal location to study the effects of global warming on the genetic diversity and structure of marine macroalgae. The east coast of Australia follows a north (equatorward) to south (poleward) gradient with a natural warm to cold water transition interface at approximately 28-30° S. The East Australian Current is a poleward flowing current and serves as the main conduit for gene flow for many sessile marine species within coastal ecosystems (Coleman *et al.* 2011b). It is suggested that rear-edge populations found closer to the equator lack genetic diversity as the EAC flows polewards and restricts gene flow (Coleman *et al.* 2011b). The EAC has been found to serve as a good facilitator of dispersal for subtidal species of macroalgae, but perhaps not for intertidal species, including *Hormosira banksii* (Coleman *et al.* 2011a).

Hormosira banksii is the dominant habitat-forming intertidal macroalga found in temperate rocky reefs in Australia and New Zealand (Millar & Kraft 1994; Osborn 1948; Womersley 1987). Its distribution follows a latitudinal gradient with its warm (equatorward) biogeographic limit at a cold to warm interface at ~28 °S. In related intertidal taxa (e.g. *Fucus*) gene flow has been suggested to be limited due to gamete release being restricted to calm waters at high tide (Serrão *et al.* 1996), or low, neap tides (Pearson & Brawley 1996) with eggs being negatively buoyant and male gametes being negatively phototactic resulting in rapid fertilisation (Pearson & Serrão 2006). Due to this, gametes and zygotes of fucoids are often only dispersed within meters (Brawley 1992; Dudgeon *et al.* 2001; Pearson & Brawley 1996; Serrão *et al.* 1996) with *H. banksii* reported to typically disperse their gametes less than

10 m (Bellgrove *et al.* 1997, 2004). Additionally, cell walls secrete extracellular substances which facilitates rapid adhesion to the substrate (Dimartino *et al.* 2016; Taylor & Schiel 2003). Although these reproductive strategies ensure 100% fertilisation success, gene flow can often be limited (Coleman & Brawley 2005a, 2005b). Small dispersal scales may result in inbreeding (Coleman & Kelaher 2009; Muhlin *et al.* 2008), loss of genetic variation and reduced potential for evolutionary rescue by tolerant genotypes.

In this study, genetic diversity and structure of the habitat-forming macroalga, *H. banksii*, is characterised at different spatial scales ranging from 100s km to 10s meters. Specifically, this study aims to characterise genetic diversity and structure in *H. banksii* regionally from the edge of this species distribution (marginal populations) versus "central populations" 100s km apart, and well as among locations (10s km apart) and locally between tidal heights (10s of m). It was hypothesised that due to putative poor dispersal capabilities of *H. banksii,* genetic diversity would be lower in marginal populations compared to central populations and that *H. banksii* would become more genetically differentiated towards biogeographic range edges. Similarly, at local scales, it was predicted that genetic diversity would differ between vertical shore heights with less genetic diversity found in higher shore heights than low shore heights. This study will aid in our understanding of the response of this species and its potential resilience to global warming on local and regional scales.

4.2. METHODS

4.2.1. Study species

Hormosira banksii is a dioecious macroalga endemic to Australia and New Zealand and is dominant on wave-swept platforms of the rocky intertidal. Due to its autogenic role of facilitating biodiversity and modifying environmental stressors, it is an ecologically important

species. It has been recorded in Australia from Albany, Western Australia to Lennox Head (personal observation J. Clark), New South Wales, Tasmania, New Zealand as well as Lord Howe Is., Norfolk Is., and the Kermadec and Chatham Islands (Millar & Kraft 1994; Osborn 1948; Womersley 1987). On the east coast of Australia, the marginal population of *H. banksii* occurs at Lennox Head where thalli are generally found only within rock pools. In contrast, at Angourie, the population just south of Lennox Head and elsewhere in the distribution, *H. banksii* also resides on exposed rocky platforms. Given that gene flow and genetic structuring has been found to be restricted among rockpools (Coleman & Brawley 2005b; Engel *et al.* 2004), *H. banksii* thalli were only sampled on exposed rocky platforms at all locations. *H. banksii* has been suggested to be reproductive throughout the year and potentially reproduce (broadcast spawn gametes) at every low tide (Gunthorpe *et al.* 1997), however, like some fucoids, reproduction may be restricted to calm, low tides following a period when thalli are exposed to light and desiccation (Pearson & Brawley 1996; Serrão *et al.* 1996).

4.2.2. Collection of samples and microsatellite amplification

Samples were collected from four locations within two climate regions in south-eastern Australia: two locations from the northern warm-edge of its range (marginal), Angourie and Minnie Water, and two temperate locations, Pearl Beach and Bilgola Beach which are situated towards the centre of its range (centre; Fig. 4.1). These locations are exposed headlands comprising rocky reef substrates where *H. banksii* is the dominant intertidal macroalga. Within each location 32 thalli (approximately 1 m apart) were haphazardly sampled from the low and high shore. High shore thalli were selected based on tidal exposure, local topography and drainage patterns to ensure they contrasted with low shore thalli, which were immediately adjacent to the water's edge. Low and high tidal heights were separated by ~ 5 m. Extraction of genomic DNA was conducted for a total of 235 individuals. Samples

comprised of unfouled apical segments that were washed in freshwater to remove salts and epiphytes, snap frozen in liquid nitrogen before storing in a -80 °C freezer until use. Before DNA extraction, samples were freeze-dried overnight.

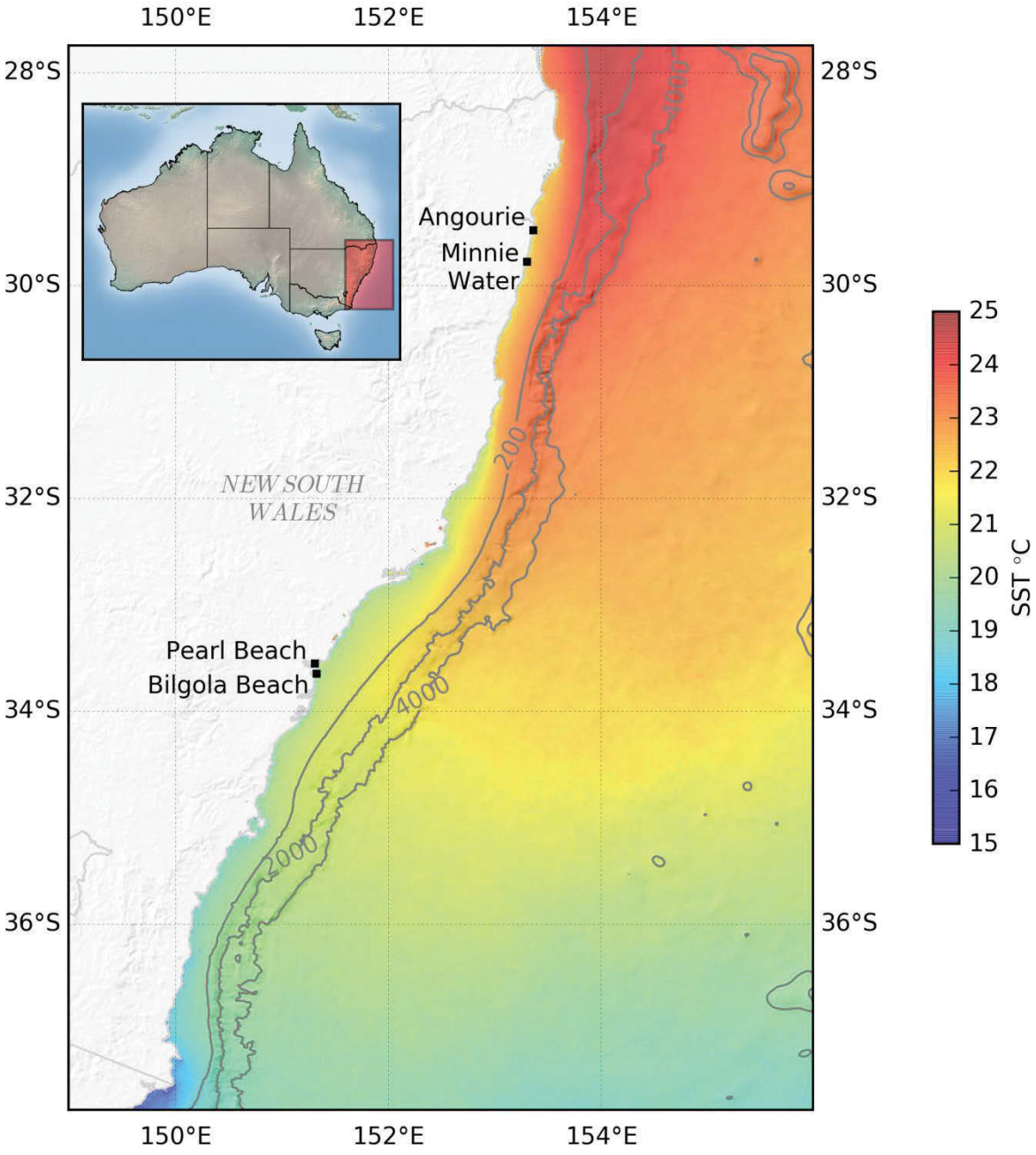

Figure 4.1: Map of samples collected from New South Wales, Australia. Sea surface Temperature (SST) data reflect average SST taken from 2003 - 2015 from the Integrated Marine Observing System (IMOS).

Genomic DNA was isolated from 20 – 30 mg of freeze dried tissue using the Nucleospin® 96 Plant II DNA extraction kit (Machery-Nagel, AGRF). Individuals were genotyped at 10 microsatellite loci (HB03, HB05, HB16, HB21, HB36, HB41, HB42, HB57, HB61, HB70; Bellgrove *et al.* 2016). Microsatellites are neutral genetic markers that can be used to infer information about genetic diversity and gene flow. Microsatellite regions were amplified in four multiplexing PCR reactions (Multiplex 1: HB03, HB21; multiplex 2: HB16, HB36, HB42; multiplex 3: HB05, HB41; multiplex 4: HB57, HB61, HB70; Bellgrove *et al.* 2016. Each PCR reaction was set up in 11 µL which consisted of 5 µL 2 x Multiplex Mastermix (Qiagen), 4 µL Primer mastermix and 2 µL of 1 in 20 diluted genomic DNA. Primer mastermix consisted of 10 µM reverse primer, 10 µM forward primer and unique 10µM fluorophores (FAM, VIC, NED, PET) which tagged the flanking region of the microsatellites.

PCR reactions were run on a Veriti 96-well thermal cycler (Applied Biosystems) with the PCR conditions of 95°C for 15 min for denaturing, followed by 40 cycles of 94°C for 30 s, 59°C for 90s, 72°C 60 s, and a final elongation step at 60°C for 30 mins as per the protocol described in Blacket *et al.* (2012). PCR products were checked for amplification using 1.5% agarose gel before fragment separation was conducted using ABI Genescan 3730 using the size standard LIZ500 (AGRF). Polymorphisms and allele sizes were visualised and determined manually using GeneMapper (v 4.0, Applied Biosystems)

4.2.3. Estimates of genetic diversity and structure

Prior to analyses, genotyping errors such as null alleles, stuttering, dropped alleles, and typographic errors were checked using MICROCHECKER (Van Oosterhout *et al.* 2004 www.microchecker.hull.ac.uk/index.jsp). Estimates of allelic frequencies, observed (H_O) and expected (H_E) heterozygosity and departures from Hardy-Weinberg equilibrium were

conducted in GENETIX (v 4.05.2, Belkhir *et al.* 2000). Analysis of variances (ANOVA) were conducted to test for differences between locations with planned comparisons between regions (marginal and central) as well as between heights on the shore using PRIMER-E with PERMANOVA (v 6.1.16). F_{IS}, the proportion of genetic variance contained in an individual (I) relative to the variance contained in a subpopulation (S), and F_{ST}, the proportion of genetic variance contained in a subpopulation relative to total genetic variance (T) were estimated using the program FSTAT (v 2.9.3.2, Goudet 1995) where Weir & Cockerham's estimates of F_{ST} and pairwise comparisons of shore heights were calculated within each location. F_{ST} estimates the genetic differentiation among populations and ranges from 0 to 1. F_{ST} values of ~0.2 are considered moderate levels of genetic differentiation and equate to one migrant per generation (Freeland *et al.* 2011). F_{IS} estimates the amount of selfing or inbreeding occurring within a population and ranges between -1 and 1, where negative values represent an excess of heterozygotes and positive values represent an excess of homozygotes. F_{IS} estimates were tested for significance using GENETIX. Linkage equilibrium was tested using 1000 permutations in FSTAT. F_{ST} estimates among all pairs of populations were calculated in FSTAT and significance levels of pairwise comparisons were corrected using Bonferroni correction (Rice 1989). Analysis of molecular variance (AMOVA) was performed using ARLEQUINN (v 3.5.22, Excoffier *et al.* 2005) which calculated the percentage of genetic variation attributed among and within each location. This analysis was conducted twice to determine variation amongst regions, among locations with regions and within locations (among individuals). F_{ST} estimates indicated no significant differences between shore heights, therefore shore heights were pooled for the AMOVA analysis. Isolation by distance was tested using Mantel tests in IBD WebService (Jensen *et al.* 2005, http://ibdws.sdsu.edu) which tests the null hypothesis of no correlation between pairwise geographic distance and genetic distance matrices.

To determine the number of distinct genetic clusters (K) in the locations sampled, the assignment of genotypes to clusters was inferred through STRUCTURE 2.3.3 (Pritchard *et al.* 2000). The number of possible clusters (K) was 1-9 (the maximum number of populations plus one) and assessed using 20 independent runs using a 5000 burn in time with a Markov Chain Monte Carlo iteration of 50,000. This was conducted on a dataset which allowed admixture, assuming correlated allele frequencies and assumed no prior information about populations.

Delta K was used to predict the number of real clusters (K) which is an ad hoc statistic based on the rate of change in the log probability of data between successive K values (Evanno *et al.* 2005). The modal value of the distribution, calculated as the Delta (K) was considered the uppermost hierarchical level of structuring and calculated as per Evanno *et al.* (2005) (Fig. 4.5). The software CLUMPP (Jakobsson & Rosenberg 2007) was then used to find the optimal alignment of multiple replicate analyses of each K. Population assignment was then graphically displayed using the program DISTRUCT (Rosenberg 2004).

To detect whether bottlenecks recently occurred in the populations sampled, the software BOTTLENECK 1.2.0.2 (Piry *et al.* 1999) was used which determined whether a temporary loss of allelic diversity occurred at a significantly faster rate than heterozygosity (Luikart & Cornuet 1998). The Two-Phase Model was used as it was deemed appropriate for use with microsatellites. For multiple microsatellite markers, step mutations were set at 0.9 and the variance of mutations set to 12 (Piry *et al.* 1999). The Wilcoxon test tested the null hypothesis of no excess heterozygosity across loci (Luikart & Cornuet 1998).

4.3. RESULTS

4.3.1. Genetic diversity and structure

Null alleles were found in low and high shore populations at Minnie Water and Angourie for locus HB3 but not at any other locations or other loci. All analyses were run with and without HB3 and did not alter results, therefore the locus HB3 was kept in subsequent analyses. Linkage disequilibrium was not found for any locus and all loci were in Hardy-Weinberg equilibrium (Table 4.1). Amongst the 235 individuals collected, a total of 39 different alleles were genotyped across 10 loci (Table 4.1, Appendix B). Total mean (± SD) number of alleles across all locations and shore heights sampled was 26.00 ± 1.20 where unique alleles were found at low shore at Bilgola Beach and Minnie Water as well as amongst high shore at Angourie. The number of alleles varied across locations and shore height with low shore at Bilgola Beach thalli having the greatest number of alleles (28) and low shore at Pearl Beach having the lowest number of alleles (24; Table 4.1). The number of alleles was found to not significantly differ amongst all locations ($F_{3,79} = 0.106$, $P = 0.967$), between regions (planned comparisons, $F_{1,79} = 0.055$, $P = 0.820$), or between heights on the shore ($F_{1,79} = 0.053$, $P = 0.814$). Genetic diversity was determined by the expected heterozygosity (H_E) which tended to be lower in marginal populations (Table 4.1). Positive F_{IS} values indicated inbreeding amongst marginal populations while, significant negative F_{IS} values were found from low shore at Pearl Beach, indicating an excess of heterozygotes (Table 4.1).

Table 4.1: Descriptive statistics for *Hormosira banksii* among 4 locations, and 2 shore heights within each location. Number of individuals sampled (*n*), Total number of alleles (*a*), mean number of alleles (± SD), unique alleles, observed heterozygosity (H_O) and expected heterozygosity (H_E) for each height on the shore within each location.

Location	Height on the shore	*n*	Total number of alleles (***a***)	Mean number of alleles	Unique alleles	H_O	H_E
Angourie	High	27	26	2.60 ± 1.17	2	0.189	0.256
	Low	32	25	2.50 ± 0.97	0	0.166	0.250
Minnie Water	High	32	26	2.60 ± 1.07	0	0.201	0.269
	Low	32	26	2.60 ± 1.26	1	0.197	0.278
Pearl Beach	High	32	27	2.70 ± 0.67	0	0.299	0.310
	Low	32	24	2.40 ± 0.97	0	0.375	0.315
Bilgola Beach	High	27	26	2.60 ± 0.70	0	0.400	0.396
	Low	25	28	2.80 ± 0.79	2	0.403	0.399

Table 4.2: F_{IS} estimates for each locus for each shore height and location as well as across all locations of *H. banksii*. Overall F_{ST} overall is also shown.

Location	Shore height	HB03	HB05	HB16	HB21	HB36	HB41	HB42	HB57	HB61	HB70	All
Angourie	High	0.557	0.353	0.000	1.000	-0.111	-0.227	0.000	0.801	0.000	-0.198	0.282
	Low	0.536	-0.019	0.000	0.919	-0.098	-0.047	0.000	0.636	0.000	-0.224	0.355
Minnie Water	High	0.361	0.225	-0.008	0.736	-0.061	-0.130	0.000	0.564	0.000	-0.125	0.284
	Low	0.525	0.295	-0.008	0.761	0.133	-0.195	0.000	0.492	0.000	-0.071	0.312
Pearl Beach	High	0.000	-0.283	-0.039	-0.024	-0.051	-0.104	-0.299	0.643	0.000	0.051	0.055
	Low	0.000	-0.314	-0.017	-0.040	0.151	-0.209	-0.206	-0.236	0.000	-0.173	**-0.168**
Bilgola Beach	High	0.000	-0.079	0.086	0.082	-0.179	0.085	0.066	-0.031	-0.020	0.185	0.008
	Low	0.000	-0.260	0.303	0.158	0.177	-0.098	-0.062	0.076	-0.049	-0.212	0.010
F_{IS} Overall		0.478	-0.115	0.082	0.575	0.004	-0.130	-0.139	0.370	-0.035	-0.085	0.121
F_{ST} Overall		0.215	0.159	0.287	0.493	0.311	0.026	0.327	0.211	0.026	0.176	0.256

Bold denotes significance at $P< 0.05$

The overall F_{ST} estimate overall amongst all spatial scales tested was 0.256 indicating high genetic structure (Table 4.3). There was pronounced and significant genetic structure between pairs of central and marginal populations with F_{ST} values ranging between 0.274 - 0.413 (Table 4.3). Between central locations (Bilgola and Pearl Beach) there was moderate and significant genetic structure with pairwise F_{ST} values ranging from 0.020 – 0.188. Similarly, there was low but significant genetic structure between locations within the marginal region (Minnie Water and Angourie; F_{ST} = 0.002 - 0.105 (Table 4.3)). Pairwise F_{ST} estimates between vertical shore heights were not significantly different at any location (Table 4.3).

Table 4.3: Pairwise F_{ST} estimates between all pairs of shore heights (L and H) and locations Bilgola Beach (BB), Pearl Beach (PB), Minnie Water (MW), and Angourie (ANG). Significant values are highlighted in bold and the adjusted p-value using Bonferroni Correction (P < 0.002). Grey shading shows comparisons between central and marginal regions.

		Marginal				Central			
		ANGL	ANGH	MWL	MWH	PBL	PBH	BBL	BBH
Marginal	ANGL								
	ANGH	0.002							
	MWL	**0.066**	**0.105**						
	MWH	0.032	**0.075**	0.000					
Central	PBL	**0.374**	**0.413**	**0.276**	0.302				
	PBH	**0.358**	**0.392**	**0.286**	**0.303**	0.020			
	BBL	**0.375**	**0.387**	**0.318**	**0.347**	**0.150**	**0.188**		
	BBH	**0.338**	**0.355**	**0.274**	**0.309**	**0.150**	**0.190**	0.000	

A large (25.694 %) and significant amount of genetic variation was explained between regions, (marginal vs central). Similarly, a large and significant amount of genetic variation (8.03 %) was explained among locations within regions, with the largest amount of genetic variation (66.33 %) amongst individuals within each location (F_{ST} = 0.337, P < 0.000001; Table 4.4). When shore heights were pooled within each location, a separate AMOVA

revealed a significant amount of variation was explained at the scale of locations (27.49 %) and amongst individuals within each location (72.51 %; F_{ST} = 0.275, P < 0.00001; Table 4.4). There were strong significant relationships between geographic and genetic distance across all locations of *H. banksii* (Mantel test: Z = 2620.41, r = 0.924, P = 0.002, Fig. 4.2) but not within central (Mantel test: Z = 7.56, r = 0.976, P = 0.113, Fig. 4.2) or marginal regions (Mantel test: Z = 6.90, r = 0.831, P = 0.250, Fig. 4.2).

Table 4.4: Analysis of molecular variance (AMOVA) among regions: central (BB, PB) and marginal (MW, ANG) among locations within regions and within locations. A second AMOVA tested variance among and within locations (BB, PB, MW, ANG) with shore heights pooled. Bold denotes significance at P < 0.05.

Source of Variation	df	SS	Variance component	% Variation
Among regions	1	42.30	0.152	**25.64**
Among locations within regions	2	12.10	0.048	**8.03**
Within locations (among individuals)	472	185.95	0.394	**66.33**
Total	475	240.34	0.594	
Among locations (shore heights pooled)	3	54.38	0.150	**27.49**
Within locations (among individuals)	472	185.95	0.394	**72.51**
Total	475	240.34	0.543	

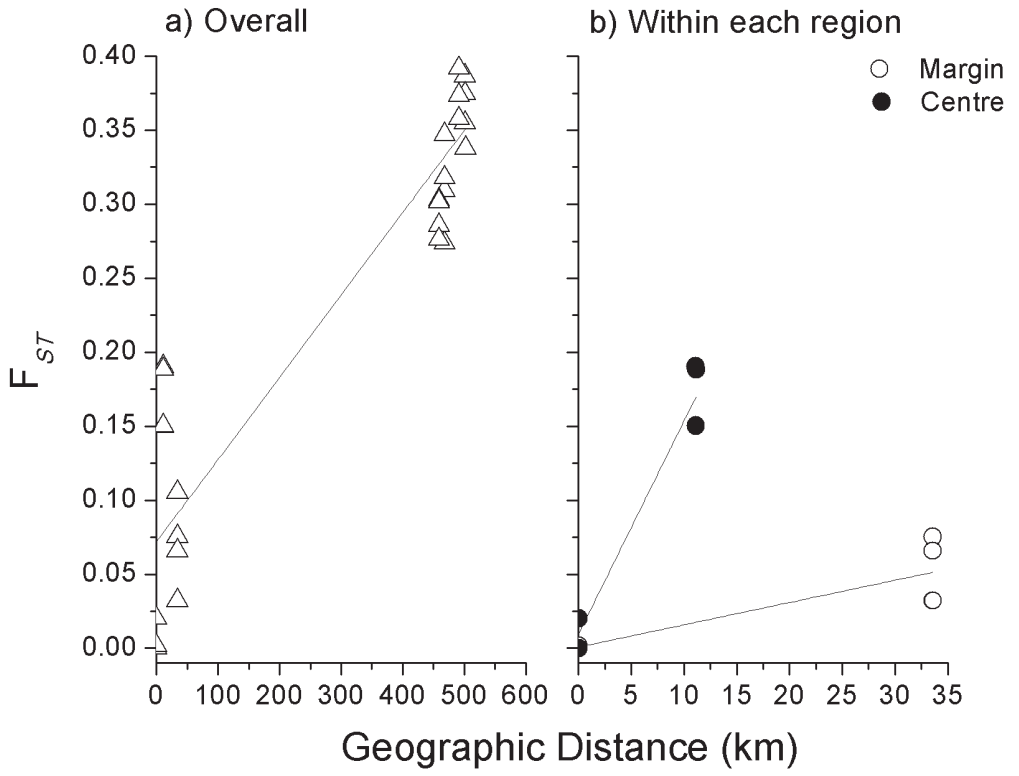

Figure 4.2: Patterns of isolation by distance (pairwise genetic differentiation F_{ST} estimates relative to geographic distances) at multiple spatial scales: a) over all locations and shoreheights as well as b) between locations within a region.

The results from STRUCTURE revealed that the greatest level of structure is K = 2 which separates the marginal (ANG and MW) from the central populations (PB and BB; Fig. 4.3, Table S4.2). K = 3 was also assessed but the large decrease to K = 3 when comparing Delta K to K was substantial, therefore genetic clusters remained at K = 2. The results from BOTTLENECK revealed that the Wilcoxon test of the null hypothesis of no excess heterozygosity across loci was non-significant which indicates that there were no bottlenecks evident for any population sampled (one tailed test, $P > 0.05$ for all populations sampled).

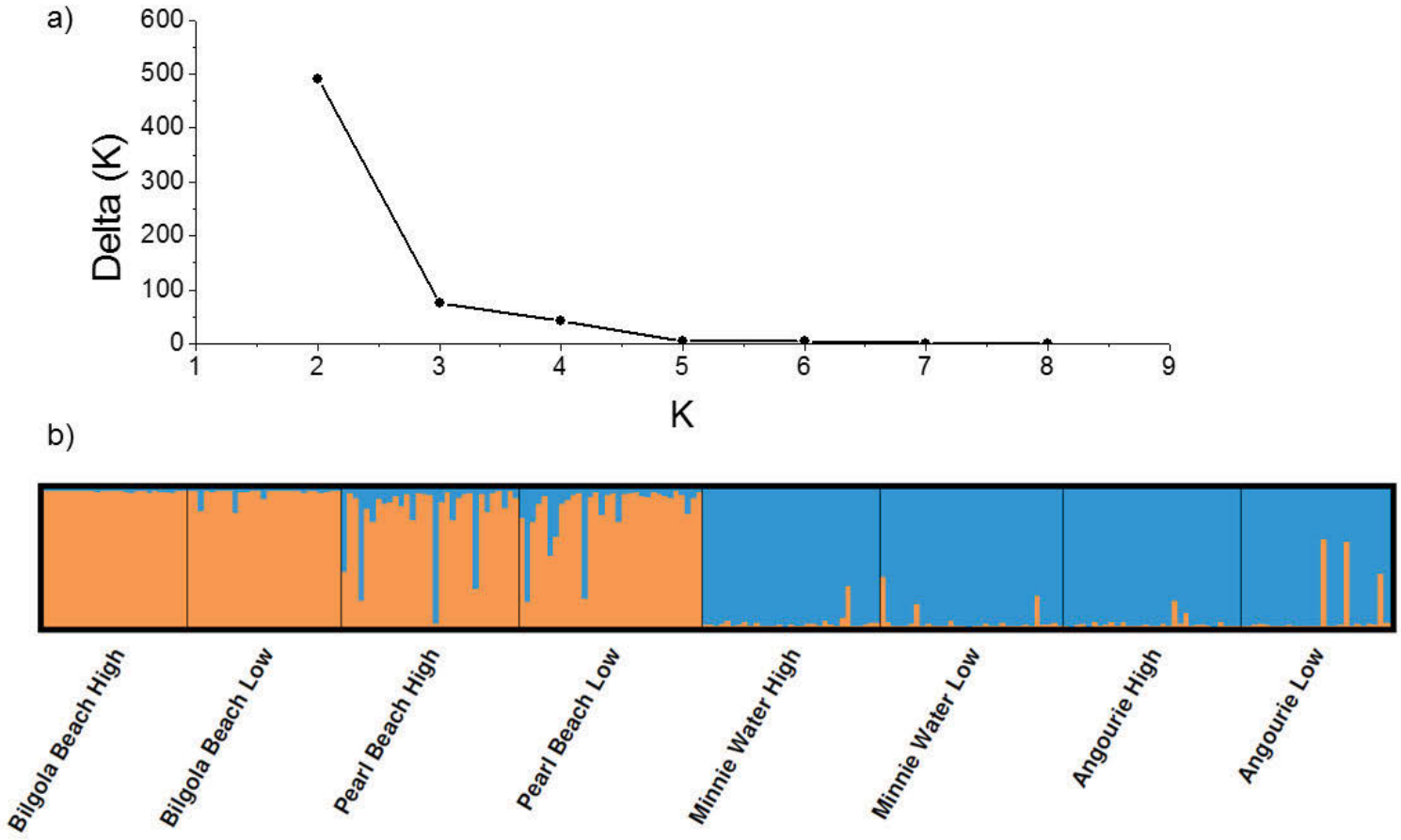

Figure 4.3: a) Plot of Delta (K) where the modal value of the distribution is considered to be the highest level of structuring which in this case = 2. b) Genetic subdivision of *H. banksii* based on STRUCTURE. The proportions of individual multilocus genotypes assigned to K=2 virtual clusters are indicated by the grayscale. Bilgola Beach represents central populations while Angourie represents marginal populations (north).

4.4. DISCUSSION

Climatic changes are already having impacts on ecosystems globally, causing redistribution of species and changes in physiology, resulting in range shifts and fragmentation of populations (Parmesan and Yohe 2003). The exact mechanisms of change, however, are often unclear. Genetic diversity, connectivity and gene flow may confer resilience to populations against perturbations in climate. Here, the genetic diversity and genetic structure of the habitat forming alga, *H. banksii*, was examined in warm range edge and central populations. Results revealed lower genetic diversity towards the marginal range edge but no variation in genetic diversity and a lack of genetic structure between vertical heights on the intertidal shore.

Despite the East Australian Current serving to facilitate gene flow and connectivity in many planktonic species and subtidal macroalgae (Coleman *et al.* 2011a, 2011b; Coleman & Kelaher 2009), it does not appear to facilitate long-distance dispersal of *H. banksii*. The strong genetic structure among locations (~ 500 km apart) and isolation by distance suggests that dispersal capacity is limited across long distances as well as between neighbouring populations (< 50 km; Fig 4.2, Table 4.3). The results of STRUCTURE also revealed two genetic clusters between marginal and central populations, supporting that dispersal is limited between the two regions. Gene flow, however, is evident between populations of *H. banksii* separated by < 50 km, particularly between marginal populations (average F_{ST} = 0.026). Dispersal of gametes or zygotes is not likely method of long-distance dispersal as fertilised zygotes sink to the substrate and adhere within hours of fertilisation (Dimartino *et al.* 2016). Rather, rafting of buoyant dislodged adult thalli which drift with ocean currents with the aid of air bladders or vesicles has been suggested as the most likely method of long-distance dispersal and has been evident amongst different macroalgal species (Bussolini & Waters

2015; Hobday 2000; Muhlin *et al.* 2008; Valero *et al.* 2011) including *H. banksii* (McKenzie & Bellgrove 2008). Drifting thalli have been found to be reproductively viable for up to 8 weeks and could potentially release viable gametes or propagules into distant populations (McKenzie & Bellgrove 2008). There is limited empirical evidence that supports whether floating thalli contribute to long distance gene flow, and thalli often end up on unsuitable habitat such as beaches (McKenzie and Bellgrove 2008). Furthermore, thalli of *H. banksii* need to arrive in an already established or reproductively viable population and require another individual of the opposite sex for successful genetic recombination to occur.

The moderate but significant levels of genetic structure between neighbouring populations within both central and marginal regions may show restriction in dispersal possibly due to the existence of physical barriers. It has been suggested that sandy beaches, and mouths of estuaries may serve as barriers to gene flow in other macroalgae (Billot *et al.* 2003; Coleman & Kelaher 2009). Central populations of Bilgola and Pearl Beach are separated by complex landscape topography including the large mouth of an estuary (e.g. Patonga Estuary), a sheltering island (Lion Island), headlands (e.g. Barrenjoey Head) as well as several sandy beaches which may account for higher genetic structure between these areas. In contrast, the coastline separating the marginal populations of Minnie Water and Angourie is characterised by only a few sandy beaches which may explain why Minnie Water and Angourie are more genetically similar compared to central populations.

Reproductive strategies in many species of *Fucuceae* may also contribute to limited gene flow between neighbouring populations. Although female eggs are viable for 6-7 hours (Muhlin *et al.* 2008; Serrão *et al.* 1996), eggs are negatively buoyant and fertilisation and attachment to

the substrate occurs over a few hours (6 hours; Dimartino *et al.* 2015). Similarly, male gametes are mobile and can disperse up to 2 m, however, are negatively phototactic and generally only viable for a few hours (Muhlin *et al.* 2008). As reproductive cues (low tide, calm water, slack tide) ensure 100% fertilisation success in many Fucales (Pearson & Serrão 2006), it is likely that these strategies by female and male gametes may also contribute to limited dispersal. Thus, dispersal of gametes and zygotes may occur freely within a shore, but be rare between neighbouring populations (km apart) where dispersal of fertile drift may be more important. Timing of reproduction can also be asynchronous among neighbouring populations which may also temporally limit gene flow (Muhlin *et al.* 2011). For example, gamete release among neighbouring populations of *F. vesiculosus* was found to vary with wind speed, light intensity and water motion, suggesting that the aspect and location of different platforms could cause populations to release their gametes at different times (Muhlin *et al.* 2011), resulting in a temporal limitation of gene flow.

Gene flow of *H. banksii* was not restricted between vertical heights on the intertidal shore (Table 3) which agrees with other studies on macroalgae (Engel *et al.* 2004; Tatarenkov *et al.* 2007; Teixeira *et al.* 2016; Zardi *et al.* 2011, Bellgrove *et al.* 2016). The intertidal is characterised by steep environmental gradients suggesting selection for stress-tolerant genotypes on high shores may be an important driver of genetic structure. This has been demonstrated amongst barnacles (Schmidt & Rand 2001) and gastropods (Johannesson 2009) as well as amongst the hybrids of *Fucus vesiculosus* and *Fucus spiralis* (Zardi *et al.* 2011). Nonetheless, the lack of small scale genetic structure between shore heights (5 m) found in this study, suggests that gene flow is unobstructed and that zygotes and gametes may be readily dispersed across these distances (Dudgeon *et al.* 2001). Water motion of the incoming tide could potentially carry zygotes to other parts of the intertidal platform before zygotes

fully adhere to the substrate (Dudgeon *et al.* 2001). Furthermore, water temperature can retard zygote attachment with colder temperatures in winter months potentially facilitating longer distance dispersal with the incoming tide (Pearson & Brawley 1996, Coleman & Brawley 2005). Studies on the attachment strength of *H. banksii* zygotes have found that adhesion to the substrate is not at maximum strength until 24 h after fertilisation suggesting that zygotes could potentially be dislodged and recruit elsewhere (Dimartino *et al.* 2015).

Specific habitat types related to strong environmental gradients within the intertidal have been found to influence phenotypic divergence independently of genetic structure (Engel *et al.* 2004; Zardi *et al.* 2013). Lack of differences at small scales suggest that *H. banksii* may survive living in different environmental gradients through phenotypic plasticity rather than genetic differentiation as documented in *F. vesiculosus* (Zardi *et al.* 2013). This suggests that exposure within the intertidal may not necessarily select for different genotypes but perhaps genotypes that are highly plastic. An alternative explanation is that genetic differentiation between tidal heights does exist, but is not apparent in our neutral markers (which only show variation due to dispersal and connectivity, not selection). Testing this idea would require use of markers such as SNPs which examine portions of the genome under selection.

A trend for lower estimates of genetic diversity towards distributional edges found in this study is in accordance with biogeographic patterns found in previous research (Coleman *et al.* 2011a; Faugeron *et al.* 2004; Teixeira *et al.* 2016). The observed patterns of lower genetic diversity at marginal populations is suggested to be the result of reduced gene flow and connectivity which is pronounced particularly with the poleward flow of the East Australian Current (Coleman *et al.* 2011b). Decreased gene flow and connectivity can create isolation among populations which can reduce within population genetic diversity (Hampe & Petit

2005). As distributional limits often represent the physiological limits of a species, environmental conditions would impose strong selection pressure which would decrease the effective population sizes as environmental conditions and habitat become less optimal. This suggests that only tolerant genotypes can persist at range edges (Frankham 2005). I found no evidence for this, however, with adults and early life stages of *H. banksii* from marginal populations being physiologically sensitive to increased temperatures compared to populations found within the centre of its range (adults Chapter 2, early life stages Chapter 3). Thus, the result of lower neutral genetic diversity at range edges is likely a result of decreased dispersal and gene flow, rather than selection.

Similar to previous studies, (Coleman *et al.* 2011a) evidence of inbreeding was found in populations of *H. banksii* and particularly pronounced amongst marginal populations. This is not surprising as these populations are at the edge of their equatorward distribution, where populations are more fragmented, conditions are not optimal and macroalgal populations are therefore at their physiological threshold. The lower genetic diversity found at these populations and increased susceptibility to inbreeding suggests that these populations may lack the potential to adapt to future warming and be particularly vulnerable to extreme climate events (i.e. heat waves). With marginal populations also exhibiting a narrower thermal niche (Chapter 3), these populations may not be physiologically buffered to sudden changes in local climates. Previous studies have already shown local extinction in marginal populations of macroalgal populations with extreme climate events which may be a consequence of a smaller gene pool (Araújo & Williams 2001; Smale & Wernberg 2013).

Although a slight excess in heterozygotes is expected for dioecious species (Cockerham 1973) excesses were particularly high at Pearl Beach. Bottlenecks were not found in this population

and therefore cannot explain the significant excess heterozygosity. Excess heterozygosity may arise from reproductive scenarios such as a small number of individuals contributing to reproduction at any one point in time. For example, a "risky day" in which low tides occur in the middle of the day (Mislan *et al.* 2009) when conditions are extreme, or an extreme climate event (e.g. heat wave) could restrict only tolerant genotypes to spawn during unfavourable conditions as other genotypes may require more energy for repair. Therefore, a cohort of recruits could have a separate genetic signature. Second, rafting of fertile material could create cohorts of recruits arising from different matings between localised adults and distant populations (Coleman & Brawley 2005b; Engel *et al.* 2004; Muhlin *et al.* 2008). Finally, excess of heterozygotes could also be caused through microscopic stages of macroalgae or "seed banks" which are equivalent to terrestrial plant seed banks. This could cause a cohort of recruits of different genetic signature to be maintained through suspended growth until more favourable conditions arise (Carney & Edwards 2006; Hampe & Petit 2005; Hoffmann & Santelices 1991). Although *H. banksii* does not have an alternation of generations life history, it has been suggested that the germinating portion of thalli could persist through harsh conditions, effectively acting as a seedbank to regenerate from the holdfast (Underwood 1998).

4.4.1. Conclusions

In summary, this chapter characterised the genetic diversity and structure of *H. banksii* at three spatial scales to determine patterns of gene flow and dispersal. Within different tidal heights gene flow was found to be unobstructed suggesting that the intertidal landscape does not limit dispersal between heights on the shore for *H. banksii*. With on-going warming and increases in extreme climate events (heatwaves, storm surges), perturbations will most likely alter fecundity and subsequently gene flow. The results of this study also found that dispersal in *H. banksii* is limited over longer distances (~50 to 500 km) which is evident in its

pronounced genetic structure and strong patterns of isolation by distance. Low estimates of genetic diversity towards marginal range edges also supported limited dispersal capacity of *H. banksii* within a poleward flowing current. Marginal populations of macroalgae in subtropical climates will most likely be affected by thermal perturbations with biogeographic ranges of macroalgae already shifting poleward (Lima *et al.* 2007; Phillips 2001; Smale & Wernberg 2013; Wernberg, Russell, Thomsen*, et al.* 2011). If marginal populations have lower genetic diversity, then there are fewer genotypes that can provide functional responses to perturbations (Al-Janabi *et al.* 2016; Reusch *et al.* 2005) potentially decreasing the ability of populations to persist. Marginal populations of *H. banksii* have already been found to be physiologically sensitive to increased temperatures (Chapter 2 and Chapter 3) and have narrower thermal breadths compared to central populations (Chapter 3). This suggests that along with low genetic diversity and the slim possibility of tolerant genotypes dispersing northward, marginal populations of *H. banksii* may be at risk of extirpation with climate change. The loss of this ecologically important species will have cascading and long-lasting effects on intertidal biodiversity and ecosystem services (Schiel & Lilley 2011).

4.5. SUPPLEMENTARY TABLE

Table S4.2: Calculation of inference of K using the Delta (K) method as per Evanno *et al.* (2005).

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	20	-2878.695	0.100			
2	20	-2363.580	0.752	515.115	369.085	491.092
3	20	-2217.550	1.220	146.030	92.280	75.625
4	20	-2163.800	2.118	53.750	89.315	42.163
5	20	-2199.365	5.135	-35.565	23.830	4.641
6	20	-2211.100	5.453	-11.735	23.845	4.373
7	20	-2246.680	12.931	-35.580	9.285	0.718
8	20	-2272.975	32.704	-26.295	15.059	0.460
9	21	-2314.329	22.771	-41.354		

Chapter 5

Is bigger better? Reproductive investment and maternal effects on the performance of early life stages of a habitat-forming macroalga

5. ABSTRACT

Maternal provisioning is increasingly recognised as an important contribution to the phenotype of an offspring and may give early life stages and following generations advantages in performance. Organisms face well known life history trade-offs between allocating resources to growth or reproduction and between many larger and fewer smaller offspring, however, less is known about maternal provisioning within foundation species such as benthic macroalgae. This study investigated whether variation in reproductive investment (reproductive biomass, fecundity and egg size) and maternal provisioning of an intertidal habitat-forming macroalga, *Hormosira banksii*, occurred amongst individuals from different heights on the shore, and whether differences in egg size had subsequent effects on embryo growth and development under different temperatures. Investment in reproductive biomass and fecundity was found not to vary amongst different females and heights on the shore. Females found high on the shore, however, had larger eggs than females found low on the shore, suggesting that fertilised eggs could have different development trajectories. A trade-off between larger eggs for decreased number of eggs (fecundity) was not found. Furthermore, egg size did not correlate with rate of embryo growth or the timing of when the germinating cells morphologically differentiated in length, however, it did correlate with the timing of when germinating cells grew, with smaller embryos increasing in width earlier than bigger embryos. This may have important implications for recruitment and subsequent development of juveniles, but overall, the results of this study suggest that variation in maternal provisioning among individuals has only subtle effects on the development of early life stages of *H. banksii.*

5.1. INTRODUCTION

Understanding how events in the early life history of organisms contribute to population dynamics and tolerance to stress is important if we are to predict and manage how populations will respond to a changing environment. Non-genetic sources of variation in offspring performance, such as parental environmental effects, are increasingly becoming recognised as important contributors to adaptive evolution (Galloway 2005; Mousseau & Fox 1998; Wolf & Wade 2009). Specifically, maternal effects in which the maternal environment affects the phenotype of her offspring, may be adaptive, producing offspring well suited to their local environment (Mousseau & Fox 1998; Stearns 1976). Furthermore, the local environment that a mother experiences can influence the amount of resources available for reproduction, which can profoundly influence the number, growth and survival of her offspring (Chambers & Leggett 1996; Roach & Wulff 1987; Stanton 1984). Studies on terrestrial plants (Bischoff & Müller-Schärer 2010; Galloway 2005), marine invertebrates (Allen & Marshall 2014; Marshall 2008) and vertebrates (Donelson *et al.* 2009; Semlitsch & Gibbons 1990), have demonstrated that larger offspring often have higher fitness and advantages in early life development. However, due to finite resources that mothers can allocate to reproduction, there are well known trade-offs between resources allocated to maternal fitness and offspring, and between production of fewer larger sized offspring and greater numbers of smaller offspring (Smith & Fretwell 1974).

Sessile organisms typically experience a single environment within their lifetime, and this environment can have substantial effects on the size and physiology of mothers, the amount of resources allocated to reproduction, and their offspring (Roach & Wulff 1987; Wolf & Wade 2009). For example, in terrestrial plants grown in different light environments, the maternal environment can influence the allocation of resources to roots and shoots in

offspring as well as seed size (Galloway *et al.* 2009). Less is known about parental effects in marine organisms such as benthic macroalgae. Brown macroalgae have complex and diverse life histories with some groups having both haploid and diploid generations, in which sporophytes give rise to gametophytes living as separate entities (e.g. kelps in the Laminariales). Other groups, including brown algae in the order Fucales, feature only diploid life cycles with diploid gametophytes and lack the alternation of generations (Osborn 1948). Members of the Fucales are well known for their poor dispersal capacity due to reproductive strategies that are thought to maximise fertilisation success (Brawley 1992; Pearson & Brawley 1996; Serrão *et al.* 1996). Eggs are often negatively buoyant while sperm are phototactic and gametes produced by separate male and female individuals are often dispersed only within 10 m of their parents (Serrão *et al.* 1997). Consequently, offspring are likely to experience the same environment as their parents, suggesting that maternal provisioning could give an advantage to early life stages if phenotypes are produced that are best suited to maternal environments (Galloway 2001; Roach & Wulff 1987).

Hormosira banksii is an abundant habitat-forming macroalga from the order Fucales found on wave-swept intertidal rock platforms in temperate Australia and New Zealand (Womersley 1987). The intertidal is a highly dynamic environment, characterised by steep environmental gradients that cause both thermal and desiccation stress (Davison & Pearson 1996). It is an ideal environment to examine the role of maternal effects as these environmental gradients impose strong selective pressure on individuals to effectively allocate resources amongst growth, survival and reproduction (Araújo *et al.* 2015). Quantitative genetic breeding designs have demonstrated interactions between female genotype and environment for growth and ontogenetic development of *H. banksii* embryos (Clark *et al.* 2013; Chapter 3), indicating that female identity has a significant contribution to phenotypic variation in thermal tolerance

traits of embryos. Thalli exposed to emersion stress higher on the shore have been suggested to be physiologically stressed, where individuals may allocate more resources towards photoprotection and repair rather than growth and reproduction (Davison & Pearson 1996). Trade-offs in allocation of resources within thalli and amongst individuals have been documented in macroalgae (Araújo *et al.* 2015; Chu *et al.* 2011; Gillespie & Critchley 2001; Viejo *et al.* 2011), however, it remains to be determined how stress imposed on female *H. banksii* in the intertidal affects the size and number of eggs they produce, and whether there are transgenerational effects manifested in offspring.

This chapter investigates whether egg size and reproductive investment differs between females high on the shore compared to low on the shore, and whether a trade-off occurs between egg size and fecundity. As environmental stress increases with tidal height, it was hypothesised that 1) female thalli from high on the shore would have a lower reproductive investment in terms of reproductive biomass, fecundity and egg size than thalli low on the shore, 2) larger, more well provisioned eggs would produce embryos that grow faster than smaller eggs and 3) these maternal effects would be temperature dependent, with maternal provisioning being more important in more stressful temperatures.

5.2. METHODS

5.2.1. Sample collection and variation in reproductive investment

Thalli were collected from high and low shore at Pearl Beach (S 33º32’57.70”, E 151º18’32.36”) in austral winter and kept at 4 °C for 48 hours prior to being used in experiments. Entire thalli were weighed (total wet weight) using a digital balance (NewClassic MF ML3002/01, Mettler Toledo, Switzerland) then rinsed in room temperature tap water and blotted dry before being placed into individual containers (n=20). Gametes

were exuded from conceptacles within 20–30 minutes. Once gametes were exuded, females and males were identified (olive green for females and orange for males; Osborn 1948), filtered seawater (30 mL) was added to each container and gently swirled to release gametes from conceptacles. All thalli were dried at 60 °C over 3 days to determine dry weight biomass.

5.2.2. Temperature effects on egg fertilisation and embryo growth

To determine whether maternal provisioning had an effect on early life stage development, embryos were incubated at three different temperatures (22, 26, 30 °C) that were representative of temperatures experienced in the field (determined in Chapter 3). Embryos were incubated in different temperature treatments to test whether provisioning had the same outcome at all temperatures, or whether embryos grew optimally in the environment most commonly experienced by their mother (22 °C).

The egg solution was filtered into individual centrifuge tubes using 100 μm nylon mesh and sperm solution was filtered using 40 μm nylon mesh. An aliquot of each egg solution from each female was taken and preserved using glutaraldehyde (1% v/v final concentration) for fecundity and egg size analysis. Eggs and sperm were quickly viewed under an inverted microscope to ensure eggs were released from the mucus envelope and sperm were active. Gametes were then pooled into one container according to sex. The sperm solution was added to the egg solution in the ratio of 50:1. The mixed gamete solution was then dispensed into a 12 well-plate (4 plates per height on the shore) and allocated randomly into 3 different temperature treatments, 22 °C, 26 °C, 30 °C with a decrease of 5 °C overnight, and incubated at a 12:12 light cycle at ~30 μmol photon m^{-2} s^{-1}.

To quantify growth and ontogenetic development, images of embryos were taken daily with a high content analyser (Incell Analyzer 2200 Cell Imaging System, GE Healthcare; Fig 5.1). Individual embryos were tracked over seven days, with the first image taken 24 hours after fertilisation. Fertilisation success was determined at each temperature by assessing 400 embryos per temperature treatment using the fluorescent marker Calcofluor (0.001% v/v final concentration) which stains cellulose and alginate and fluoresces white when excited by UV light (Hughes & McCully 1975). Embryos that were stained white were counted as fertilised. Images were captured daily to assess growth and embryo ontogenic development over a total of 7 days using FIJI imaging software (Image J v 1.51d, National Institute of Health, USA).

To determine whether initial egg size influenced embryo development, a descriptive model was used to characterise the development of the embryos at different temperatures. Embryo metrics were quantified by first determining the length and width of the germinating cell (orthogonal red and green lines in Fig. 5.2) and by the length of the rhizoid (red and blue splines (smoothed curves) Fig. 5.2). The measurements were performed in FIJI by a user-guided script which models the embryos using 'control points'. The germinating cell was modelled as two circles of differing radius which requires three control points where each rhizoid is modelled as a Cat-Mull spline defined by between 2-7 points. The script then refined the location by detecting the edges of embryo. From these control points the length and width of the germinating cell and the length of the rhizoid were calculated.

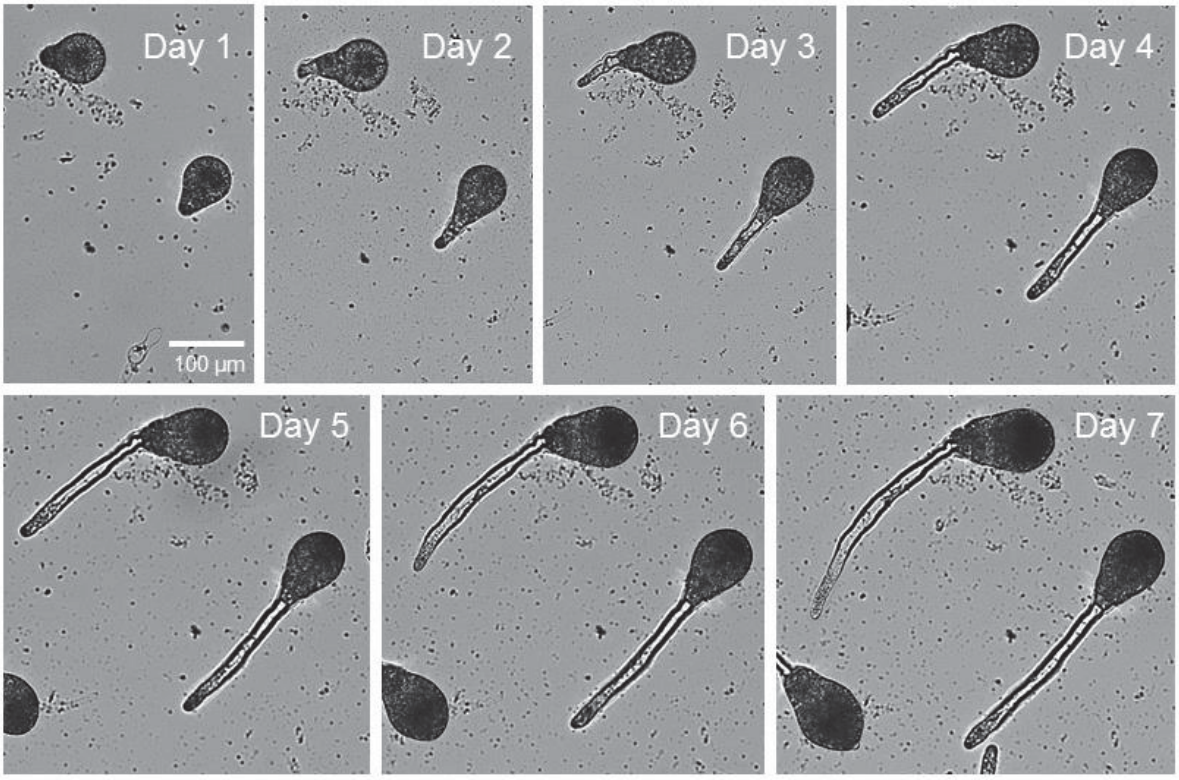

Figure 5.1: Example of embryo development at 22 °C over 7 days post-fertilisation. Images were captured using a high content analyser (Incell Analyzer 2200, GE Healthcare.)

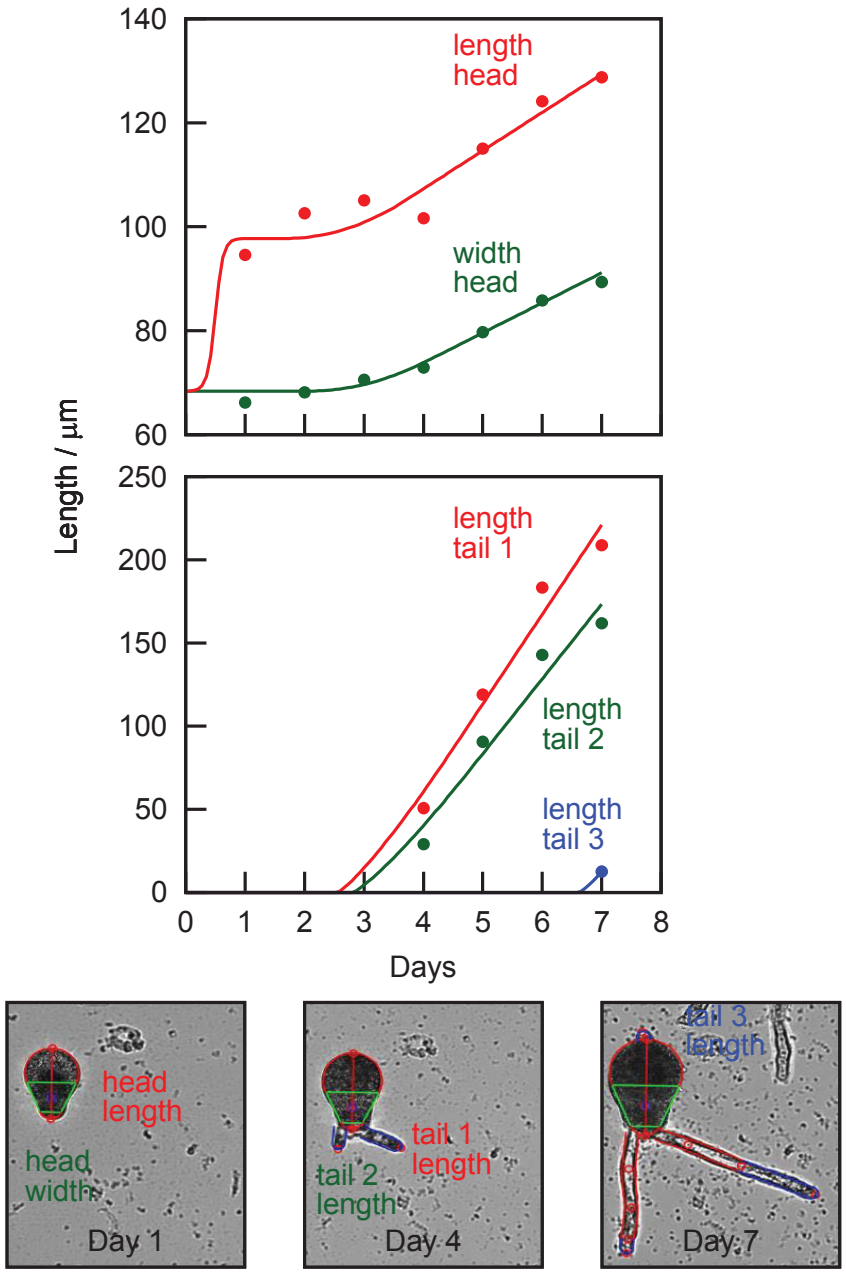

Figure 5.2: An example of the development of embryo over 7 days in terms of measured traits. Lines represent embryo width (green) and length (red) of the germinating cell, where red and blue spline (smooth curve) represents length of rhizoids.

A simple descriptive model was built to analyse the development of the embryo. The descriptive model described all dimensions relative to the initial width of the egg. The initial development was modelled as a logistic function and the subsequent growth as a linear function:

$$L(t) = W_0\left(1 + f_L \,\text{logistic}\left(t, t_{L0}, 2\right) + r_{L1}\,\text{linear}\left(t, t_{L1}, t_{L1} + 2, 2\right)\right)$$

where:

$$\text{logistic}\left(t, t_h, r\right) = \frac{1}{1 + \exp\left(-r\left(t - t_h\right)\right)}$$

The linear function was designed to transition smoothly from a constant to linear growth between time t_0 and t_1:

$$\text{linear}\left(t, t_0, t_1, \alpha\right) = \begin{cases} 0 & \text{if } t < t_0 \\ \dfrac{(t_1 - t_0)}{\alpha}\left(\dfrac{t - t_0}{t_1 - t_0}\right)^{\alpha} & \text{if } t_0 < t < t_1 \\ \left(t_1 - t_0\right)\left(\dfrac{t - t_0}{t_1 - t_0} + 1 + \dfrac{1}{\alpha}\right) & \text{if } t_1 < t \end{cases}$$

The initial development, as described by Clarke & Womersley (1981), was described by a logistic function and f_L logistic was used to describe the development of the circular egg into the fertilised pear shape. In most cases, morphological differentiation (the time of when change in dimensions from spherical shape to pear-shaped with the appearance of the rhizoid) occurred before the first image was captured 24 hours after fertilisation, so only the fractional increase in the length could be determined.

From this, the rate of growth of the width and length of the germinating cell could be determined as well as the rate of growth of the first, second and third rhizoid. Similarly, the time when morphological differentiation (both in length and width) occurred and when growth was initiated in the rhizoid could also be determined. Due to embryos generally not growing at 30 °C, growth and timing of morphological differentiation was not analysed in this treatment.

5.2.3. Statistical analysis

All data were analysed using Origin Pro8 (v8.0724 (B724) Origin Lab Corporation, USA) and PRIMER-E +PERMANOVA (v 1.06) and checked for assumptions using residual plots and Levene's test for homogeneity of variances. One-way analysis of variance (ANOVA) was used to contrast fertilisation success, fecundity (normalised to biomass and expressed as total eggs g^{-1} dry biomass), embryo length and rates of growth for width, length of germinating cell and growth of the first rhizoid for embryos across temperatures (22 and 26 °C). A two- way ANOVA was used to contrast egg volume (μm^3) across heights on the shore (fixed factor) and individual females (random factor). Analyses of covariance (ANCOVA) were used to contrast each embryo metric (rate of growth and time of morphological differentiation of the width and length of the germinating cell and appearance of first rhizoid) with initial egg size as the covariate and temperature as the categorical predictor variable.

5.3. RESULTS

5.3.1. Variation in reproduction investment

Thalli collected from high and low on the shore did not differ in their reproductive investment in terms of total dry biomass and fecundity. Thalli had similar total dry biomass between heights on the shore ($F_{1,18} = 0.64$ $P = 0.434$, data not shown). Fecundity was highly variable,

however, there was no significant difference in the number of eggs per gram of dry biomass between thalli from low and high on the shore ($F_{1,19} = 4.27$ $P = 0.05$, Fig. 5.3a).

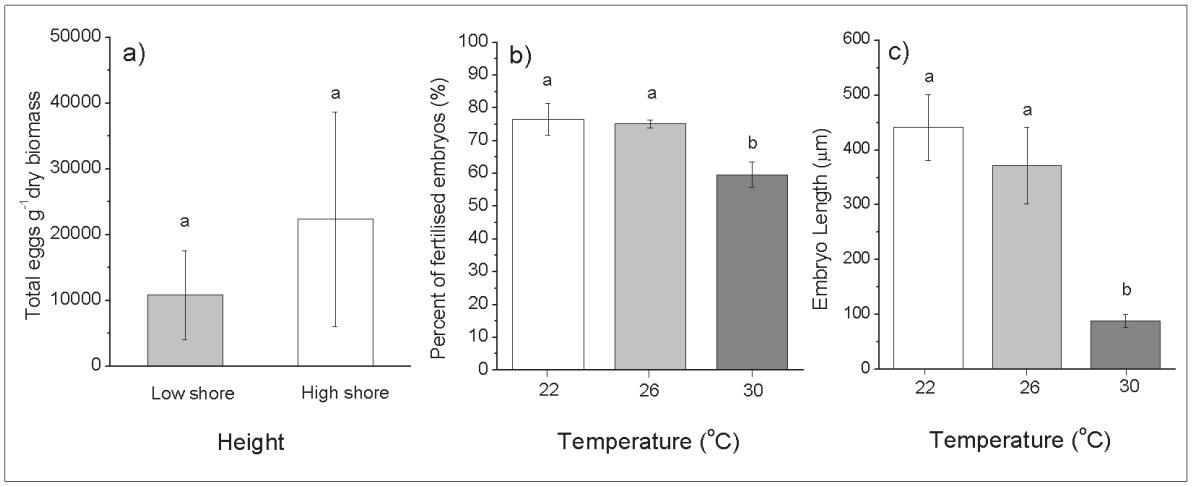

Figure 5.3: Mean (±SD) of a) fecundity measured as total eggs g-1 dry biomass b) percent of fertilised embryos and c) embryo length at day 7 amongst different temperatures 22, 26, 30 °C at low and high on the shore. Letters signify significant differences at P<0.05

Mean egg volume significantly differed among individual females ($F_{9,399} = 9.63$, $P < 0.001$, Fig. 5.4a, b) and between heights on the shore ($F_{1,399} = 8.23$, $P = 0.01$, Fig. 5.4c) with significantly larger egg volume in thalli from high on the shore than low on the shore ($F_{1,380} = 11.84$, $P= 0.001$, Fig. 5.4c). Egg volume, however, did not significantly correlate with fecundity for thalli found low and high on the shore (low shore: $F_{1,9} = 2.629$, $P = 0.144$, $R^2 = 0.153$; high shore: $F_{1,9} = 1.370$, $P = 0.280$, $R^2 = 0.044$, Fig. 5.5).

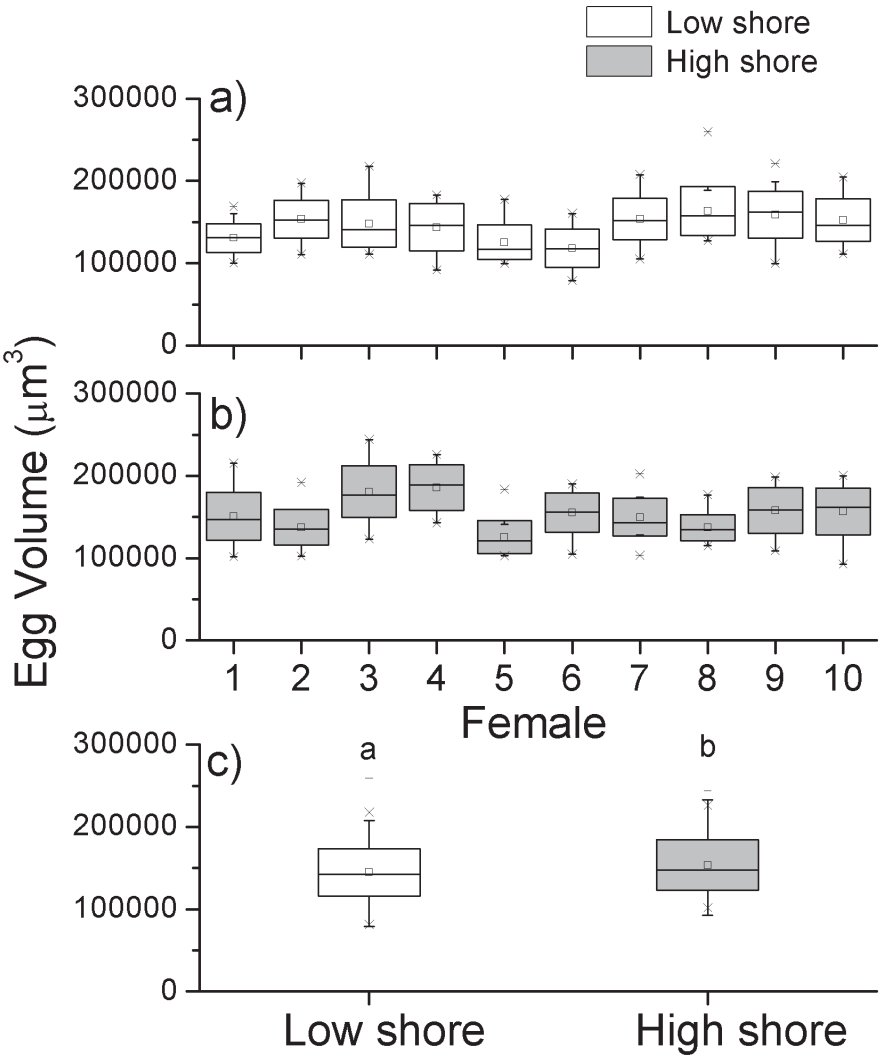

Figure 5.4: Boxplots of mean (±SD) of total egg volume of female *H. banksii* thalli from different heights on the shore. Grey boxes represent eggs collected from females from a) low and b) high on the shore and c) pooled on different heights on the shore. Letters signify differences.

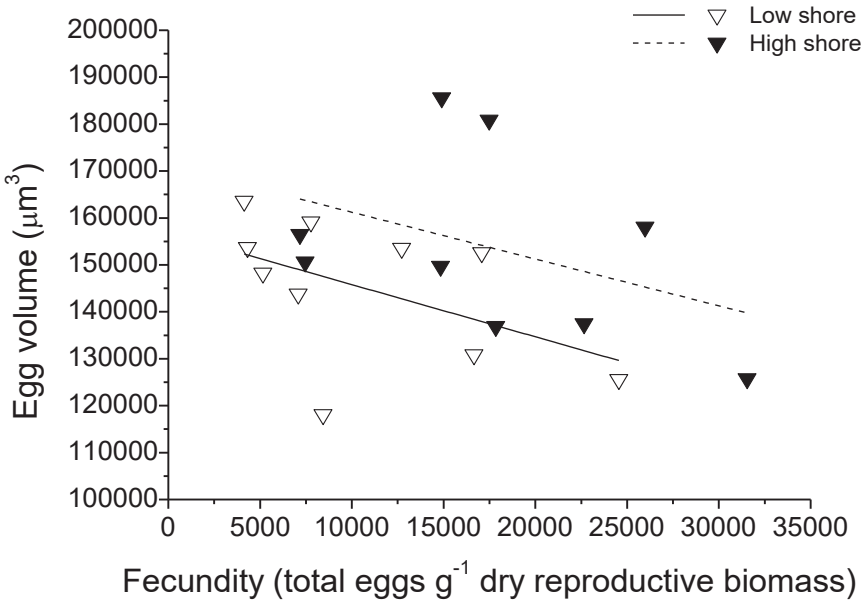

Figure 5.5: Relationship between egg volume and fecundity expressed as total eggs g^{-1} dry reproductive biomass amongst females low and high on the shore.

5.3.2. Temperature effects on embryo growth

Temperature had an effect on the fertilisation success and development of embryos (Fig. 5.3 b, c). At 22 °C and 26 °C, 75% of eggs were fertilised (as indicated by the Calcofluor stain). The percentage of fertilised eggs decreased to 59% at 30 °C and many embryos at this temperature became dislodged over time (Fig 5.3b). As a result, tracking embryos over the course of the 7-day experiment became difficult in this treatment.

Temperature also influenced the growth of embryos, with embryos at 22 °C having the greatest rhizoid extension after 7 days (441 ± 60 μm) followed by 26 °C (372 ± 70 μm) and then 30 °C (89 ± 12 μm) ($F_{2,219}$ = 888.04, P < 0.001, Fig. 5.3c, 5.6). The growth rate of rhizoids was similar between 22 °C and 26 °C ($F_{1,58}$ = 3.13 P = 0.082, Fig. 3c). Within the first 24 h, embryos incubated at 22 °C and 26 °C changed from eggs, as symmetrical spheres, to pear-shape through the development of the initial rhizoid. The germinating cell

differentiated lengthwise earlier at 22 °C and 26 °C compared to 30 °C and this occurred before it widened (Fig. 5.7). Embryos growing at 22 °C tended to develop a single rhizoid with development of second and third rhizoids staggered after the previous rhizoid was established (Fig. 5.1, 5.6a). Within the 26 °C treatment, the development of rhizoids was much more variable with 2 or 3 rhizoids growing simultaneously creating many short rhizoids (Fig. 5.6b). Embryos incubated at 30 °C remained spherical or only developed into a pear shape (Fig. 5.6c).

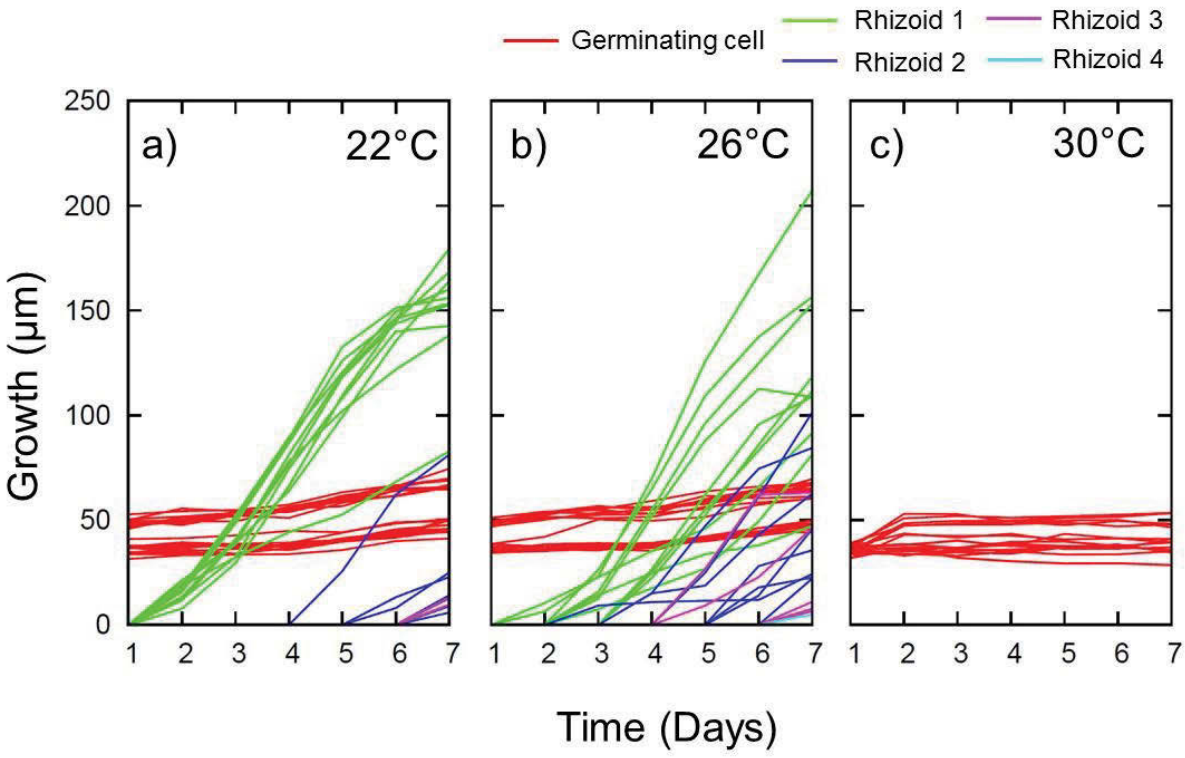

Figure 5.6: Growth of individual embryos in 3 temperature treatments a) 22 °C b) 26 °C and c) 30 °C over 7 days post-fertilisation. Lines represent growth over time of the germinating cell (red), and rhizoid 1, 2, 3 and 4 (n=30).

Variation in initial egg size did not have a significant effect on the development of early life stages. There were no significant correlations between the initial egg diameter and the growth rate of the length and width of the germinating cell and the growth rate of the first rhizoid at both 22 °C and 26 °C (Fig. 5.7, Table 5.1). Initial egg diameter only correlated with the timing of when the width of the germinating cell differentiated morphologically (Fig. 5.7, Table 5.1). Increased temperature reduced the growth of the germinating cell and affected the timing of development of the initial rhizoid (Fig. 5.7, Table 5.1). There were no significant interactions found between initial egg size and temperature suggesting that effect of maternal provisioning is not temperature dependent (Table 5.1).

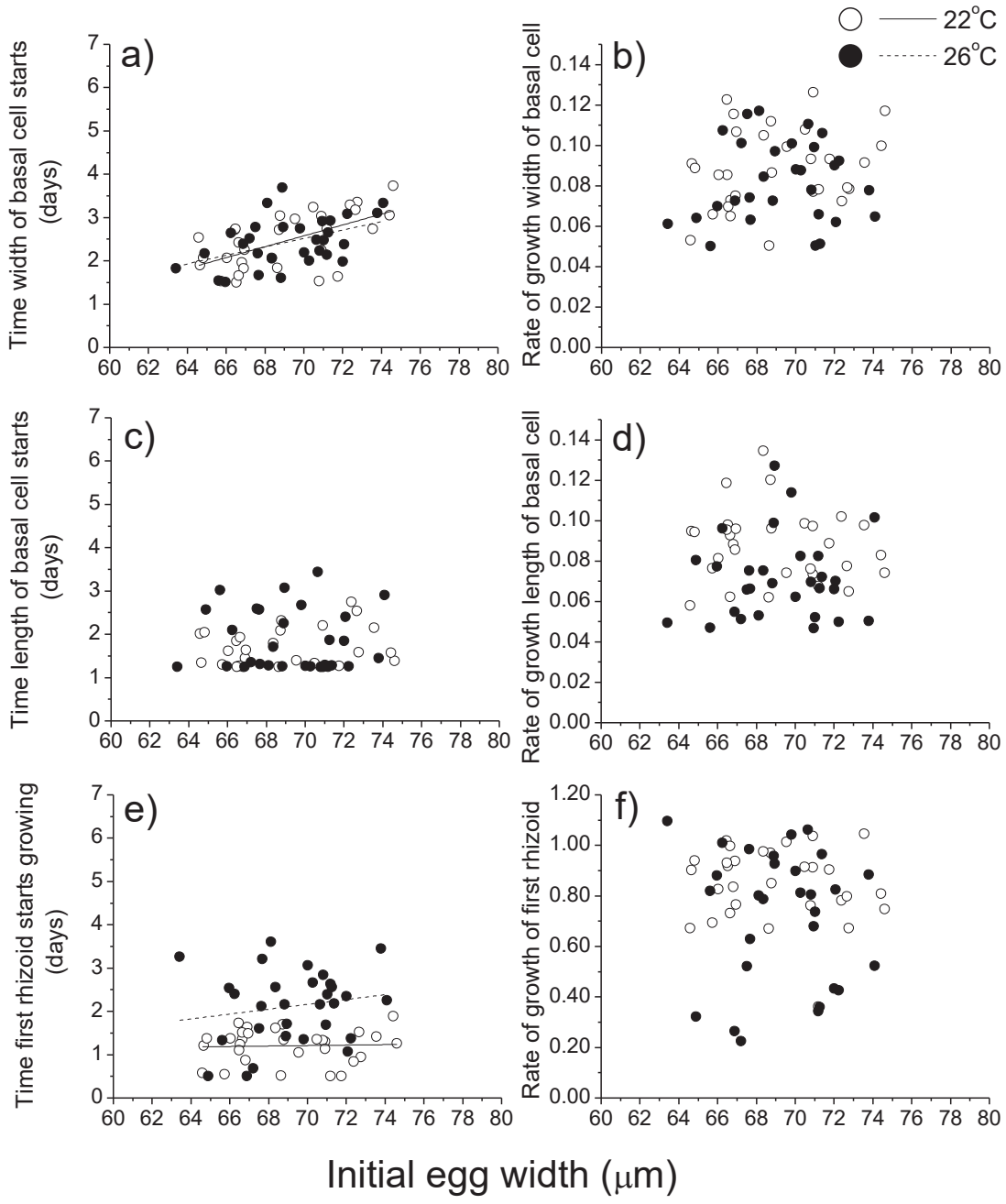

Figure 5.7: The relationship of embryo metrics and initial embryo width and time (days) when differentiation occurs of a) width and b) length of germinating cell start to grow and c) appearance of first rhizoid. Rate of growth of d) width and e) length of germinating cell and f) first rhizoid of two temperatures, 22 °C and 26 °C contrasted with initial embryo width (n=30). Embryo metrics are not presented for 30 °C as embryos did grow at this temperature). Lines of best fit present significant effects of the ANCOVA.

Table 5.1: Result of analysis of covariance (ANCOVA) of rate of growth and time of differentiation of the width and length of the germinating cell as well as the first rhizoid (n= 30). Temperature is the categorical variable and initial egg size is the covariate for each of the traits tested. The probabilities of F values from ANCOVAs were calculated using 9999 permutations.

		Rate of growth								
		Width of germinating cell			Length of germinating cell			Rhizoid 1		
Source	**df**	***F***	***P***	**R^2**	***F***	***P***	**R^2**	***F***	***P***	**R^2**
Initial										
Width	1	0.854	0.354	0.025	0.000	0.992	0.076	0.708	0.406	0.061
Temperature	1	0.608	0.443		4.700	**0.036**		2.922	0.094	
Error	56									
		Time of differentiation								
		Width of germinating cell			Length of germinating cell			Rhizoid 1		
Source	**df**	***F***	***P***	**R^2**	***F***	***P***	**R^2**	***F***	***P***	**R^2**
Initial			<							
Width	1	22.684	**0.001**	**0.288**	0.160	0.692	0.030	1.315	0.258	0.344
Temperature	1	0.087	0.758		1.612	0.211		28.427	**< 0.001**	
Error	56									

Bold denotes significance at *P* = 0.05

5.4. DISCUSSION

Despite environmental pressures imposed on *H. banksii* that inhabit the intertidal environment, reproductive investment in terms of total biomass and fecundity was similar amongst heights on the shore. Variation in egg size among individual females, and between individuals collected from different heights on the shore did differ, however, generally did not correlate with embryo development within the first 7 days. Initial egg size only showed a positive correlation with the timing of when embryos increased the width of their germinating cell. This egg size effect was independent of temperature (non-significant temperature effect), suggesting that larger eggs were better at growing regardless of temperatures. A trade-off between egg size and fecundity for females was not evident suggesting that high or low shore individuals are not limited in resources for reproduction.

5.4.1. Reproductive investment

This study estimated reproductive investment by identifying the proportion of adult tissue that appeared to release gametes. While only a snapshot view, and an imperfect one (because apparently sterile tissue may have yielded gametes on a different occasion), the hypothesis that thalli found high on the shore would have a lower reproductive investment (fecundity) was not supported by this study. The lack of differences in total thallus biomass, and fecundity between heights on the shore suggests that *H. banksii* may not re-allocate resources for reproduction because of their position on the shore, despite the differences in these local environments (Chapter 3). Previous studies with brown macroalgae have found that reproductive investment varies with wave exposure as well as between the centre and edges of a species distribution (Araújo *et al.* 2015; Chu *et al.* 2011). For instance, *Sargassam thumbergerii* allocated more resources to reproductive structures during its peak reproductive period which decreased the amount of resources used for vegetative regeneration in wave

sheltered locations (Chu *et al.* 2011). In *Ascophyllum nodosum,* individuals in edge populations had a greater investment of resources to reproduction compared to thalli in central populations (Araújo *et al.* 2015). It was suggested that the increased reproductive allocation in edge populations contributes to the availability of recruits and maintenance of population size over time (Araújo *et al.* 2015; Kendrick & Walker 1994). In contrast, Viejo *et al.* (2011) found reproductive investment was lower in marginal populations of, *Fucus serratus,* with an overall smaller size at maturity and reproduction occurring much earlier than central populations, which coincided with changes in sea surface temperature. This suggests that life history and changes in environment may be important to the viability of future populations with global warming. Furthermore, species that develop reproductive structures such as receptacles or sori (i.e., kelps, Mohring *et al* 2012) and have a defined reproductive period tend to show differences in resource allocation. As *H. banksii* seem to be fertile throughout the year and potentially spawns at each low tide (Gunthorpe *et al.* 1997), this suggests that a relatively small proportion of their energy is directed into reproductive structures and gamete production. The lack of a trade-off found between egg size and fecundity also further supports that *H. banksii* may not be limited in resources for reproduction and that maternal provisioning may not be critical for early life stage development.

5.4.2. Relationship of egg size with embryo development

Egg size varied amongst individual females but also among individuals collected from different heights on the shore, with females from high on the shore producing larger eggs (Fig. 5.4). Differences in macroalgal egg size have been found to reflect differences in lipid allocation, with extensive energy reserves potentially allowing larger eggs to be viable for longer (Clayton 1992). The advantage of longer viability may be to allow larger eggs to disperse further (Clayton 1992; Hoffmann 1987), possibly to other favourable areas within the

rock platform, and may be important in gene flow and maintenance of population densities (Kendrick & Walker 1994).

The positive correlation of initial egg size and timing of when the width of the embryo germinating cell increased indicates that different sized eggs have different development trajectories. A delay in the development of large embryos suggests *H. banksii* have the potential to disperse further, as they could potentially be dislodged more easily, disperse and re-attach at a greater distance from their parents (Dudgeon *et al.* 2001; Muhlin *et al.* 2008; Pearson & Brawley 1996). Adhesion to the substrate of early life stages can also vary in different species macroalgae (Dimartino *et al.* 2015; Taylor & Schiel 2003). In a study by Pearson and Brawley (1996), zygotes of *Fucus distichus* remained unattached for a single tidal cycle. In *H. banksii* embryos, adhesion to the substrate requires 6 h for a firm attachment but 24 h for maximum attachment, whereas *Durvillaea antarctica* can adhere firmly within the first hour (Dimartino *et al.* 2015; Taylor & Schiel 2003). It is currently unclear what the downstream impact of delayed development would have on embryos.

The timing of when the length of germinating cell morphologically differentiates and when the first rhizoid appeared had no relationship with initial egg size. This lack of difference in the timing of embryo differentiation suggests that embryo performance may more strongly reflect their genotype rather than environment within the first stages of development. Additionally, previous studies have found that development in fucoid embryos 7-10 days old was light-independent (using photosynthate produced by the Calvin Cycle and stored, rather than photosynthesis), suggesting that development could occur independently of photosynthesis with reserves of mannitol used instead (Davison *et al.* 1993; Major & Davison 1998). In any case, fucoid embryos are equipped with functioning chloroplasts which they

can potentially use to photosynthesise within the first 24 h (Clark *et al.* 2013), unlike developing terrestrial seedlings that must utilise reserves of endosperm until they germinate and can photosynthesise (Galloway 2001). Thus, variation in maternal provisioning of eggs of *H. banksii* may not be as important for development in early life stages.

5.4.3. Effect of egg size on embryo metrics at variable temperatures

The absence of a relationship between initial egg size and the rate of growth at either 22 or 26 °C suggests that maternal provisioning was no more important at stressful versus ambient temperature, limiting the potential for adaptive maternal effects to give offspring an advantage in environments that match the maternal environment. The lack of advantageous maternal provisioning would potentially allow offspring to function in a variety of habitats rather than be limited physiologically to the same environment as their parents. Although fucoids are suggested to have limited dispersal capacity (< 10 m; (Bellgrove *et al.* 1997, 2004; Serrão *et al.* 1997), differences in a few meters within the intertidal environment can have vast impacts on the thermal exposure of an organism due to topography, wave splash, substrate orientation and light intensity (Harley 2008). Therefore, the probability of an embryo settling and growing in an environment similar to its parents may be highly variable, depending on the spatial and temporal heterogeneity of the local habitat.

An alternative explanation for the absence of a maternal provisioning effect is that it was not found in the traits tested or expressed within the 7 days of development (note that female x temperature interactions were found within 5 days in Clark *et al.* 2013 and in Chapter 3). Temperature, however, did affect fertilisation success and embryo growth rates, with almost no growth at 30 °C. Fertilisation success in fucoids has been reported to be close to 100% (Brawley *et al.* 1999; Pearson & Brawley 1996; Serrão *et al.* 1996) although success rates as

low as 75% have been also been documented. The decrease in fertilisation success of embryos at 30 °C suggests that temperature had physiological effects on sperm, where temperatures experienced outside optimal temperatures could diminish their swimming efficiency. There are limited studies, however, that have analysed the effects of sperm viability with increased temperature in macroalgae.

5.4.4. Conclusions

This chapter was a preliminary study which investigated maternal provisioning in a brown macroalga. It revealed that maternal provisioning in *H. banksii* does not give early life stages an advantage in growth or development within the first 7 days of development, and that *H. banksii* females may not be restricted in the amount of resources they invest in sexual reproduction. Egg size did not generally correlate with embryo metrics suggesting that provisioning may not be required in early life stages of *H. banksii*. Differences in the timing of development of different sized eggs, however, may aid in dispersal of embryos to more favourable environments.

In other sessile organisms, maternal provisioning has been observed in variable light and nutrient environments, suggesting that parents can regulate resource allocation to offspring and reproduction (Galloway 2005; Roach & Wulff 1987). As early life stages are considered to be the most vulnerable stages in macroalgal development (Brawley & Johnson 1991) and important in the maintenance of future populations (Kendrick & Walker 1994), further research is required to investigate whether provisioning will aid in tolerance and evolutionary adaptation in a changing environment.

Chapter 6

General Discussion and Conclusions

6. Overview

Hormosira banksii, is a temperate, intertidal macroalga and important foundation species. The goal of this thesis was to determine the vulnerability of *H. banksii* to warming at two different spatial scales (regional and local). Examination of adult physiology, thermal performance curves of multiple embryo traits, genetic diversity and transgenerational effects allowed me to address the following questions:

- **What scale of exposure is most physiologically important in populations of *H. banksii*?**
- **What are the physiological tolerances of *H. banksii* at different spatial scales?**
- **Does the life history of *H. banksii* make it more vulnerable to climate warming?**
- **What is the adaptive capacity of *H. banksii* to climate warming?**

Below I synthesise the main results of this thesis in regards to these fundamental questions.

6.1.1. What scale of exposure is more physiologically important in populations of H. banksii*?*

Temperature fluctuations within *H. banksii* populations sampled in this study were highly variable between regional and local spatial scales (Fig 3.1). The warm rear-edge population had lower annual temperature fluctuations and experienced fewer days over 35 °C compared to the population located more centrally (Fig. 3.1 b). This pattern of exposure is consistent with the climate variability hypothesis, which states that lower latitudes experience less variable temperatures compared to higher latitudes (Stevens 1989; Sunday *et al.* 2011).

Local scale effects such as differences in topography (mounds and crevices), aspect, tidal regime and local weather can all have effects on the exposure at the individual level and may have more influence on the physiology and govern the thermal niche of *H. banksii* than larger scale effects (Harley & Helmuth 2003; Helmuth *et al.* 2010). Difference in thermal exposure at the local scale, i.e., between heights on the shore, revealed that thalli higher on the shore of some populations are exposed to greater thermal variation than thalli lower on the shore (Fig. 3.1 c-f). At central populations *H. banksii* from high on the shore had a wider thermal niche compared to thalli low on the shore which peaked at a single thermal optimum (Fig. 3.2 b). At the rear-edge population there were no differences in thermal niche between heights on the shore, suggesting that individuals from this population experience similar temperature fluctuations despite position on the shore (Fig. 3.2 a). Local scale effects at rear-edge populations reduced the exposure of high shore thalli so they have similar physiology to low shore thalli (Fig. 3.2 a). As stated in Chapter 2 and 3, the rock platform at Minnie Water is characterised by boulders with thalli inhabiting areas where water pools between boulders during low tide. The effect of the boulders may minimise stress otherwise experienced by thalli exposed to a flat topography, and essentially buffers individuals from the full magnitude of regional climate effects. These local scale effects created substantial differences in physiology and morphology in the Minnie Water population as determined in both adult (Chapter 2) and early life stages (Chapter 3). The high plasticity in morphology (Chapter 2) and varying thermal niche (Chapter 3) of *H. banksii* sampled at different spatial scales in this thesis suggests that physiology may be governed more by local scale effects than larger regional scale climate effects. These findings agree with a previous study by Helmuth *et al.* (2006) which revealed body temperatures of an intertidal mussel were higher in some locations found more poleward compared to locations found equatorward, which was due to local scale effects particularly timing of tidal regime, topography and aspect.

Experimental temperature exposures were specifically designed to be environmentally realistic, and hence appropriate for determining differences in the physiology of different *H. banksii* populations. For example, to assess photosynthetic function and water retention of adults in relation to their morphology, adult thalli were subjected to either 20 °C or 30 °C for 4 h to simulate tidal exposure. As Figure 3.1 (c-d) shows, a 10 °C elevation in exposure during tidal emersion is entirely feasible for intertidal organisms. To assess the physiological, ontogenetic and fitness responses of early life stages to temperature, exposures were considerably longer (5 to 7 days), and incorporated a diel component. Thus, for the 28 °C temperature treatment, the thermal exposure during the day was 28 °C, and at night it was 23 °C. This study also highlighted the need to incorporate thermal variation in experimental designs as the results of this thesis, indicates that thermal variation in natural populations can produce different thermal tolerances and different optima which will affect how organismal response.

In view of both the in situ and experimental temperature exposures, this study has provided evidence to show that **small scale effects at the level of the individual are potentially more important to the populations of *H. banksii* investigated in this thesis compared to regional scales**. Although regional scale climate may set both hot and cold thermal limits which govern species distribution, it is the local scale effects of weather and topography that will either enhance or buffer the effects of climate change. Thus, when making predictions of how organisms will respond to future warming, local exposure of different populations needs to be considered, as the results of this thesis suggest that populations can behave differently, which is supported by ongoing research by Helmuth *et al.* (2006, 2010, 2014). Long-term monitoring and analyses of thermal exposure of other populations throughout the distribution

of *H. banksii* would be needed to determine the full extent of how local scale effects will buffer or enhance climate stressors on the physiology and distribution of *H. banksii.*

6.1.2. What are the physiological tolerances of H. banksii *at different spatial scales?*

The range of temperatures that govern physiological tolerances (thermal niche) are proportional to the magnitude of temperature variation experienced throughout a species' life history (Stevens 1989; Sunday *et al.* 2011). In this study, physiological tolerances of *H. banksii* were investigated for two life history stages: adults (Chapter 2) and embryos (Chapter 3). Thermal performance was assessed amongst numerous traits in embryos (growth, photosynthetic efficiency of PSII, ontogenic development) and in adults (photosynthetic efficiency of PSII, relative water content).

A common finding for both adult and early life stages was that marginal populations were sensitive to elevated temperatures beyond temperatures experienced in their natural environment. Early life stages of *H. banksii* (Chapter 3) showed distinct thermal performance curves which differed between regions and between tidal heights (Fig. 3.2). Embryos from the rear-edge population had lower thermal optima (24 – 26 °C, Fig. 3.2) and a narrower range of temperature over which growth was maximal (22–26 °C) compared to the central population that had a wider thermal breadth and higher thermal optimum. This finding was quite surprising as previous studies investigating thermal tolerances of intertidal species (crustaceans, algae) across distributions have found rear-edge (warm) populations are more thermally tolerant than populations in higher latitudes (Kelly *et al.* 2012; Martínez *et al.* 2012; Pearson *et al.* 2009; Stillman & Somero 2000; Zardi *et al.* 2013). The lower thermal optimum and narrower thermal niche of embryos from the marginal population of *H. banksii* may reflect local habitat buffering and lower seasonal temperature fluctuations experienced in

lower latitudes. It also suggests that a thermal specialist strategy may be utilised by *H. banksii* early life history stages, as it is more energetically costly to grow across all temperatures, therefore growth is maximised at temperatures frequently experienced (Gilchrist 1995). Divergence in thermal performance was also observed at the local scale in which embryos from low on the shore within marginal and central populations grew optimally in a narrower range of temperature compared to high shore embryos (Fig. 3.2 a, b). This suggests that embryos low on the shore are more sensitive to warming, irrespective of geographic location, due to the lower variation in temperatures experienced.

Adult stages of *H. banksii* (Chapter 2) at the northern-most populations sampled (warm-edge, Angourie) were sensitive to both 20 °C and 30 °C as shown by the rapid decline in photosynthetic efficiency of PSII in both temperature treatments (Fig. 2.4). As 20 °C represents temperatures commonly experienced within this region (Fig. 3.1), such thermal sensitivity suggests that adults at rear edge populations rapidly redirect energy away from photochemistry to minimise damage to their photosystems. Implementation of this strategy may be favourable for these individuals as the relatively small vesicle morphology of adults from this location suggests they have relatively small capacity for water retention. As revealed in Chapter 2, vesicle morphology had a strong influence on photosynthetic efficiency of PSII through the retention of water within vesicles, in line with other studies (Dring & Brown 1982; Oates 1985). Sensitivity to both ambient (20 °C) and increased temperatures (30 °C) could also suggest an additive effect of thermal and desiccation stress with light intensity on the physiology of individuals inhabiting this location (Ferreira *et al.* 2014; Martínez *et al.* 2012). The additive effects of light and temperature have been observed in other species of fucoids in which light intensity was found to strongly correlate with position within the shore (Dromgoole 1987; Gómez *et al.* 2004; Williams & Dethier 2005). Other macroalgal species

found towards the equator had an increased ability to recover from thermal and desiccation stress than species living at higher latitudes (Ferreira *et al.* 2014). This was not the case for *H. banksii* in the rear-edge population (Angourie) as thalli were unable to recover photosynthesis in either temperature treatment. The lack of full recovery of F_V/F_M suggests that photosystem II was irreversibly damaged or thalli were unable to repair proteins of PSII (Allakhverdiev *et al.* 2008). Further research would be needed to determine the additive effects of light and temperature on marginal populations on *H. banksii*.

It is generally thought that early life stages are more susceptible to environmental and anthropogenic stress than adult forms as they lack mechanisms that allow resistance against environmental stress (such as morphological structures that aid in water retention, Chapter 2) (Brawley & Johnson 1991; Coelho *et al.* 2000; Nielsen *et al.* 2014b). Physiological tolerances were variable in both life stages of *H. banksii* investigated. While not directly comparable, treatment temperatures overlapped in adults and embryos, where 30 °C was lethal for adult thalli and embryos from the marginal population (Minnie Water). For adult thalli desiccated at 30 °C, *H. banksii* was not able to recover photophysiologically at any location investigated. Embryos from the warm rear-edge population however, showed decreased photosynthetic efficiency at 30 °C which retarded growth and development whereas some embryos from cool central population were able to grow at this temperature. Despite the different time scales of experimentation (< 24 h for adults, 5 days for embryos), these laboratory data suggest that embryos are potentially less sensitive to thermal exposure compared to adults, however, early life stages have been found to grow in temperatures several degrees above adult forms (Chu *et al.* 2012), but may not survive in later life. These findings would predict that *H. banksii* would not be found at temperatures exceeding 30 °C, however, natural populations are exposed to such temperatures (J. Clark personal observation; Chapter 3). The dynamic nature of the

intertidal with changes in tidal cycle, aspect, wind speed, humidity and cloud cover, ensure that temperatures as high as 30 °C are not usually sustained over long periods. Embryos may also inhabit sites beneath parental thalli or small cracks or crevices that would buffer temperatures experienced during low tide (Brawley & Johnson 1991).

Although upper thermal limits are important to understand as species are being pushed to their physiological limits with global warming, cold tolerances have also been suggested to be more of an influence on physiological traits in macroalgae (Zardi *et al.* 2015). Although not investigated here, cold tolerances are suggested to be more influential on traits than upper thermal limits, with the tolerance generally decreasing with latitude. It is suggested that upper thermal limits are more conservative and depend on the acclimatory abilities of different species (Addo-Bediako *et al.* 2000; Araújo *et al.* 2013; Sunday *et al.* 2014; Wernberg *et al.* 2016; Zardi *et al.* 2015). Further research on the cold tolerances of *H. banksii* are also needed to determine the full thermal range which *H. banksii* can tolerate.

The results of this study suggest that ***H. banksii*** **inhabiting central locations and higher on the shore and with more of a generalist thermal strategy may be the 'winners' in future warming, as they are essentially 'buffered' to a range of temperatures and have a larger thermal breadth in which individuals can functionally respond** (Ketola *et al.* 2013; Somero 2010). In contrast, *H. banksii* inhabiting warm rear-edge locations and lower on the shore are living in proximity to their physiological limits and may be susceptible to future warming as they have narrower thermal safety margins. These individuals may potentially be unable to acclimate to further increases in temperature, as suggested by other studies (Huey *et al.* 2012; Janzen 1967; Somero 2010).

6.1.3. Does the life history of H.banksii *make it more vulnerable to climate warming?*

The framework developed by Williams et al (2008), states that resilience encompasses life history traits and dispersal capacity that would enable species to recover from perturbations. Within this study, increased temperature mostly had a negative effect on the photophysiology of adults and embryos, fertilisation success (in the central population Pearl Beach) and recruitment (embryo attachment), however, had context-specific effects (+/-) on growth and ontogenic development (Fig. 6.1). These context specific effects of increased temperature can either have a positive or negative effect on growth and ontogenic development and depend on what part of the species distribution it occurs in. Small increases in temperature may increase fitness in colder poleward populations, however, increased temperatures at marginal populations closer to the equator can have negative impacts (Somero 2002). The increase in mean temperature and alterations in seasonal variation associated with global warming may have major effects on life history processes of *H. banksii,* as found in other members of the Fucaceae such as growing season, phenology (synchronising of reproduction; Muhlin *et al.* 2008; Zardi *et al.* 2015) as well as successful recruitment for persistence and maintenance of future populations (Kendrick & Walker 1994). Many macroalgal species use environmental cues including spectral or changes in light intensity, sharp temperature or salinity change, nutrient limitation, low water motion (Muhlin *et al.* 2008; Pearson & Serrão 2006) to trigger gamete release. Global warming may alter these environmental cues and timing for spawning events resulting in temporal variability and asynchronous reproduction (Muhlin *et al.* 2008).

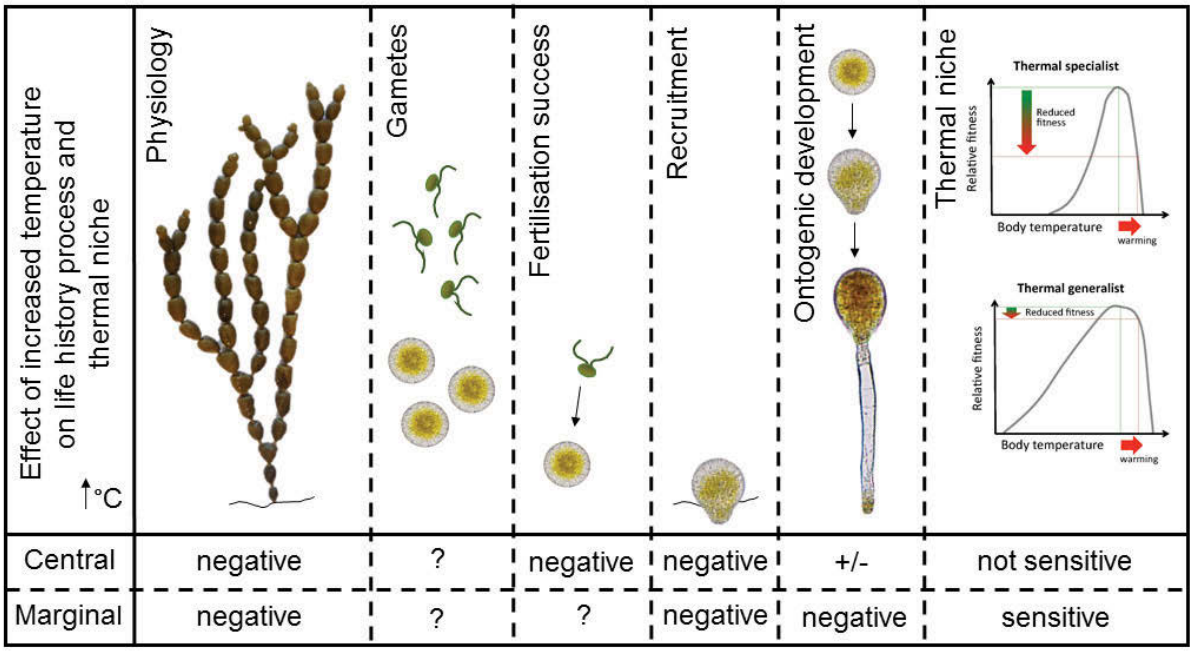

Figure 6.1: Conceptual diagram summarising the effects of increased temperatures on life history processes and thermal niche as revealed in this thesis. Question marks are life history processes that were not assessed in this thesis, whereas +/- symbol is context specific. Diagram was adapted from Harley *et al.* (2012).

With respect to thermal impacts on early life stage development, the results of Chapter 5 revealed little evidence of maternal provisioning giving an advantage to 1-7 day old embryos. Significantly larger eggs were found in females from high on the shore, however, larger eggs were not fitter – i.e., they did not grow faster than smaller eggs, and therefore did not give any apparent fitness advantage in early life. Timing of when eggs morphologically differentiated correlated positively with egg size suggesting that larger eggs developed more slowly than smaller eggs which may be important in the dispersal capacity of *H. banksii* (rather than being advantageous in the fitness of offspring). Furthermore, as some embryos from Pearl Beach appeared to have a broad thermal niche and were limited in their dispersal capacity to small scales (within a single platform, Chapter 4), it is most likely that embryos would experience a similar environment as their parents, therefore any transgenerational plasticity may be selected for. Thus, in some cases, maternal provisioning could be an important source of

variation if other sources (such as genotypic diversity) are not available (Chapter 5, Mousseau & Fox 1998; Wolf & Wade 2009).

Life history traits predicted to promote resilience and recovery from natural and anthropogenic disturbances and reduce extinction rate include short life span, rapid maturation, high reproductive rates, large population sizes and capacity to disperse over long distances (McKinney 1997; Williams *et al.* 2008). *H. banksii* is a long-lived species known for its slow vegetative growth and longevity (> 4 years), with reproductive maturation occurring at 11 to 14 months (Kain 2015). Species with short life spans and fast maturation times are suggested to be more resilient to climate impacts, as evolutionary adaptation occurs over many generations (McKinney 1997). *H. banksii* is also a broadcast spawner, and reproductive throughout the year (Gunthorpe *et al.* 1995) and it is therefore suggested to have high reproductive rates. High reproductive rates may ensure persistence of populations or increase population density in *H. banksii* by increasing the chances of successful recruitment. Although *H. banksii* has been suggested to be fertile throughout the year, there were multiple occasions during this study when *H. banksii* did not produce gametes, or produced them but they were not viable, particularly during the height of the austral summer (Dec-Feb), and for a few months leading into austral autumn (Mar-April). Members of the Fucaceae family are known to have a sterile summer period (Coelho *et al.* 2000), that could be related to energy being redirected towards photoprotection and repair at higher temperatures (Allakhverdiev *et al.* 2008). **As such, the results suggest that the life history traits of *H. banksii* such as limited dispersal capacity and gene flow (Chapter 4), and lack of transgenerational effects through maternal provisioning (Chapter 5) may potentially make them vulnerable to global warming**.

6.1.4. What is the adaptive capacity of H. banksii *to global warming?*

One of the most important findings of this study was that *H. banksii* has lower genetic diversity in marginal populations compared to central populations (Table 4.1, 4.3) and has limited gene flow, contributing to increased genetic structure over increased geographical distances (Fig. 4.2). Furthermore, moderate levels of genetic structure were found between neighbouring populations within each region, with marginal populations being more genetically related than their central counterparts. This has important implications for the adaptive capacity of *H. banksii* to global warming, as higher genetic diversity within a population provides a greater range of genotypes, likely associated with a greater range of functional responses, thus increasing the probability of recovery and resilience to perturbations (Al-Janabi *et al.* 2016; Reusch *et al.* 2005). A lower number of genotypes within the warm edge population suggests that *H. banksii* adaptation will need to be underpinned by plasticity of adults to tolerate changes in environmental conditions, genetic and epigenetic changes leading to increased fitness, rather than 'shuffling' the relative abundance of different genotypes already present (Litchman *et al.* 2012). Further, the extirpation of marginal populations may also cause the loss of unique alleles that may provide tolerance to populations further south (Assis *et al.* 2013). However, in the case of *H. banksii*, genotypes inhabiting marginal populations were revealed to be sensitive to increased temperatures (Chapter 2 and Chapter 3) and have a narrower thermal niche (Chapter 3), which may not aid the resilience of southern populations to global warming.

The limited gene flow of *H. banksii* found in this study agrees with other findings that species in the Fucaceae have reproductive strategies which ensure increased fertilisation success (i.e., close to 100%; (Pearson & Brawley 1996; Pearson & Serrão 2006; Serrão *et al.* 1996) and recruitment of embryos close to parent thalli. Despite this, the results suggest that gene flow is

still evident between neighbouring populations over 10 km (F_{ST} value of 0.2 suggests one migrant per generation), therefore transport of reproductively viable floating thalli is most likely the method of *H. banksii* dispersal over long distances (McKenzie & Bellgrove 2008). Evolutionary rescue through gene flow by tolerant genotypes from central populations is problematic for northern marginal populations due to the poleward flow of the East Australian Current, the prevailing transport vector in the region. Although northward flowing counter-currents from the EAC have been found to occur seasonally and could potentially bring floating wrack nearer to the coast, they occur on much rarer occasions (Coleman *et al.* 2011b). Consequently, *H. banksii* show patterns of genetic differentiation over geographic distances, which over time could cause outbreeding depression (i.e., decrease in fitness with mating of geographically distant individuals; (Waser & Price 1994)) due to limited dispersal of propagules (McKenzie and Bellgrove 2006, Coleman *et al* 2011a). No evidence of outbreeding depression in *H. banksii* has been found, suggesting that *H. banksii* has not been isolated long enough for genetic divergence to occur (McKenzie & Bellgrove 2006) or that there is gene flow occurring between populations that is preventing genetic isolation, or both. In contrast, the reduced gene flow and the increased susceptibility to inbreeding at marginal populations may pose a threat to the persistence of these populations through the inability to adapt to warming climates with a reduced range of responses due to low genetic variation.

A challenge in determining the adaptive capacity of a species, is determining whether response to environmental stress is a genetically fixed trait or whether it reflects phenotypic plasticity. Plasticity in thermal traits of embryos from the same families were identified in Chapter 3. The thermal specialist phenotype of 5-day old embryos at Minnie Water suggests that this marginal population has lower plasticity in thermal tolerance traits, compared to embryos at Pearl Beach that show a thermal generalist phenotype, however, it is not known

whether this pattern is consistent between other populations within the rear-edge and central populations. Furthermore, genotype x environment interactions (male x temperature interactions, Table 3.2) were found for ontogenic development, suggesting that heritable genetic variation in traits related to thermal tolerance are found in populations of *H. banksii*. Future populations at the locations investigated in this study therefore have heritable genetic variation for selection to act upon, suggesting that thermal tolerance of *H. banksii* may be enhanced over time.

Given the local-scale differences in thermal exposure of *H. banksii* (Fig. 3.1) but absence of genetic structure between heights on the shore (Table 4.2), this suggests that plasticity may play an important role in stress tolerance of *H. banksii* over relatively small (cm to m) space and time scales, without genetic variation in functional responses. Phenotypic divergence in morphotypes has been found for other species of macroalgae, without differences in the underlying genetic structure (Zardi *et al.* 2015). This suggests that environmental variation in the intertidal results in small scale differences in morphology and physiology of *H. banksii,* however, the counteracting effects of gene flow eliminates genetic structuring. The differences in morphology found in Chapter 2 most likely reflect plasticity, however, common garden experiments, in which single genotypes are raised in multiple environments, is required to establish that the variation in morphological traits is entirely plastic. Another common finding throughout this thesis was that although temperature had a significant effect on photosynthesis, it did not interact with either the regional or local spatial scale tested at either life stage (Chapter 2, Chapter 3) and suggests that photosystems have a high degree of plasticity (Clark *et al.* 2013). This is perhaps not surprising, as intertidal species must contend with a highly stressful and variable environment including fluctuating light intensity related to

diel and tidal cycles, as well as local climate (Davison & Pearson 1996; Dring & Brown 1982; Gómez *et al.* 2004).

6.2. Implications of this study

Global warming is already having profound effects on many species, including macroalgae, through effects on physiology, abundance and shifting distributions (Lima *et al.* 2007; Parmesan & Yohe 2003; Poloczanska *et al.* 2011; Smale & Wernberg 2013; Wernberg, Russell, Thomsen*, et al.* 2011). **The findings of this thesis suggest that populations of *H. banksii* vary in their vulnerability to warming, but rear-edge warm marginal populations may be particularly vulnerable.** Marginal populations nearing their distributional edge were sensitive to increased temperatures at both life stages (Chapter 2 and 3), have narrow thermal safety margins (Chapter 3), have limited gene flow (Chapter 4) and lower genetic diversity (Chapter 4).

Increased temperatures will likely have significant effects on the persistence of marginal populations of *H. banksii* in the future. The predicted increases in annual mean temperatures associated with climate change (IPCC 2014) may not cause mortality on the temperate populations investigated, as they experience greater daily and annual fluctuations in temperature than the projected 2-3 °C increase for 2030. However, the increase in average temperatures will most likely have carry-on effects on cool climate populations as elevated temperatures may cause physiological effects such as reduced growth and damage to photosystems which can in turn affect fecundity, recruitment and potentially gene flow. For marginal populations, which are close to their thermal limits, the predicted increase may push them beyond their physiological limits. In either case, the predicted increase in frequency and intensity of extreme climate events such as heat waves and storm surges (IPCC 2014) is likely

to impact *H. banksii* populations, particularly populations with lower genetic diversity and resilience such as rear-edge populations, as found in *Scythothalia dorycarpa*, a subtidal macroalga in Western Australia following a marine heat wave (Smale & Wernberg 2013). The loss of a functionally and ecologically important species will have cascading effects on the communities it supports.

The findings of this thesis indicate that local habitat effects may be more important in governing physiological tolerances than regional effects by buffering the full extent of global warming (local tidal regime, topography, i.e. boulders at Minnie Water). Previous studies have revealed that due to local habitat effects, we will most likely see declines in abundance and distribution as mosaics of "hot spots" and "cold spots" rather than a linear shift in distribution (Helmuth *et al.* 2006; Helmuth *et al.* 2002). Given this, physiological tolerances can differ between populations (see Chapter 3), and thus the vulnerability of *H. banksii* should be assessed on a population by population basis.

6.2.1. Suggestions for management of H. banksii in Australia and future directions

The wider significance of these results may aid in providing science-based evidence for policy development as well as for management of ecosystems and conservation efforts (Riebesell & Gattuso 2015). Ultimately, the global reduction of greenhouse gas emissions is needed in order to reduce further increases in ocean temperatures. Given that environmental change due to ocean warming will not be modified in the immediate future, the results of this thesis suggests that adaptive management of Australia's coastal environments should focus efforts on mitigating stress of populations most at risk. The lower resilience of marginal populations of *H. banksii* to temperature may make them susceptible to other anthropogenic stressors such as coastal development, pollution, disease or competition from invasive species

(Wernberg *et al.* 2010). Furthermore, as *H. banksii* is found in the mid-intertidal area, individuals are also more prone to human impacts such as trampling (Keough & Quinn 1998) and sewage effluent (Bellgrove *et al.* 1997), therefore resource management would be required to minimise these effects on vulnerable populations. Maintenance of marine reserves is also required to ensure adequate connectivity and gene flow to populations more susceptible to inbreeding such as marginal edges or populations separated by geographical barriers (Coleman *et al.* 2011a). For more susceptible populations, transplantation or restoration of new genotypes that increase genetic diversity has shown to increase the chances of recovery from disturbance (Al-Janabi *et al.* 2016; Evans *et al.* 2016; Reusch *et al.* 2005). For example, by introducing genetically diverse individuals of seagrass into a population, it was found that meadows were more productive, contained more trophic levels and were more resilient to perturbation; (heat wave, Reusch *et al.* 2005). The restoration of *Phyllospora comosa,* a subtidal brown macroalga that has been absent in areas around Sydney Harbour has been successful and may aid in the connectivity and recruitment back into the urban shores of Sydney (Campbell *et al.* 2014). Implementation of such strategies may aid *H. banksii* to recover similarly in the future.

The research presented within this thesis has provided a better understanding of the differences in vulnerability of *H. banksii* populations to thermal stress. The findings of this thesis are significant as they have identified traits such as limited gene flow (dispersal capacity), lower genetic diversity and distinct thermal niches in different populations that may lead to vulnerability to warming oceans. Moreover, it has identified populations vulnerable to extirpation from global warming (warm - marginal populations Angourie and Minnie Water) to concentrate conservation efforts. To conclude, this thesis has highlighted the importance of considering exposure and various species traits that collectively determine the physiological

sensitivity, resilience and adaptive capacity of macroalgal species which is particularly important in maintaining the biodiversity of rocky shores in subtropical-temperate Australia.

APPENDIX A

List of Supplementary of Figure and Table Captions

Figure S2.1: Pilot study that determined stressful light intensities for desiccation treatments. PI curves showed that 400 µmol photons m^{-2} s^{-1} was close to the saturation region and therefore an effective light intensity to use for examining stress responses. L1-4 are low shore thalli and H1-4 are high shore thalli

Figure S2.2: Mean ± SE of thallus morphometric traits collected from 4 locations (ANG = Angourie, MW = Minnie Waters, PB = Pearl Beach, and BB = Bilgola Beach) and two heights on the shore (n = 10).

Figure S2.3: Mean ± SE of vesicle morphometric traits collected from 4 locations (ANG, MW, PB, and BB) and two shore heights (black= low shore, grey= high shore; n = 40).

Figure S3.1: Mean (± SD) maximum quantum yield of PSII (F_V/F_M) of embryos from Minnie Water (a) and Pearl Beach (b) after incubation for 5 days at three temperatures: 24, 28, 32 °C. Closed circles (●) indicate F_V/F_M measured from embryos low on the shore and open circles (○) indicate F_V/F_M measured from embryos high on the shore.

Figure S3.2: Mean (± SD) effective quantum yield of PSII (photosynthetic efficiency) of embryos measured under low light (LYII; 32 µmol photons m^{-2} s^{-1}) and high light (HYII; 113 µmol photons m^{-2} s^{-1}) from Minnie Water (a, b) and Pearl Beach (c, d) after 5 days incubation at three temperatures: 24, 28, 32 °C. Closed circles (●) indicate Y(II) of embryos low on the shore and open circles (○) indicate Y(II) of embryos high on the shore (n=6).

Figure S3.3: Mean (±SD) baseline chlorophyll-a fluorescence (F_t) of embryos from Minnie Water (a) and Pearl Beach (b) after incubation for 5 days at three temperatures: 24, 28, 32 °C. Closed circles (●) indicate F_t measured from embryos low on the shore and open circles (○) indicate F_t measured for embryos high on the shore.

Figure S3.4: The habitat of *H. banksii* at the marginal location, Minnie Water (top), and central location, Pearl Beach (bottom) indicating differences in local-scale topography.

Table S2.1: Mean (± SE) of thallus and vesicle morphometrics of *H. banksii* collected from low and high on the shore from four locations on Australia's east coast: Angourie, Minnie Waters, Pearl Beach and Bilgola Beach.

Table S2.1: Mean (± SE) of thallus and vesicle morphometrics of *H. banksii* collected from low and high on the shore from four locations on Australia's east coast: Angourie, Minnie Waters, Pearl Beach and Bilgola Beach.

Table S4.2: Calculation of inference of K using the Delta (K) method as per Evanno *et al.* (2005).

APPENDIX B

Table S4.3: Expected (H_E) and observed (H_O) heterozygosity estimates and number of occurrences of each allele for each locus from each shore height (L and H) and across locations Bilgola Beach (BB), Pearl Beach (PB), Minnie Water (MW), and Angourie (ANG). N denotes sample size.

LOCATION		BBH	BBL	PBH	PBL	MWH	MWL	ANGH	ANGL
LOCUS									
HB03									
N		27	25	29	28	28	31	25	28
Allele	121	0.982	1.000	0.983	1.000	0.679	0.790	0.560	0.554
	127	0.019	0.000	0.000	0.000	0.321	0.210	0.400	0.411
	129	0.000	0.000	0.017	0.000	0.000	0.000	0.040	0.036
H_E		0.036	0.000	0.034	0.000	0.436	0.331	0.525	0.524
H_O		0.037	0.000	0.035	0.000	0.286	0.161	0.240	0.250
HB21									
N		27	25	30	27	31	30	26	30
Allele	145	0.130	0.120	0.017	0.000	0.774	0.717	0.885	0.733
	147	0.815	0.840	0.950	0.944	0.210	0.283	0.115	0.250
	151	0.056	0.040	0.033	0.056	0.016	0.000	0.000	0.017
H_E		0.316	0.278	0.096	0.105	0.356	0.406	0.204	0.399
H_O		0.296	0.240	0.100	0.111	0.097	0.100	0.000	0.033
HB16									
N		27	25	32	31	31	32	26	32
Allele	109	0.000	0.000	0.000	0.000	0.016	0.016	0.000	0.016
	115	0.519	0.400	0.828	0.774	0.968	0.969	0.981	0.984
	117	0.019	0.020	0.047	0.097	0.016	0.016	0.019	0.000
	119	0.463	0.580	0.125	0.129	0.000	0.000	0.000	0.000
H_E		0.517	0.503	0.296	0.375	0.063	0.061	0.038	0.031
H_O		0.482	0.360	0.313	0.387	0.065	0.063	0.039	0.031
HB36									
N		27	25	32	30	30	29	21	29
Allele	199	0.241	0.300	0.938	0.867	0.417	0.345	0.095	0.172
	208	0.426	0.340	0.000	0.000	0.050	0.155	0.024	0.035
	211	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.000
	219	0.000	0.000	0.000	0.000	0.017	0.017	0.000	0.000
	220	0.333	0.360	0.063	0.133	0.517	0.483	0.833	0.793
H_E		0.650	0.665	0.117	0.231	0.557	0.624	0.294	0.340
H_O		0.778	0.560	0.125	0.200	0.600	0.552	0.333	0.379

HB42									
N		27	25	32	30	31	31	27	32
Allele	165	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000
	168	0.630	0.460	0.516	0.567	1.000	1.000	1.000	1.000
	171	0.370	0.520	0.484	0.433	0.000	0.000	0.000	0.000
H_E		0.466	0.518	0.500	0.491	0.000	0.000	0.000	0.000
H_O		0.444	0.560	0.656	0.600	0.000	0.000	0.000	0.000
HB41									
N		27	25	31	28	32	31	26	26
Allele	102	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000
	105	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000
	111	0.000	0.000	0.048	0.036	0.016	0.032	0.019	0.019
	115	0.167	0.080	0.000	0.000	0.000	0.016	0.000	0.000
	121	0.833	0.860	0.742	0.786	0.859	0.774	0.769	0.923
	124	0.000	0.060	0.210	0.179	0.125	0.161	0.192	0.058
H_E		0.278	0.250	0.403	0.350	0.246	0.373	0.371	0.144
H_O		0.259	0.280	0.452	0.429	0.281	0.452	0.462	0.154
HB05									
N		27	24	31	27	32	32	26	28
Allele	154	0.296	0.396	0.000	0.019	0.047	0.031	0.096	0.000
	156	0.500	0.500	0.742	0.704	0.938	0.906	0.904	0.964
	158	0.093	0.083	0.226	0.241	0.016	0.063	0.000	0.036
	160	0.111	0.021	0.032	0.037	0.000	0.000	0.000	0.000
H_E		0.641	0.586	0.398	0.445	0.119	0.174	0.174	0.069
H_O		0.704	0.750	0.516	0.593	0.094	0.125	0.115	0.071
HB57									
N		27	25	19	12	9	17	21	23
Allele	255	0.222	0.360	0.211	0.333	0.000	0.000	0.000	0.000
	257	0.000	0.000	0.421	0.000	0.333	0.294	0.714	0.609
	261	0.000	0.000	0.000	0.000	0.056	0.000	0.048	0.087
	271	0.667	0.560	0.132	0.250	0.278	0.353	0.119	0.152
	273	0.111	0.080	0.237	0.417	0.333	0.353	0.119	0.152
H_E		0.494	0.550	0.705	0.653	0.698	0.664	0.459	0.576
H_O		0.519	0.520	0.263	0.833	0.333	0.353	0.095	0.217
HB61									
N		27	25	32	29	29	31	25	30
Allele	129	0.037	0.060	0.016	0.000	0.000	0.000	0.000	0.000
	131	0.963	0.920	0.984	1.000	1.000	1.000	1.000	1.000
	133	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000
H_E		0.071	0.150	0.031	0.000	0.000	0.000	0.000	0.000
H_O		0.074	0.160	0.031	0.000	0.000	0.000	0.000	0.000

HB70									
N		27	25	30	27	28	31	23	25
Allele	114	0.574	0.580	0.400	0.389	0.000	0.000	0.261	0.140
	118	0.426	0.420	0.567	0.593	0.875	0.919	0.652	0.740
	124	0.000	0.000	0.033	0.019	0.125	0.081	0.087	0.120
H_E		0.489	0.487	0.518	0.497	0.219	0.148	0.499	0.418
H_O		0.407	0.600	0.500	0.593	0.250	0.161	0.609	0.520

REFERENCES

Åberg, P. 1992, 'A demographic study of two populations of the seaweed *Ascophyllum nodosum*', *Ecology*, vol. 73, no. 4, pp. 1473-1487.

Addison, P.F.E., Koss, R.S. & O'Hara, T.D. 2008, 'Recreational use of a rocky intertidal reef in victoria: Implications for ecological research and management', *Australasian Journal of Environmental Management*, vol. 15, no. 3, pp. 169-179.

Addo-Bediako, A., Chown, S.L. & Gaston, K.J. 2000, 'Thermal tolerance, climatic variability and latitude', *Proceedings of the Royal Society B: Biological Sciences*, vol. 267, no. 1445, pp. 739-745.

Al-Janabi, B., Kruse, I., Graiff, A., Karsten, U. & Wahl, M. 2016, 'Genotypic variation influences tolerance to warming and acidification of early life-stage *Fucus vesiculosus* L. (Phaeophyceae) in a seasonally fluctuating environment', *Marine Biology*, vol. 163, no. 1, pp. 1-15.

Alestra, T. & Schiel, D.R. 2015, 'Impacts of local and global stressors in intertidal habitats: Influence of altered nutrient, sediment and temperature levels on the early life history of three habitat-forming macroalgae', *Journal of Experimental Marine Biology and Ecology*, vol. 468, pp. 29-36.

Allakhverdiev, S.I., Kreslavski, V.D., Klimov, V.V., Los, D.A., Carpentier, R. & Mohanty, P. 2008, 'Heat stress: An overview of molecular responses in photosynthesis', *Photosynthesis Research*, vol. 98, no. 1-3, pp. 541-50.

Allen, R.M. & Marshall, D. 2014, 'Egg size effects across multiple life-history stages in the marine annelid *Hydroides diramphus*', *Plos One*, vol. 9, no. 7, p. e102253.

Araújo, M.B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P.A., Valladares, F. & Chown, S.L. 2013, 'Heat freezes niche evolution', *Ecology Letters*, vol. 16, no. 9, pp. 1206-1219.

Araújo, M. B. and Williams, P. H. (2001), The bias of complementarity hotspots toward marginal populations. *Conservation Biology*, 15: 1710–1720.

Araújo, R., Serrão, E.A., Sousa-Pinto, I. & Åberg, P. 2011, 'Phenotypic differentiation at southern limit borders: The case study of two fucoid macroalgal species with different life-history traits1', *Journal of Phycology*, vol. 47, no. 3, pp. 451-462.

Araújo, R., Serrão, E.A., Sousa-Pinto, I. *et al.* 2015, 'Trade-offs between life-history traits at range-edge and central locations', *Journal of Phycology*, vol. 51, no. 4, pp. 808-818.

Assis, J., Coelho, N.C., Alberto, F., Valero, M. & Raimondi, P. 2013, 'High and distinct range-edge genetic diversity despite local bottlenecks', *Plos One*, vol. 8, no. 7, pp. 1-11.

Bates, A.E., Pecl, G.T., Frusher, S. *et al.* 2014, 'Defining and observing stages of climate-mediated range shifts in marine systems', *Global Environmental Change*, vol. 26, pp. 27-38.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. & Bonhomme, F. 2000, 'Genetix, a windowstm based software for population genetic analyses. Laboratoire génome, populations, interactions cnrs umr 5000', *Université de Montpellier II, Montpellier, France. http://www. univ-montp2. fr*genetix/genetix.htm*.

Bell, E. 1995, 'Environmental and morphological influences on thallus temperature and desiccation of the intertidal alga *Mastocarpus papillatus* Kützing', *Journal of Experimental Marine Biology and Ecology*, vol. 191, no. 1, p. 2955.

Bell, E.C. 1993, 'Photosynthetic response to temperature and desiccation of the intertidal alga *Mastocarpus papillatus*', *Marine Biology*, vol. 117, no. 2, pp. 337-346.

Bellgrove, A., van Rooyen, A., Weeks, A.R., Clark, J.S., Doblin, M.A. & Miller, A.D. 2016, 'New resource for population genetics studies on the australasian intertidal brown alga,

hormosira banksii: Isolation and characterization of 15 polymorphic microsatellite loci through next generation DNA sequencing', *Journal of Applied Phycology*, pp. 1-7.

Bellgrove, A., Clayton, M.N. & Quinn, G.P. 1997, 'Effects of secondarily treated sewage effluent on intertidal macroalgal recruitment processes', *Marine and Freshwater Research*, vol. 48, no. 2, pp. 137-146.

Bellgrove, A., Clayton, M.N. & Quinn, G.P. 2004, 'An integrated study of the temporal and spatial variation in the supply of propagules, recruitment and assemblages of intertidal macroalgae on a wave-exposed rocky coast, Victoria, Australia', *Journal of Experimental Marine Biology and Ecology*, vol. 310, no. 2, pp. 207-225.

Bellgrove, A., McKenzie, P.F., McKenzie, J.L. & Sfiligoj, B.J. 2010, 'Restoration of the habitat-forming fucoid alga *Hormosira banksii* at effluent-affected sites: Competitive exclusion by coralline turfs', *Marine Ecology-Progress Series*, vol. 419, pp. 47-56.

Bennett, S., Wernberg, T., Joy, B., de Bettignies, T. & Campbell, A.H. 2015, 'Central and rear-edge populations can be equally vulnerable to warming', *Nature Communications*, vol. 6, no. 10280, pp. 1-7.

Berger, D., Walters, R.J. & Blanckenhorn, W.U. 2014, 'Experimental evolution for generalists and specialists reveals multivariate genetic constraints on thermal reaction norms', *Journal of Evolutionary Biology*, vol. 27, no. 9, pp. 1975-1989.

Bergquist, P. 1959, 'A statistical approach to the ecology of *Hormosira banksii*', *Botanica Marina*, vol. 1, no. 1-2, pp. 22-53.

Billot, C., Engel, C.R., Rousvoal, S., Kloareg, B. & Valero, M. 2003, 'Current patterns, habitat discontinuities and population genetic structure: The case of the kelp *Laminaria digitata* in the English channel', *Marine Ecology Progress Series*, vol. 253, pp. 111-121.

Bischoff, A. & Müller-Schärer, H. 2010, 'Testing population differentiation in plant species - how important are environmental maternal effects', *Oikos*, vol. 119, no. 3, pp. 445-454.

Blacket, M.J., Robin, C., Good, R.T., Lee, S.F. & Miller, A.D. 2012, 'Universal primers for fluorescent labelling of pcr fragments-an efficient and cost-effective approach to genotyping by fluorescence', *Molecular Ecology Resources*, vol. 12, no. 3, pp. 456-63.

Blanchette, C.A. 1997, 'Size and survival of intertidal plants in response to wave action: A case study with fucus gardneri', *Ecology*, vol. 78, no. 5, pp. 1563-1578.

Bolton, J. 1994, 'Global seaweed diversity: Patterns and anomalies', *Botanica Marina*, vol. 37, no. 3, pp. 241-246.

Brawley, S.H. 1992, 'Fertilization in natural-populations of the dioecious brown alga *Fucus-ceranoides* and the importance of the polyspermy block', *Marine Biology*, vol. 113, no. 1, pp. 145-157.

Brawley, S.H. & Johnson, L.E. 1991, 'Survival of fucoid embryos in the intertidal zone depends upon developmental stage and microhabitat1', *Journal of Phycology*, vol. 27, no. 2, pp. 179-186.

Brawley, S.H., Johnson, L.E., Pearson, G.A., Speransky, V., Li, R. & Serrão, E. 1999, 'Gamete release at low tide in fucoid algae: Maladaptive or advantageous?', *American Zoologist*, vol. 39, no. 2, pp. 218-229.

Bridle, J.R. & Vines, T.H. 2007, 'Limits to evolution at range margins: When and why does adaptation fail?', *Trends in Ecology & Evolution*, vol. 22, no. 3, pp. 140-147.

Brown, J.H. 1984, 'On the relationship between abundance and distribution of species', *The American Naturalist*, vol. 124, no. 2, pp. 255-279.

Brown, M. 1987, 'Effects of desiccation on photosynthesis of intertidal algae from a southern New Zealand shore', *Botanica Marina*, vol. 30, no. 2, pp. 121-128.

Buchanan, J. & Zuccarello, G.C. 2012, 'Decoupling of short- and long-distance dispersal pathways in the endemic New Zealand seaweed *Carpophyllum maschalocarpum* (Phaeophyceae, Fucales)', *Journal of Phycology*, vol. 48, no. 3, pp. 518-529.

Bussolini, L.T. & Waters, J.M. 2015, 'Genetic analyses of rafted macroalgae reveal regional oceanographic connectivity patterns', *Journal of Biogeography*, vol. 42, no. 7, pp. 1319-1326.

Campbell, A.H., Marzinelli, E.M., Vergés, A., Coleman, M.A. & Steinberg, P.D. 2014, 'Towards restoration of missing underwater forests', *Plos One*, vol. 9, no. 1, p. e84106.

Carney, L.T. & Edwards, M.S. 2006, 'Cryptic processes in the sea: A review of delayed development in themicroscopic life stages of marine macroalgae', *Algae*, vol. 21, no. 2, pp. 161-168.

Chambers, R.C. & Leggett, W.C. 1996, 'Maternal influences on variation in egg sizes in temperate marine fishes', *American Zoologist*, vol. 36, pp. 180-196.

Chapman, A.R.O. 1995, 'Functional ecology of fucoid algae: Twenty-three years of progress', *Phycologia*, vol. 34, no. 1, pp. 1-32.

Charrier, B., Le Bail, A. & de Reviers, B. 2012, 'Plant proteus: Brown algal morphological plasticity and underlying developmental mechanisms', *Trends in Plant Science*, vol. 17, no. 8, pp. 468-477.

Chen, I.C., Hill, J.K., Ohlemüller, R., Roy, D.B. & Thomas, C.D. 2011, 'Rapid range shifts of species associated with high levels of climate warming', *Science*, vol. 333, no. 6045, pp. 1024-1026.

Chu, S., Zhang, Q., Liu, S., Tang, Y., Zhang, S., Lu, Z. & Yu, Y. 2012, 'Tolerance of *Sargassum thunbergii* germlings to thermal, osmotic and desiccation stress', *Aquatic Botany*, vol. 96, no. 1, pp. 1-6.

Chu, S., Zhang, Q., Liu, S., Zhang, S., Tang, Y., Lu, Z. & Yu, Y. 2011, 'Trade-off between vegetative regeneration and sexual reproduction of *Sargassum thunbergii*', *Hydrobiologia*, vol. 678, no. 1, pp. 127-135.

Clark, J.S., Poore, A.G.B., Ralph, P.J., Doblin, M.A. & Harley, C. 2013, 'Potential for adaptation in response to thermal stress in an intertidal macroalga', *Journal of Phycology*, vol. 49, no. 4, pp. 630-639.

Clarke & Womersley, H.B.S. 1981, 'Cross-fertilization and hybrid development of forms of the brown alga *Hormosira banksii* (Turner) Decaisne', *Australian Journal of Botany*, vol. 29, no. 4, pp. 497-505.

Clayton, M.N. 1992, 'Propagules of marine macroalgae: Structure and development', *British Phycological Journal*, vol. 27, no. 3, pp. 219-232.

Cockerham, C.C. 1973, 'Analyses of gene frequencies', *Genetics*, vol. 74, no. 4, pp. 679-700.

Coelho, S.M., Rijstenbil, J.W. & Brown, M.T. 2000, 'Impacts of anthropogenic stresses on the early development stages of seaweeds', *Journal of Aquatic Ecosystem Stress and Recovery*, vol. 7, no. 4, pp. 317-333.

Coleman, M.A. & Brawley, S.H. 2005a, 'Are life history characteristics good predictors of genetic diversity and structure? A case study of the intertidal alga *Fucus spiralis* (Heterokontophyta; Phaeophyceae)', *Journal of Phycology*, vol. 41, no. 4, pp. 753-762.

Coleman, M.A. & Brawley, S.H. 2005b, 'Spatial and temporal variability in dispersal and population genetic structure of a rockpool alga', *Marine Ecology-Progress Series*, vol. 300, pp. 63-77.

Coleman, M.A. & Brawley, S.H. 2005c, 'Variability in temperature and historical patterns in reproduction in the *Fucus distichus* complex (Heterokontophyta; Phaeophyceae): Implications for speciation and the collection of herbarium specimens1', *Journal of Phycology*, vol. 41, no. 6, pp. 1110-1119.

Coleman, M.A., Chambers, J., Knott, N.A., Malcolm, H.A., Harasti, D., Jordan, A. & Kelaher, B.P. 2011a, 'Connectivity within and among a network of temperate marine reserves', *Plos One*, vol. 6, no. 5. e20168.

Coleman, M.A. & Kelaher, B.P. 2009, 'Connectivity among fragmented populations of a habitat-forming alga, *Phyllospora comosa* (Phaeophyceae, Fucales) on an urbanised coast', *Marine Ecology-Progress Series*, vol. 381, pp. 63-70.

Coleman, M.A., Roughan, M., Macdonald, H.S., Connell, S.D., Gillanders, B.M., Kelaher, B.P. & Steinberg, P.D. 2011b, 'Variation in the strength of continental boundary currents determines continent-wide connectivity in kelp', *Journal of Ecology*, vol. 99, no. 4, pp. 1026-1032.

Costanza, R., d'Arge, R., de Groot, R. *et al.* 1997, 'The value of the world's ecosystem services and natural capital', *Nature*, vol. 387, no. 6630, pp. 253-60.

Crain, C., Kroeker, K. & Halpern, B.S. 2008, 'Interactive and cumulative effects of multiple human stressors in marine systems', *Ecology Letters*, vol. 11, no. 12, pp. 1304-1315.

Davison, I.R. 1991, 'Environmental effects on algal photosynthesis: Temperature', *Journal of Phycology*, vol. 27, no. 1, pp. 2-8.

Davison, I.R., Johnson, L.E. & Brawley, S.H. 1993, 'Sublethal stress in the intertidal zone: Tidal emersion inhibits photosynthesis and retards development in embryos of the brown alga *Pelvetia fastigiata*', *Oecologia*, vol. 96, no. 4, pp. 483-92.

Davison, I.R. & Pearson, G.A. 1996, 'Stress tolerance in intertidal seaweeds', *Journal of Phycology*, vol. 32, no. 2, pp. 197-211.

Dayton, P.K. 1972, 'Toward an understanding of community resilience and the potential effects of enrichments to the benthos at Mcmurdo Sound, Antarctica', *Proceedings of the colloquium on conservation problems in Antarctica. Allen Press, Lawrence, Kansas, USA*, pp. 81-95.

Demes, K.W., Harley, C.D.G., Anderson, L.M., Carrington, E. & Nilsson, C. 2013, 'Shifts in morphological and mechanical traits compensate for performance costs of reproduction in a wave-swept seaweed', *Journal of Ecology*, vol. 101, no. 4, pp. 963-970.

Deutsch, C., Ferrel, A., Seibel, B., Pörtner, H.-O.O. & Huey, R.B. 2015, 'Ecophysiology. Climate change tightens a metabolic constraint on marine habitats', *Science (New York, N.Y.)*, vol. 348, no. 6239, pp. 1132-1135.

Diaz-Pulido, G., Villamil, L. & Almanza, V. 2007, 'Herbivory effects on the morphology of the brown alga *Padina boergesenii* (Phaeophyta)', *Phycologia*, vol. 46, no. 2, pp. 131-136.

Dimartino, S., Mather, A.V., Alestra, T., Nawada, S. & Haber, M. 2015, 'Experimental and computational analysis of a novel flow channel to assess the adhesion strength of sessile marine organisms', *Interface Focus*, vol. 5, no. 1.

Dimartino, S., Savory, D.M., Fraser-Miller, S.J., Gordon, K.C. & McQuillan, A.J. 2016, 'Microscopic and infrared spectroscopic comparison of the underwater adhesives produced by germlings of the brown seaweed species durvillaea antarctica and hormosira banksii', *Journal of The Royal Society Interface*, vol. 13, no. 117, p. 20151083.

Doblin, M.A. & Clayton, M.N. 1995, 'Effects of secondarily-treated sewage effluent on the early life-history stages of two species of brown macroalgae: *Hormosira banksii* and *Durvillaea potatorum'*, *Marine Biology*, vol. 122, pp. 689 – 698.

Donelson, J.M., Munday, P.L. & McCormick, M.I. 2009, 'Parental effects on offspring life histories: When are they important?', *Biology Letters*, vol. 5, no. 2, pp. 262-5.

Doney, S.C., Ruckelshaus, M., Emmett Duffy, J. *et al.* 2012, 'Climate change impacts on marine ecosystems', *Annual Review of Marine Science*, vol. 4, no. 1, pp. 11-37.

Dring, M. & Brown, F. 1982, 'Photosynthesis of intertidal brown algae during and after periods of emersion: A renewed search for physiological causes of zonation', *Marine Ecology Progress Series*, vol. 8, pp 301-308.

Dromgoole, F. 1980, 'Desiccation resistance of intertidal and subtidal algae', *Botanica Marina*, vol. 23, no. 3, pp. 149-160.

Dromgoole, F.I. 1987, 'Photosynthesis of marine algae in fluctuating light. I. Adjustment of rate in constant and fluctuating light regimes', *Functional Ecology*, vol. 1, no. 4, pp. 377-386.

Dudgeon, S., Kübler, J.E., Wright, W.A., Vadas, R.L. & Petraitis, P.S. 2001, 'Natural variability in zygote dispersal of *Ascophyllum nodosum* at small spatial scales', *Functional Ecology*, vol. 15, no. 5, pp. 595-604.

Dudgeon, S.R., Kübler, J., Vadas, R. & Davison, I.R. 1995, 'Physiological responses to environmental variation in intertidal red algae: Does thallus morphology matter?', *Marine Ecology-Progress Series*, vol. 117, no. 40546, p. 193.

Eckert, C.G., Samis, K.E. & Lougheed, S.C. 2008, 'Genetic variation across species' geographical ranges: The central–marginal hypothesis and beyond', *Molecular Ecology*, vol. 17, no. 5, pp. 1170-1188.

Eggert, A. 2012, 'Seaweed responses to temperature', in C. Wiencke & K. Bischof (eds), *Seaweed biology: Novel insights into ecophysiology, ecology and utilization*, Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 47-66.

Engel, C.R., Destombe, C. & Valero, M. 2004, 'Mating system and gene flow in the red seaweed *Gracilaria gracilis*: Effect of haploid-diploid life history and intertidal rocky shore landscape on fine-scale genetic structure', *Heredity (Edinb)*, vol. 92, no. 4, pp. 289-298.

Evanno, G., Regnaut, S. & Goudet, J. 2005, 'Detecting the number of clusters of individuals using the software structure: A simulation study', *Molecular Ecology*, vol. 14, no. 8, pp. 2611-2620.

Evans, S.M., Sinclair, E.A., Poore, A.G., Bain, K.F. & Vergés, A. 2016, 'Genotypic richness predicts phenotypic variation in an endangered clonal plant', *PeerJ*, vol. 4, p. e1633.

Excoffier, L., Laval, G. & Schneider, S. 2005, 'Arlequin (version 3.0): An integrated software package for population genetics data analysis', *Evolutionary bioinformatics online*, vol. 1, p. 47.

Falace, A. & Bressan, G. 2006, 'Seasonal variations of cystoseira barbata (stackhouse) c. Agardh frond architecture', *Hydrobiologia*, vol. 555, pp. 193-206.

Falkowski, P.G. & Raven, J.A. 2013, *Aquatic photosynthesis*, Princeton University Press.

Faugeron, S., Martínez, E.A., Correa, J.A., Cardenas, L., Destombe, C. & Valero, M. 2004, 'Reduced genetic diversity and increased population differentiation in peripheral and overharvested populations of *Gigartina skottsbergii* (Rhodophyta, Gigartinales) in southern chile', *Journal of Phycology*, vol. 40, no. 3, pp. 454-462.

Ferreira, J.G., Arenas, F., Martínez, B., Hawkins, S.J. & Jenkins, S.R. 2014, 'Physiological response of fucoid algae to environmental stress: Comparing range centre and southern populations', *New Phytologist*, vol. 202, no. 4, pp. 1157-1172.

Foo, S.A., Dworjanyn, S.A., Khatkar, M.S., Poore, A.G. & Byrne, M. 2014, 'Increased temperature, but not acidification, enhances fertilization and development in a tropical urchin: Potential for adaptation to a tropicalized eastern australia', *Evolutionary Applications*, vol. 7, no. 10, pp. 1226-1237.

Foo, S.A., Dworjanyn, S.A., Poore, A.G. & Byrne, M. 2012, 'Adaptive capacity of the habitat modifying sea urchin *Centrostephanus rodgersii* to ocean warming and ocean acidification: Performance of early embryos', *Plos One*, vol. 7, no. 8, pp. e42497-e.

Fowler-Walker, M.J., Wernberg, T. & Connell, S.D. 2006, 'Differences in kelp morphology between wave sheltered and exposed localities: Morphologically plastic or fixed traits?', *Marine Biology*, vol. 148, no. 4, pp. 755-767.

Frankham, R. 2005, 'Stress and adaptation in conservation genetics', *Journal of Evolutionary Biology*, vol. 18, no. 4, pp. 750-755.

Freeland, J.R., Kirk, H. & Petersen, S.D. 2011, *Molecular ecology*, Wiley-Blackwell, West Sussex, United Kingdom.

Fuller, A., Dawson, T., Helmuth, B., Hetem, Robyn S., Mitchell, D. & Maloney, Shane K. 2010, 'Physiological mechanisms in coping with climate change', *Physiological and Biochemical Zoology*, vol. 83, no. 5, pp. 713-720.

Fusi, M., Giomi, F., Babbini, S., Daffonchio, D., McQuaid, C.D., Porri, F. & Cannicci, S. 2015, 'Thermal specialization across large geographical scales predicts the resilience of mangrove crab populations to global warming', *Oikos*, vol. 124, no. 6, pp. 784-795.

Galletly, B.C., Blows, M.W. & Marshall, D.J. 2007, 'Genetic mechanisms of pollution resistance in a marine invertebrate', *Ecological Applications*, vol. 17, no. 8, pp. 2290-2297.

Galloway, L.F. 2001, 'The effect of maternal and paternal environments on seed characters in the herbaceous plant *Campanula americ*ana (Campanulaceae)', *American Journal of Botany*, vol. 88, no. 5, pp. 832-840.

Galloway, L.F. 2005, 'Maternal effects provide phenotypic adaptation to local environmental conditions', *New Phytologist*, vol. 166, no. 1, pp. 93-99.

Galloway, L.F., Etterson, J.R. & McGlothlin, J.W. 2009, 'Contribution of direct and maternal genetic effects to life-history evolution', *New Phytologist,* vol. 183, pp 826-838.

Gerard, V.A. & Bois, D.K.R. 1988, 'Temperature ecotypes near the southern boundary of the kelp *Laminaria saccharina'*, *Marine Biology*, vol. 97, pp. 575-580.

Gilchrist, G.W. 1995, 'Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity', *The American Naturalist*, vol. 146, no. 2, pp. 252-270.

Gillespie, R. & Critchley, A. 2001, 'Reproductive allocation and strategy of *Sargassum elegans suhr* and *Sargassum incisifolium* (Turner) c. Agardh from Reunion Rocks, Kwazulu-Natal, South Africa', *Botanica Marina*, vol. 44, no. 3, pp. 231-5.

Gómez, I. & Huovinen, P. 2011, 'Morpho-functional patterns and zonation of south Chilean seaweeds: The importance of photosynthetic and bio-optical traits', *Marine Ecology Progress Series*, vol. 422, pp. 77-91.

Gómez, I., López-Figueroa, F., Ulloa, N., Morales, V., Lovengreen, C., Huovinen, P. & Hess, S. 2004, 'Patterns of photosynthesis in 18 species of intertidal macroalgae from southern Chile', *Marine Ecology Progress Series*, vol. 270, pp. 103-116.

Goudet, J. 1995, 'Fstat (version 1.2): A computer program to calculate f-statistics', *Journal of heredity*, vol. 86, no. 6, pp. 485-486.

Graham, M.H. & Edwards, M.S. 2001, 'Statistical significance versus fit: Estimating the importance of individual factors in ecological analysis of variance', *Oikos*, vol. 93, no. 3, pp. 505-513.

Gunthorpe, L., Nottage, M., Palmer, D. & Wu, R. 1995, 'The development of a fertilisation inhibition assay using gametes of the brown alga *Hormosira banksii*', *Australasian Journal of Ecotoxicology*, vol. 1, no. 1, pp. 25-31.

Gunthorpe, L., Nottage, M., Palmer, D. & Wu, R. 1997, 'Development and validation of a fertilization inhibition bioassay using gametes of *Hormosira banksii*. National pulp mills research program technical report series no. 21', CSIRO, Canberra.

Guntrip, J. & Sibly, R.M. 1998, 'Phenotypic plasticity, genotype-by-environment interaction and the analysis of generalism and specialization in *Callosobruchus maculatus*', *Heredity*, vol. 81, no. 2, pp. 198-204.

Hairston, N.G., Ellner, S.P., Geber, M.A., Yoshida, T. & Fox, J.A. 2005, 'Rapid evolution and the convergence of ecological and evolutionary time', *Ecology Letters*, vol. 8, no. 10, pp. 1114-1127.

Hampe, A. & Petit, R.J. 2005, 'Conserving biodiversity under climate change: The rear edge matters', *Ecology Letters*, vol. 8, no. 5, pp. 461-7.

Hanelt, D., Wiencke, C., Karsten, U. & Nultsch, W. 1997, 'Photoinhibition and recovery after high light stress in different developmental and life-history stages of *Laminaria saccharina* (Phaeophyta)', *Journal of Phycology*, vol. 33, no. 3, p. 387-395.

Harley, C.D.G. 2008, 'Tidal dynamics, topographic orientation, and temperature-mediated mass mortalities on rocky shores', *Marine Ecology Progress Series*, vol. 371, pp. 37-46.

Harley, C.D.G., Anderson, K.M., Demes, K.W., Jorve, J.P., Kordas, R.L., Coyle, T.A. & Graham, M.H. 2012, 'Effects of climate change on global seaweed communities', *Journal of Phycology*, vol. 48, no. 5, pp. 1064-1078.

Harley, C.D.G. & Helmuth, B.S.T. 2003, 'Local- and regional-scale effects of wave exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal zonation', *Limnology and Oceanography*, vol. 48, no. 4, p. 1498-1508.

Harley, C.D.G., Randall Hughes, A., Hultgren, K.M. *et al.* 2006, 'The impacts of climate change in coastal marine systems', *Ecology Letters*, vol. 9, no. 2, pp. 228-241.

Hawkins, S. & Hartnoll, R. 1985, 'Factors determining the upper limits of intertidal canopy-forming algae', *Marine ecology. Progress series*, vol. 20, no. 3, pp. 265-271.

Helmuth, B. 2009, 'From cells to coastlines: How can we use physiology to forecast the impacts of climate change?', *Journal of Experimental Biology*, vol. 212, no. 6, pp. 753-760.

Helmuth, B., Broitman, B.R. & Blanchette, C.A. 2006, 'Mosaic patterns of thermal stress in the rocky intertidal zone: Implications for climate change', *Ecological Monographs,* vol. 76, no. 4, pp. 461-479

Helmuth, B., Broitman, B.R., Yamane, L., Gilman, S.E., Mach, K., Mislan, K.A.S. & Denny, M.W. 2010, 'Organismal climatology: Analyzing environmental variability at scales relevant to physiological stress', *Journal of Experimental Biology*, vol. 213, no. 6, pp. 995-1003.

Helmuth, B., Harley, C.D.G., Halpin, P.M., O'Donnell, M., Hofmann, G.E. & Blanchette, C.A. 2002, 'Climate change and latitudinal patterns of intertidal thermal stress', *Science*, vol. 298, no. 5595, pp. 1015-1017.

Helmuth, B., Russell, B.D., Connell, S.D. *et al.* 2014, 'Beyond long-term averages: Making biological sense of a rapidly changing world', *Climate Change Responses*, vol. 1, no. 1, p. 6.

Helmuth, B., Yamane, L., Lalwani, S., Matzelle, A., Tockstein, A. & Gao, N. 2011, 'Hidden signals of climate change in intertidal ecosystems: What (not) to expect when you are expecting', *Journal of Experimental Marine Biology and Ecology*, vol. 400, no. 1-2, pp. 191-199.

Helmuth, B.S. & Hofmann, G.E. 2001, 'Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone', *The Biological bulletin*, vol. 201, no. 3, pp. 374-84.

Hobday, A.J. 2000, 'Abundance and dispersal of drifting kelp *Macrocystis pyrifera* rafts in the southern california bight', *Marine Ecology Progress Series*, vol. 195, pp. 101-16.

Hoegh-Guldberg, O. & Bruno, J.F. 2010, 'The impact of climate change on the world's marine ecosystems', *Science*, vol. 328, pp. 1523-1528.

Hoffmann, A. 1987, 'The arrival of seaweed propagules at the shore: A review', *Botanica Marina*, vol. 30, no. 2, pp. 151-166.

Hoffmann, A.A. & Sgrò, C.M. 2011, 'Climate change and evolutionary adaptation', *Nature*, vol. 470, no. 7335, pp. 479-485.

Hoffmann, A.A., Shirriffs, J. & Scott, M. 2005, 'Relative importance of plastic vs genetic factors in adaptive differentiation: Geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia', *Functional Ecology*, vol. 19, no. 2, pp. 222-227.

Hoffmann, A.J. & Santelices, B. 1991, 'Banks of algal microscopic forms: Hypotheses on their functioning and comparisons with seed banks', *Marine Ecology Progress Series,* vol. 79, pp. 185-194.

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A., Jess, M. & Williams, S.E. 2012, 'Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation', *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, vol. 367, no. 1596, pp. 1665-1679.

Huey, R.B. & Kingsolver, J.G. 1989, 'Evolution of thermal sensitivity of ectotherm performance', *Trends in Ecology & Evolution*, vol. 4, no. 5, pp. 131-135.

Hughes, J. & McCully, M.E. 1975, 'The use of an optical brightener in the study of plant structure', *Stain technology*, vol. 50, no. 5, pp. 319-329.

Intergovernmental Panel of Climate Change. 2014, 'Climate change 2014: Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change [core writing team, R.K. Pachauri, L.A. Meyer (eds.)] 'IPCC, Geneva, Switzerland.

Jakobsson, M. & Rosenberg, N.A. 2007, 'CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure', *Bioinformatics (Oxford, England)*, vol. 23, no. 14, pp. 1801-1806.

Janzen, D.H. 1967, 'Why mountain passes are higher in the tropics', *The American Naturalist*, vol. 101, no. 919, pp. 233-249.

Jensen, J.L., Bohonak, A.J. & Kelley, S.T. 2005, 'Isolation by distance, web service', *BMC Genetics*, vol. 6, no. 1, p. 1.

Johannesson, K. 2009, 'Inverting the null-hypothesis of speciation: A marine snail perspective', *Evolutionary Ecology*, vol. 23, no. 1, pp. 5-16.

Jones, C.G., Lawton, J.H. & Shachak, M. 1994, 'Organisms as ecosystem engineers', *Ecosystem Management*, Springer, pp. 130-147.

Jump, A.S. & Penuelas, J. 2005, 'Running to stand still: Adaptation and the response of plants to rapid climate change', *Ecology Letters*, vol. 8, no. 9, pp. 1010-1020.

Kain, J.M. 2015, '*Hormosira banksii* (Phaeophyceae): A tough survivor in the harsh conditions of high intertidal pools in southeast Australia', *European Journal of Phycology*, vol. 50, no. 8, pp. 408-421.

Kassen, R. 2002, 'The experimental evolution of specialists, generalists, and the maintenance of diversity', *Journal of Evolutionary Biology*, vol. 15, no. 2, pp. 173-190.

Kawamitsu, Y., Driscoll, T. & Boyer, J.S. 2000, 'Photosynthesis during desiccation in an intertidal alga and a land plant', *Plant and Cell physiology*, vol. 41, no. 3, pp. 344-53.

Kelly, M.W., Sanford, E. & Grosberg, R.K. 2012, 'Limited potential for adaptation to climate change in a broadly distributed marine crustacean', *Proc Biol Sci*, vol. 279, no. 1727, pp. 349-356.

Kelly, R.P. & Palumbi, S.R. 2010, 'Genetic structure among 50 species of the northeastern pacific rocky intertidal community', *Plos One*, vol. 5, no. 1.

Kendrick, G.A. & Walker, D.I. 1994, 'Role of recruitment in structuring beds of *Sargassum spp.*(Phaeophyta) at Rottnest Island, Western Australia', *Journal of Phycology*, vol. 30, no. 2, pp. 200-208.

Keough, M.J. & Quinn, G.P. 1998, 'Effects of periodic disturbances from trampling on rocky intertidal algal beds', *Ecological Applications*, vol. 8, no. 1, pp. 141-161.

Kerr, R.A. 2011, 'Humans are driving extreme weather; time to prepare', *Science*, vol. 334, no. 6059, p. 1040.

Ketola, T., Mikonranta, L., Zhang, J. *et al.* 2013, 'Fluctuating temperature leads to evolution of thermal generalism and preadaptation to novel environments', *Evolution*, vol. 67, no. 10.

Koehl, M.A.R., Silk, W.K., Liang, H. & Mahadevan, L. 2008, 'How kelp produce blade shapes suited to different flow regimes: A new wrinkle', *Integrative and Comparative Biology*, vol. 48, no. 6, pp. 834-851.

Kramer, D.M., Johnson, G., Kiirats, O. & Edwards, G.E. 2004, 'New fluorescence parameters for the determination of qa redox state and excitation energy fluxes', *Photosynthesis Research*, vol. 79, pp. 209-218.

Kübler, J.E. & Dudgeon, S.R. 1996, 'Temperature dependent change in the complexity of form of *Chondrus crispus* fronds', *Journal of Experimental Marine Biology and Ecology*, vol. 207, no. 1, pp. 15-24.

Ladah, L.B. & Zertuche-González, J.A. 2007, 'Survival of microscopic stages of a perennial kelp (*Macrocystis pyrifera*) from the center and the southern extreme of its range in the northern hemisphere after exposure to simulated El Niño stress', *Marine Biology*, vol. 152, no. 3, pp. 677-686.

Lilley, S.A. & Schiel, D.R. 2006, 'Community effects following the deletion of a habitat-forming alga from rocky marine shores', *Oecologia*, vol. 148, no. 4, p. 672-681.

Lima, F.P., Ribeiro, P.A., Queiroz, N., Hawkins, S.J. & Santos, A.M. 2007, 'Do distributional shifts of northern and southern species of algae match the warming pattern?', *Global Change Biology*, vol. 13, no. 12, pp. 2592-2604.

Litchman, E., Edwards, K.F., Klausmeier, C.A. & Thomas, M.K. 2012, 'Phytoplankton niches, traits and eco-evolutionary responses to global environmental change', *Marine Ecology Progress Series*, vol. 470, pp. 235-248.

Littler, M.M. & Littler, D.S. 1980, 'The evolution of thallus form and survival strategies in benthic marine macroalgae: Field and laboratory tests of a functional form model', *American Naturalist*, pp. 25-44.

Lough, J. 2009, 'Temperature. In ‘a Marine climate change impacts and adaptation report card for Australia 2009’.(eds E.S. Poloczanska, A.J. Hobday and A.J. Richardson.) NCCARF publication 05/09'ISBN 978-1-921609-03-9. Available at www.oceanclimatechange.org.au [verified 2 June 2011].

Lough, J.M. & Hobday, A.J. 2011, 'Observed climate change in australian marine and freshwater environments', *Marine and Freshwater Research*, vol. 62, no. 9, p. 984.

Luikart, G. & Cornuet, J.M. 1998, 'Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data', *Conservation Biology*, vol. 12, no. 1, pp. 228-237.

Lüning K, Yarish C, Kirkman H. 1990, *Seaweeds: their environment, biogeography, and ecophysiology*. John Wiley, New York, NY.

Lynch, M. & Walsh, B. 1998, *Genetics and analysis of quantitative traits*, vol. 1, Sinauer Sunderland, MA.

Macinnis-Ng, C.M., Morrison, D.A. & Ralph, P.J. 2005, 'Temporal and spatial variation in the morphology of the brown macroalga *Hormosira banksii* (Fucales, Phaeophyta)', *Botanica Marina*, vol. 48, no. 3, pp. 198-207.

Madsen, T.V. & Maberly, S.C. 1990, 'A comparison of air and water as environments for photosynthesis by the intertidal alga *Fucus spiralis* (Phaeophyta)1', *Journal of Phycology*, vol. 26, no. 1, pp. 24-30.

Major, K. & Davison, I. 1998, 'Influence of temperature and light on growth and photosynthetic physiology of *Fucus evanescens* (Phaeophyta) embryos', *European Journal of Phycology*, vol. 33, no. 2, pp. 129-138.

Marshall, D.J. 2008, 'Transgenerational plasticity in the sea: Context-dependent maternal effects across the life history', *Ecology,* vol. 89, no. 2, pp. 418-427.

Martínez, B., Arenas, F., Rubal, M. *et al.* 2012, 'Physical factors driving intertidal macroalgae distribution: Physiological stress of a dominant fucoid at its southern limit', *Oecologia*, vol. 170, no. 2, pp. 341-353.

McKenzie, P.F. & Bellgrove, A. 2006, 'No outbreeding depression at a regional scale for a habitat-forming intertidal alga with limited dispersal', *Marine and Freshwater Research*, vol. 57, no. 6, p. 655.

McKenzie, P.F. & Bellgrove, A. 2008, 'Dispersal of *Hormosira banksii* (Phaeophyceae) via detached fragments: Reproductive viability and longevity', *Journal of Phycology*, vol. 44, no. 5, pp. 1108-1115.

McKinney, M.L. 1997, 'Extinction vulnerability and selectivity:Combining ecological and paleontological views', *Annual review of ecology and systematics*, vol. 28, no. 1, pp. 495-516.

Millar, A. & Kraft, G. 1994, 'Catalogue of marine brown algae (Phaeophyta) of New South Wales, including Lord Howe Island, south-western Pacific', *Australian Systematic Botany*, vol. 7, no. 1, pp. 1-47.

Miller, A.D., Van Rooyen, A., Sweeney, O.F., Whiterod, N.S. & Weeks, A.R. 2013, 'The development of 10 novel polymorphic microsatellite markers through next generation sequencing and a preliminary population genetic analysis for the endangered Glenelg spiny crayfish, *Euastacus bispinosus*', *Molecular Biology Reports*, vol. 40, no. 7, pp. 4415-4419.

Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K. & Relyea, R.A. 2005, 'Ecological consequences of phenotypic plasticity', *Trends in Ecology & Evolution*, vol. 20, no. 12, pp. 685-92.

Mislan, K.A.S., Wethey, D.S. & Helmuth, B. 2009, 'When to worry about the weather: Role of tidal cycle in determining patterns of risk in intertidal ecosystems', *Global Change Biology*, vol. 15, no. 12, pp. 3056-3065.

Mousseau, T.A. & Fox, C.W. 1998, 'The adaptive significance of maternal effects', *Trends in Evolution and Ecology,* vol. 13, no. 10, pp.403-407.

Mueller, R., Fischer, A.M., Bolch, C.J.S. & Wright, J.T. 2015, 'Environmental correlates of phenotypic variation: Do variable tidal regimes influence morphology in intertidal seaweeds?', *Journal of Phycology*, vol. 51, no. 5, pp. 859-871.

Muhlin, J.F. & Brawley, S.H. 2009, 'Recent versus relic: Discerning the genetic signature of *Fucus vesiculosus* (Heterokontophyta; Phaeophyceae) in the northwestern Atlantic', *Journal of Phycology*, vol. 45, no. 4, pp. 828-837.

Muhlin, J.F., Coleman, M.A., Rees, T.A.V. & Brawley, S.H. 2011, 'Modeling of reproduction in the intertidal macrophyte *Fucus vesiculosus* and implications for spatial subsidies in the nearshore environment', *Marine Ecology Progress Series*, vol. 440, pp. 79-94.

Muhlin, J.F., Engel, C.R., Stessel, R., Weatherbee, R.A. & Brawley, S.H. 2008, 'The influence of coastal topography, circulation patterns, and rafting in structuring populations of an intertidal alga', *Molecular Ecology*, vol. 17, no. 5, pp. 1198-1210.

Neiva, J., Pearson, G.A., Valero, M. & Serrão, E.A. 2012, 'Drifting fronds and drifting alleles: Range dynamics, local dispersal and habitat isolation shape the population structure of

the estuarine seaweed *Fucus ceranoides'*, *Journal of Biogeography*, vol. 39, no. 6, pp. 1167-1178.

Nicastro, K.R., Zardi, G.I., Teixeira, S., Neiva, J., Serrão, E.A. & Pearson, G.A. 2013, 'Shift happens: Trailing edge contraction associated with recent warming trends threatens a distinct genetic lineage in the marine macroalga fucus vesiculosus', *BMC Biol*, vol. 11, p. 6.

Nielsen, S., Nielsen, H. & Pedersen, M. 2014a, 'Juvenile life stages of the brown alga *Fucus serratus* L. are more sensitive to combined stress from high copper concentration and temperature than adults', *Marine Biology*, vol. 161, no. 8, p. 1895-1904.

Oates, B.R. 1985, 'Photosynthesis and amelioration of desiccation in the intertidal saccate alga *Colpomenia peregrina*', *Marine Biology*, vol. 89, no. 2, p. 109-119.

Osborn, J.E. 1948, 'The structure and life history of *Hormosira banksii* (Turner) Decaisne', *Transactions of the Royal Society of New Zealand*, vol. 77, J. Hughes, Printer, pp. 47-71.

Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C. & Thomas, M.B. 2013, 'Temperature variation makes ectotherms more sensitive to climate change', *Global Change Biology*, vol. 19, no. 8, pp. 2373-2380.

Palumbi, S.R. 2004, 'Marine reserves and ocean neighborhoods: The spatial scale of marine populations and their management', *Annual Review of Environment and Resource.*, vol. 29, pp. 31-68.

Pardo, L. & Johnson, L.E. 2005, 'Explaining variation in life-history traits: Growth rate, size, and fecundity in a marine snail across an environmental gradient lacking predators', *Marine Ecology Progress Series*, vol. 296, pp. 229-239.

Parmesan, C. & Yohe, G. 2003, 'A globally coherent fingerprint of climate change impacts across natural systems', *Nature*, vol. 421, no. 6918, pp. 37-42.

Pearson, G.A. & Brawley, S.H. 1996, 'Reproductive ecology, of *Fucus distichus* (Phaeophyceae): An intertidal alga with successful external fertilization', *Marine Ecology Progress Series*.

Pearson, G.A., Lago-Leston, A. & Mota, C. 2009, 'Frayed at the edges: Selective pressure and adaptive response to abiotic stressors are mismatched in low diversity edge populations', *Journal of Ecology*, vol. 97, no. 3, pp. 450-462.

Pearson, G.A. & Serrão, E.A. 2006, 'Revisiting synchronous gamete release by fucoid algae in the intertidal zone: Fertilization success and beyond?', *Integrative and Comparative Biology*, vol. 46, no. 5, pp. 587-97.

Pease, C.J., Johnston, E.L. & Poore, A.G.B. 2010, 'Genetic variability in tolerance to copper contamination in a herbivorous marine invertebrate', *Aquatic Toxicology*, vol. 99, no. 1, pp. 10-16.

Phillips, J.A. 2001, 'Marine macroalgal biodiversity hotspots: Why is there high species richness and endemism in southern Australian marine benthic flora?', *Biodiversity & Conservation*, vol. 10, no. 9, pp. 1555-1577.

Piry, S., Luikart, G. & Cornuet, J.M. 1999, 'Computer note. BOTTLENECK: A computer program for detecting recent reductions in the effective size using allele frequency data', *Journal of Heredity*, vol. 90, no. 4, pp. 502-503.

Poloczanska, E.S., Smith, S., Fauconnet, L., Healy, J., Tibbetts, I.R., Burrows, M.T. & Richardson, A.J. 2011, 'Little change in the distribution of rocky shore faunal communities on the Australian east coast after 50 years of rapid warming', *Journal of Experimental Marine Biology and Ecology*, vol. 400, no. 1-2, pp. 145-154.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000, 'Inference of population structure using multilocus genotype data', *Genetics*, vol. 155, no. 2, pp. 945-959.

Provan, J. 2013, 'The effects of past, present and future climate change on range-wide genetic diversity in northern North Atlantic marine species', *Frontiers of Biogeography*, vol 5, no. 1, pp. 60-66.

Quintero, I. & Wiens, J.J. 2013, 'Rates of projected climate change dramatically exceed past rates of climatic niche evolution among vertebrate species', *Ecology Letters*, vol. 16, pp. 1095-1103.

Raffaelli, D. & Hawkins, S.J. 2012, *Intertidal ecology*, Springer Science & Business Media.

Ralph, P.J., Morrison, D.A. & Addison, A. 1998, 'A quantitative study of the patterns of morphological variation within *Hormosira banksii* (Turner) Decaisne (Fucales: Phaeophyta) in south-eastern Australia', *Journal of Experimental Marine Biology and Ecology*, vol. 225, no. 2, pp. 285-300.

Reusch, T.B.H., Ehlers, A., Hammerli, A. & Worm, B. 2005, 'Ecosystem recovery after climatic extremes enhanced by genotypic diversity', *Proceedings of the National Academy of Sciences*, vol. 102, no. 8, pp. 2826-2831.

Rice, W.R. 1989, 'Analyzing tables of statistical tests', *Evolution*, vol. 43, no. 1, pp. 223-5.

Riebesell, U. & Gattuso, J.-P. 2015, 'Lessons learned from ocean acidification research', *Nature Climate Change*, vol. 5, no. 1, pp. 12-14.

Roach, D.A. & Wulff, R.D. 1987, 'Maternal effects in plants', *Annual Review of Ecology and Systematics*, pp. 209-235.

Roberson, L.M. & Coyer, J.A. 2004, 'Variation in blade morphology of the kelp *Eisenia arborea*: Incipient speciation due to local water motion?', *Marine Ecology Progress Series*, vol. 282, pp. 115-128.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. & Pounds, J.A. 2003, 'Fingerprints of global warming on wild animals and plants', *Nature*, vol. 421, no. 6918, pp. 57-60.

Rosenberg, N.A. 2004, 'Distruct: A program for the graphical display of population structure', *Molecular Ecology Notes*, vol. 4, no. 1, pp. 137-138.

Sagarin, R.D. & Gaines, S.D. 2002, 'The 'abundant centre' distribution: To what extent is it a biogeographical rule?', *Ecology Letters*, vol. 5, no. 1, p. 137-147.

Schiel, D.R. 2006, 'Rivets or bolts? When single species count in the function of temperate rocky reef communities', *Journal of Experimental Marine Biology and Ecology*, vol. 338, no. 2, pp. 233-252.

Schiel, D.R. & Lilley, S.A. 2011, 'Impacts and negative feedbacks in community recovery over eight years following removal of habitat-forming macroalgae', *Journal of Experimental Marine Biology and Ecology*, vol. 407, no. 1, p. 108-115.

Schmidt, P.S., Bertness, M.D. & Rand, D.M. 2000, 'Environmental heterogeneity and balancing selection in the acorn barnacle semibalanus balanoides', *Proc Biol Sci*, vol. 267, no. 1441, pp. 379-384.

Schmidt, P.S. & Rand, D.M. 2001, 'Adaptive maintenance of genetic polymorphism in an intertidal barnacle: Habitat- and life-stage-specific survivorship of mpi genotypes', *Evolution*, vol. 55, no. 7, pp. 1336-1344.

Schoenwaelder, M.E.A. 2002, 'Physode distribution and the effect of thallus sunburn in *Hormosira banksii* (Fucales, Phaeophyceae)', *Botanica Marina*, vol. 45, no. 3.

Schonbeck, M. & Norton, T.A. 1978, 'Factors controlling the upper limits of fucoid algae on the shore', *Journal of Experimental Marine Biology and Ecology*, vol. 31, no. 3, pp. 303-313.

Schreiber, U. 2004, 'Pulse-amplitude-modulation (pam) fluorometry and saturation pulse method: An overview', in G. Papageorgiou & Govindjee (eds), *Chlorophyll a fluorescence*, vol. 19, Springer Netherlands, pp. 279-319.

Seery, C., Gunthorpe, L. & Ralph, P. 2006, 'Herbicide impact on *Hormosira banksii* gametes measured by fluorescence and germination bioassays', *Environmental Pollution*, vol. 140, no. 1, pp. 43-51.

Semlitsch, R.D. & Gibbons, J.W. 1990, 'Effects of egg size on success of larval salamanders in complex aquatic environments', *Ecology*, vol. 71, no. 5, pp. 1789-1795.

Serrão, E.A., Kautsky, L., Lifvergren, T. & Brawley, S.H. 1997, 'Gamete dispersal and pre-recruitment mortality in baltic *Fucus vesiculosus*', *Phycologia*, vol. 36, pp. 101-2.

Serrão, E.A., Pearson, G. & Kautsky, L. 1996, 'Successful external fertilization in turbulent environments', *Proceedings of the National Academy of Science,* vol. 93, pp. 5286-5290.

Sideman, E.J. & Mathieson, A.C. 1985, 'Morphological variation within and between natural populations of non-tide pool *Fucus distichus* (Phaeophyta) in New England', *Journal of Phycology*, vol. 21, no. 2, pp. 250-257.

Skene, K.R. 2004, 'Key differences in photosynthetic characteristics of nine species of intertidal macroalgae are related to their position on the shore', *Canadian Journal of Botany*, vol. 82, no. 2, pp. 177-184.

Smale, D.A. & Wernberg, T. 2013, 'Extreme climatic event drives range contraction of a habitat-forming species', *Proceedings of the Royal Society B: Biological Sciences*, vol. 280, no. 1754, pp. 20122829.

Smith, C.C. & Fretwell, S.D. 1974, 'The optimal balance between size and number of offspring', *The American Naturalist*, vol. 108, no. 962, pp. 499-506.

Smith, C.M. & Berry, J.A. 1986, 'Recovery of photosynthesis after exposure of intertidal algae to osmotic and temperature stresses: Comparative studies of species with differing distributional limits', *Oecologia*, vol. 70, no. 1, pp. 6-12.

Somero, G.N. 2002, 'Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living', *Integrative and Comparative Biology*, vol. 42, pp. 780-789.

Somero, G.N. 2010, 'The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'', *Journal of Experimental Biology*, vol. 213, no. 6, pp. 912-920.

Staehr, P.A. & Wernberg, T. 2009, 'Physiological responses of *Ecklonia radiata* (Laminariales) to a latitudinal gradient in ocean temperature', *Journal of Phycology*, vol. 45, no. 1, pp. 91-99.

Stanton, M.L. 1984, 'Seed variation in wild radish: Effect of seed size on components of seedling and adult fitness', *Ecology*, vol. 65, no. 4, pp. 1105-1112.

Stearns, S.C. 1976, 'Life-history tactics: A review of the ideas', *Quarterly review of biology*, pp. 3-47.

Stengel, D. & Dring, M. 1997a, 'Morphology and in situ growth rates of plants of *Ascophyllum nodosum* (Phaeophyta) from different shore levels and responses of plants to vertical transplantation', *European Journal of Phycology*, vol. 32, no. 2, pp. 193-202.

Stevens, G.C. 1989, 'The latitudinal gradient in geographical range: How so many species coexist in the tropics', *The American Naturalist*, vol. 133, no. 2, pp. 240-256.

Stillman, J.H. & Somero, G.N. 2000, 'A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus Petrolisthes: Influences of latitude, vertical zonation, acclimation, and phylogeny', *Physiological and Biochemical Zoology*, vol. 73, no. 2, pp. 200-208.

Sultan, S.E. 2000, 'Phenotypic plasticity for plant development, function and life history', *Trends in Plant Science*, vol. 5, no. 12, pp. 537-542.

Sunday, J.M., Bates, A.E. & Dulvy, N.K. 2011, 'Global analysis of thermal tolerance and latitude in ectotherms', *Proceedings of the Royal Society of London B: Biological Sciences*, vol. 278, no. 1713, pp. 1823-1830.

Sunday, J.M., Bates, A.E. & Dulvy, N.K. 2012, 'Thermal tolerance and the global redistribution of animals', *Nature Climate Change*, vol. 2, pp. 686-690.

Sunday, J.M., Bates, A.E. & Kearney, M.R. 2014, 'Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation', *PNAS*, vol. 111, no. 15, pp. 5610-5615.

Tanaka, Y. 2000, 'Extinction of populations by inbreeding depression under stochastic environments', *Population Ecology*, vol. 42, no. 1, pp. 55-62.

Tatarenkov, A., Jönsson, R.B., Kautsky, L. & Johannesson, K. 2007, 'Genetic structure in populations of *Fucus vesiculosus* (Phaeophyceae) over spatial scales from 10 m to 800 km', *Journal of Phycology*, vol. 43, no. 4, pp. 675-685.

Taylor, D.I. & Schiel, D.R. 2003, 'Wave-related mortality in zygotes of habitat-forming algae from different exposures in southern new zealand: The importance of 'stickability'', *Journal of Experimental Marine Biology and Ecology*, vol. 290, no. 2, pp. 229-245.

Teixeira, S., Pearson, G.A., Candeias, R., Madeira, C., Valero, M. & Serrão, E.A. 2016, 'Lack of fine-scale genetic structure and distant mating in natural populations *of fucus vesiculosus', Marine Ecology Progress Series*, vol. 544, pp. 131-142.

Underwood, A. 1998, 'Grazing and disturbance: An experimental analysis of patchiness in recovery from a severe storm by the intertidal alga *Hormosira banksii* on rocky shores in New South Wales', *Journal of Experimental Marine Biology and Ecology*, vol. 231, no. 2, pp. 291-306.

Valero, M., Destombe, C., Mauger, S., Ribout, C., Engel, C.R., Daguin-Thiebaut, C. & Tellier, F. 2011, 'Using genetic tools for sustainable management of kelps: A literature review and the example of *Laminaria digitata'*, *CBM-Cahiers de Biologie Marine*, vol. 52, no. 4, p. 467.

Van Alstyne, K.L. 1989, 'Adventitious branching as a herbivore-induced defense in the intertidal brown alga *Fucus distichus*', *Marine Ecology Progress Series,* vol. 56, no. 169-176.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. 2004, 'Micro-checker: Software for identifying and correcting genotyping errors in microsatellite data', *Molecular Ecology Notes*, vol. 4, no. 3, pp. 535-538.

Viejo, R.M., Martínez, B., Arrontes, J., Astudillo, C. & Hernández, L. 2011, 'Reproductive patterns in central and marginal populations of a large brown seaweed: Drastic changes at the southern range limit', *Ecography*, vol. 34, no. 1, pp. 75-84.

Waser, N.M. & Price, M.V. 1994, 'Crossing-distance effects in delphinium nelsonii: Outbreeding and inbreeding depression in progeny fitness', *Evolution*, pp. 842-52.

Wernberg, T., Coleman, M., Fairhead, A., Miller, S. & Thomsen, M. 2003, 'Morphology of *Ecklonia radiata* (Phaeophyta: Laminarales) along its geographic distribution in south-western Australia and australasia', *Marine Biology*, vol. 143, no. 1, pp. 47-55.

Wernberg, T., de Bettignies, T., Joy, B.A. & Finnegan, P.M. 2016, 'Physiological responses of habitat-forming seaweeds to increasing temperatures', *Limnology and Oceanography*, vol. 0, pp. 00-00.

Wernberg, T., Russell, B.D., Moore, P.J. *et al.* 2011, 'Impacts of climate change in a global hotspot for temperate marine biodiversity and ocean warming', *Journal of Experimental Marine Biology and Ecology*, vol. 400, no. 1-2, pp. 7-16.

Wernberg, T., Russell, B.D., Thomsen, M.S., Gurgel, C.F.D., Bradshaw, C.J.A., Poloczanska, E.S. & Connell, S.D. 2011, 'Seaweed communities in retreat from ocean warming', *Current Biology*, vol. 21, no. 21, pp. 1828-1832.

Wernberg, T., Smale, D.A., Tuya, F. *et al.* 2012, 'An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot', *Nature Climate Change*, vol. 3, no. 1, pp. 78-82.

Wernberg, T. & Thomsen, M.S. 2005, 'The effect of wave exposure on the morphology of *Ecklonia radiata*', *Aquatic Botany*, vol. 83, no. 1, pp. 61-70.

Wernberg, T., Thomsen, M.S., Tuya, F., Kendrick, G.A., Staehr, P.A. & Toohey, B.D. 2010, 'Decreasing resilience of kelp beds along a latitudinal temperature gradient: Potential implications for a warmer future', *Ecology Letters*, vol. 13, no. 6, pp. 685-694.

Willi, Y., Van Buskirk, J. & Hoffmann, A.A. 2006, 'Limits to the adaptive potential of small populations', *Annual Review of Ecology, Evolution, and Systematics*, vol. 37, no. 1, pp. 433-458.

Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A. & Langham, G. 2008, 'Towards an integrated framework for assessing the vulnerability of species to climate change', *PLoS Biol*, vol. 6, no. 12.

Williams, S.L. & Dethier, M.N. 2005, 'High and dry: Variation in net photosynthesis of the intertidal seaweed *Fucus gardneri*', *Ecology*, vol. 86, no. 9, pp. 2373-2379.

Wolf, J.B. & Wade, M.J. 2009, 'What are maternal effects (and what are they not)?', *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 364, no. 1520, pp. 1107-1115.

Womersley, H.B.S. 1987, *The marine benthic flora of southern australia. Part II*, South Australian Government Printer, Adelaide.

Wright, J.T., Williams, S.L. & Dethier, M.N. 2004, 'No zone is always greener: Variation in the performance of *Fucus gardneri* embryos, juveniles and adults across tidal zone and season', *Marine Biology*, vol. 145, no. 6, pp. 1061-1073.

Yanagi, S.-i. & Tuda, M. 2010, 'Interaction effect among maternal environment, maternal investment and progeny genotype on life history traits in *Callosobruchus chinensis'*, *Functional Ecology*, vol. 24, no. 2, pp. 383-391.

Zardi, G.I., Nicastro, K.R., Canovas, F., Costa, J.F., Serrão, E.A. & Pearson, G.A. 2011, 'Adaptive traits are maintained on steep selective gradients despite gene flow and hybridization in the intertidal zone', *Plos One*, vol. 6, no. 6, p. e19402.

Zardi, G.I., Nicastro, K.R., Ferreira Costa, J., Serrão, E.A. & Pearson, G.A. 2013, 'Broad scale agreement between intertidal habitats and adaptive traits on a basis of contrasting population genetic structure', *Estuarine, Coastal and Shelf Science*, vol. 131, pp. 140-148.

Zardi, G.I., Nicastro, K.R., Serrão, E.A., Jacinto, R., Monteiro, C.A. & Pearson, G.A. 2015, 'Closer to the rear edge: Ecology and genetic diversity down the core-edge gradient of a marine macroalga', *Ecosphere*, vol. 6, no. 2, pp. 1-25