Local scale thermal environment and limited gene flow indicate vulnerability at warm-edge population of a habitat-forming macroalga

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Author contribution statement

JC conceived the idea for the manuscript, collected the data, curated and formally analysed the data and wrote the original manuscript. MD, AP, MC supervised the project, reviewed, wrote and edited the manuscript, MD collected some of the data and provided resources for funding the project.

Keywords

Thermal performace, Climate Change, Photosynthesis, microsatellites, Genetic diversily, quantitative breeding designs, Seaweed, Hormosira banksii

Abstract

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Species inhabiting warm-edge populations of their distribution are suggested to be at the forefront of global warming due to reduced fitness, limited gene flow and living close to their physiological thermal limits. Determining the scale that governs thermal niche and the functional responses of habitat-forming species to environmental stressors is critical for successful conservation efforts, particularly as coastal ecosystems are impacted by global change. Here, we examine the susceptibility of warm-edge populations to warming, in the habitat-forming macroalga, Hormosira banksii, from south-eastern Australia. We use a quantitative breeding design to quantify intraspecific variation in thermal performance (growth, ontogenic development and photosynthetic efficiency) of different genotypes sourced from sites at the equatorward distributional edge (warm-edge) and those towards the center of its distribution (non-edge). The genetic diversity and structure of H. banksii was also examined using microsatellite markers amongst the same sites. Our results found contrasting thermal performance in growth and development which depended on local scale thermal environment rather than distribution origin. Contrarily, warm-edge germlings grew optimally in lower temperatures and had narrower thermal breadth compared to non-edge germlings. Warm-edge germlings however, showed greater plasticity to tolerate high light indicated by a greater proportion of energy being dissipated as regulated nonphotochemical quenching (Y(NPQ)) than nonregulated nonphotochemical quenching (Y(NO)). Overall genetic diversity was lower at the warm-edge sites with evidence of increased structuring and reduced gene flow in comparison to the non-edge location. Evidence of genetic structuring was not found locally between high and low shore within sites. Together, these data suggest that non-edge populations may be "thermally buffered" from increased temperatures associated with ocean warming. Warm-edge populations of H. banksii, however, may be vulnerable to warming, due to narrower thermal breadth and sensitivity to higher temperatures, with genetic impoverishment through loss of individuals likely to further reduce population viability.

Contribution to the field

The resilience of species to persist in global warming depends on the range of functional responses produced by genotypes and phenotypes within its distribution. Previous research has identified warm-edge populations to be thermally tolerant but genetically impoverished making them more susceptible to environmental change due to the reduced range of functional responses. In marine macroalgae, there is limited research that pairs thermal tipping points with genetic diversity and determining the spatial scale in which global warming will have the greatest effect on physiology. Here, we conducted thermal performance curves using germlings from the habitat-forming, intertidal macroalga Hormosira banksii, from warm-edge and non-edge populations and multiple spatial scales (regional, local and individual) to test whether warm-edge populations are susceptible to future warming and whether this was related to population genetic structure. Contrarily, warm-edge populations presented greater thermal sensitivities and narrower thermal breadth to increased temperatures. We propose that thermal history and origin of the individual governs thermal niche, however, warm-edge populations may be facilitating their resilience to short-term environmental stress by actively dissipating a greater proportion of excess energy away from photosystems. Lower genetic diversity and gene flow at warm-edges, however, may constrain responses to environmental change over the longer term.

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Abstract

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1. Introduction

Anthropogenic mediated climate change is already having profound impacts on the physiology and distribution of many species worldwide (Pecl et al., 2017; IPCC 2018). By the end of this century, anthropogenic increases in atmospheric greenhouse gases will have increased ocean and air temperatures by 1.5 - 2 °C with global mean surface temperatures already warmed by 0.87 °C during the decade 2006 – 2015 (IPCC 2018). In the last decade, the prevalence of extreme climate events (heatwaves, droughts, floods, cold spells and storms) has caused further loss of populations and poleward shifts in distribution as species are being pushed past their physiological thresholds (Hawkins et al., 2009; Burrows et al., 2014; Poloczanska et al., 2016; Smale et al., 2019). Persistence in the face of a warming climate will require physiological plasticity and adequate genetic diversity for natural selection to act upon (Sgrò and Hoffmann, 2004; Reusch et al., 2005; Hoffmann and Sgrò, 2011; Wernberg et al., 2018; Gurgel et al., 2020).

Understanding how global warming and future extreme climate events will impact species requires knowledge of species' thermal niche and underlying genetic diversity across its distribution. Thermal niche is developed through acclimation and adaptation to temperatures experienced throughout a species life history which can vary with space and time (for a review see Bennett et al., 2015). For instance, thermal limits will differ for central and marginal populations as thermal regimes vary across a species geographical range (Sunday et al., 2012; Bennett et al., 2019). Thermal breadth is also influenced by the range of temperatures experienced throughout a species life history (Sunday et al., 2012). The climate variability hypothesis suggests that a positive relationship exists between thermal breadth of an organism and climate variability with increasing latitude. Therefore, populations in higher latitudes will have a greater thermal breadth as individuals experience a greater range in temperatures, than those closer to the equator (Stevens, 1989). Thus, cooler populations are suggested to be more resistant to warming as they have a broader thermal breadth compared to warmer populations (however see Bennett et al., 2015). As thermal limits often govern species range boundaries, and individuals are pushed beyond their physiological limits, the fitness of individuals therefore diminish towards distributional limits (Sagarin and Gaines, 2002; Thomas et al., 2004; Hampe and Petit, 2005; Pearson et al., 2009).

Reduced fitness at range margins often coincides with reduced gene flow and connectivity, habitat fragmentation (local separation of populations) and reduced effective population sizes. These can result in decreased genetic diversity (the range of functional responses provided by genotypes and phenotypes) and increase genetic differentiation between populations (Hampe and Petit, 2005; Eckert et al., 2008; Coleman et al., 2011a; Wernberg et al., 2018). The decrease in genetic diversity towards range limits has been documented extensively in plants and animals (Hampe and Petit, 2005; for a review see Eckert et al., 2008). Such patterns may also exist at the local scale over strong, but small-scale environmental gradients, where individuals live in habitat mosaics or where they may be spatially or temporally segregated. (Helmuth et al., 2006; Harley, 2008). Species inhabiting edge distributions are at the forefront of climate change, as they are already restricted by environmental factors and living close to their physiological limits (Parmesan, 2006; Smale and Wernberg, 2013; Pecl et al., 2017). Without adequate genetic diversity, potential selection for tolerant genotypes may be limited.

Investigating the spatial scale at which variation in environmental stressors will most strongly influence fitness is needed to determine impacts on biological systems (Helmuth et al., 2014). Species' distributions can often span thousands of kilometres and individuals can be exposed to wide variation in environmental regimes at different spatial scales; regionally among latitudes, and locally, among habitats within a single location. Factors such as local climate and topography within a habitat can translate to mosaics of "hotspots" and "coldspots" (Helmuth et al., 2006). For sessile organisms,

morphological differences among individuals add to local habitat topography and daily fluctuations in exposure, all of which can shape differing physiological thresholds (Helmuth and Hofmann, 2001; Harley, 2008; Clark et al., 2018). Studies have also suggested that variation at the scale of an individual can have a greater effect on physiology than broad scale differences observed over kilometres (Helmuth et al., 2002; Helmuth, 2009). Consequently, species declines in response to increasing environmental stress may not occur evenly across their range (Helmuth et al., 2006; Pearson et al., 2009; Miller et al., 2019), yet understanding intraspecific variation in tolerance is required to predict future species distributions.

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 (Stillman and Somero, 2000; Somero, 2005). 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; Bishop et al., 2009). As macroalgae are sessile organisms, they cannot move to avoid heat stress so must physiologically tolerate, adapt or perish in the face of climate change. Studies on the effects of temperature in governing species distribution and warm-edge ranges are becoming more apparent in macroalgal dominated communities (Pearson et al., 2009; Martínez et al., 2012; Ferreira et al., 2014; Bennett et al., 2015; Mota et al., 2018; King et al., 2019). 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 and Raven, 2013). Due to this, photosynthetic health has been widely used to assess thermal tolerance in photosynthetic organisms including many plants and macroalgae (Pearson et al., 2009; Smolina et al., 2016; Wernberg et al., 2016). Photosynthetic performance, in turn, can be evaluated by chlorophyll *a* fluorescence measurements, specifically the maximum quantum yield (Fv/Fm) and investigation of energy dissipation pathways (Genty et al., 1989; Kramer et al., 2004; Schreiber, 2004).

Variation in thermal response 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 (Schonbeck and Norton, 1978; Hartnoll and Hawkins, 1985; Davison and Pearson, 1996). On regional scales, individuals inhabiting warm range-edge populations closer to the equator have been shown to have greater thermal tolerances due to exposure to higher temperatures throughout their life history (Mota et al., 2018). Photosynthetic health of macroalgae at warm-range limits also reflect greater thermal tolerances indicating greater ability of warm-edge thalli to maintain maximum quantum yield of PSII (F_V/F_M) in higher temperatures than in cool-edge populations (Mota et al., 2018). Warm-edge populations are often fragmented and reduced in size (Coleman et al., 2011b, 2011a; Zardi et al., 2015), suggesting that these populations are physiologically stressed towards range limits (Araújo et al., 2011) and may be less resilient to prolonged exposure to extreme climate events (Wernberg et al., 2016; Mota et al., 2018). Variation in thermal response on the local scale, among vertical heights on the shore, is well known and is related to daily tidal regimes and topography, and the ability to effectively photosynthesize during periods of increased desiccation and thermal stress (Schonbeck and Norton, 1978; Dring and Brown, 1982; Davison and Pearson, 1996; Williams and 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. Relatively few studies have experimentally identified heritable genetic variation in seaweeds with families showing the potential for adaptation to changes in environment associated with climate change (Clark et al., 2013; Al-Janabi et al., 2019; Mabin et al., 2019).

Southeastern Australia has been identified as a climate change hotspot (Lough and Hobday, 2011; Hobday and Pecl, 2014) and is therefore an ideal location to study the effects of global warming on the genetic diversity and physiology of marine macroalgae. The east coast of Australia follows a north (equatorward) to south (poleward) thermal gradient with a natural warm to cold water transition, and serves as the main conduit for gene flow for many sessile marine species within coastal ecosystems (Coleman et al., 2011b). *Hormosira banksii,* is a dominant, habitat-forming macroalga found on rocky shores in Australia and New Zealand and spans >3000 km of coastline from the northern distributional edge Skennars Head in New South Wales to Albany in Western Australia (Womersley, 1987; Huisman, 2019) as well Tasmania, and the North and South Island of New Zealand (Nelson, 2013). Towards the equatorward distributional limits, percent cover decreases from 80% at Minnie Water to 20% at Angourie and 25% at Skennars Head, with *H. banksii* mostly found in rockpools at the distributional limits (personal observation). It is dioecious and has a monophasic life cycle with oogonium development found in mature conceptacles in every season, producing gametes potentially every low tide (Osborn, 1948). Further, gamete dispersal is less than 10 m (Bellgrove et al., 1997), and has been documented to have limited gene flow (Coleman et al., 2011a, 2019) with local adaptation recently identified in thermally different regions on the south coast of Australia (Miller et al., 2019). Previous research on thermal performance of *H. banksii* is limited, but has identified increased thermal sensitivity through decreased photosynthetic yield of PSII, smaller morphology and lower percent coverage of thalli (~20%) in adult thalli of warm-edge populations of *H. banksii* (Clark et al., 2018). Less is known about how populations of *H. banksii* within the equatorward range edge of this species will respond to elevated temperatures and extreme climate events, given limited gene flow and inhabiting physiological stressful environments.

In this study, multiple performance traits of the ecologically and functionally important intertidal macroalga, *Hormosira banksii,* were quantified to assess variation in thermal tolerance and genetic structuring at three nested spatial scales: among locations, among heights on the shore and among individual genotypes within a shore, to determine the vulnerability of warm-edge populations to climate warming. We aimed to (1) assess the thermal performance of individuals (2) determine whether genetic variation in traits are heritable, and (3) determine the genetic diversity and connectivity of warm-edge versus non-edge populations of *H. banksii* to assess the role of thermal history and adaptation in providing resilience to global warming.

2. Methods

2.1. Study locations and collection of model organism

For genetic diversity analysis, *H. banksii* populations were sampled during the austral autumn (April – May 2014) in two eastern Australian regions: a warm-edge region encompassing two locations, Angourie (29º28’41.51” S, 153º 21’ 49.53” E) and Minnie Water (29º46’34.23” S, 153º18’07.43” E) at the northern warm-edge of its distribution, and a non-edge region within the center of its range, encompassing two locations, Pearl Beach (33º32’57.70” S, 151º18’32.36” E) and Bilgola Beach (33°38’54.48” S, 151°19’39.59” E), approximately 460 km further south (Fig.1). For thermal response experiments, *H. banksii* was collected from two populations each within the mentioned regions (Minnie Water and Pearl Beach). At the time of sampling, *H. banksii* was the dominant, intertidal macroalgal species (60-80% percent cover) in all locations except Angourie which had 20% percent cover.

2.2. Thermal exposure at different spatial scales

Data from multiple sources was used to demonstrate average air and sea surface temperatures (SST) experienced at each warm-edge (Minnie Waters) and non-edge (Pearl Beach) location (Fig S2). SST detected by satellite (MODIS-Aqua), was obtained via GIOVANNI (NASA GES DISC) using a 4 km^2 area (Minnie Water -29.5 °S, 29.0 °S 153.4 °E 153.9 °E; Pearl Beach -33.844 °S, -33.441°S, 151.296

°E, 151.671 °E) for years between 2003-2014. Local weather stations were used to estimate average minimum, maximum air temperature as well as mean number of days above 35 °C at each location (Bureau of Meteorology (BOM), Australian Government, http://www.bom.gov.au; Terry Hills station from 1954-2014 and Yamba from 1877-2014)). Data reflect average temperatures recorded from when station became operational. To document the temperature variability locally within *H. banksii* beds at each location, single HOBO® pendant loggers (Onset®, USA) were drilled into the substrate at high and low tidal heights and temperatures were recorded between April and July 2014 (Fig. S3). Data only overlapped for 7 days in June 2014 due to some loggers going missing, therefore were only used to view the temperature variability within each shore height at each location and were not compared between locations.

2.3. Assessment of genetic diversity

To assess genetic structure of populations, 32 thalli (approximately 1 m apart) were haphazardly sampled from the low and high shore at each location within each of the regions. 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. Collection at low and high tidal heights were separated horizontally by ~ 5 – 10 m above the low tide mark. 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, snapped frozen in liquid nitrogen before storing in a -80 °C freezer until use. Before DNA extraction, samples were freeze-dried overnight. 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 using 10 microsatellite loci as described in Bellgrove et al. (2017). Each 11 µL PCR reaction was set up in 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 10 µM unique fluorophores (FAM, VIC, NED, PET) which tagged the flanking regions of the microsatellites.

Multiplex 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).

2.4. Effects of temperature on phenotypic traits

To assess how functional thermal responses differed within and across populations, quantitative breeding designs were set up to partition variance amongst different genotypes (families), amongst different vertical heights on the shore and between the warm and non-edge 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 vertically distance from low shore region. Thalli were transported on ice and gametes extracted within 48 hours.

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 (Doblin and Clayton, 1995). After 20 min, gametes were released from conceptacles through osmotic stress and desiccation and the sex of the thallus identified by the colour of its gametes: olive green for females and orange for males (Osborn, 1948). As in Clark et al. (2013), three males and three females from each shore height within

each location were used in a North Carolina II breeding design (Lynch and Walsh, 1998), where each male was cross fertilised with each corresponding female in a fully factorial design, yielding nine unique genotypes. Each egg and sperm solution were 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 eight glass 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 in a Climatron Plant Growth Chamber (Thermoline Scientific, Australia). These temperatures are representative of temperatures experienced by both populations (from climate weather and HOBO pendants) as well as designed to be physiologically stressful to test thermal performance of intertidal macroalgae in populations at the warmer end of the distribution. To examine thermal responses under more realistic fluctuating environments and hence estimate realized rather than fundamental thermal reaction curves, ± 5 °C diel cycle was implemented (Paaijmans et al., 2013). This temperature regime was determined from examining field data from the HOBO® pendant loggers in a pilot study. Germlings were incubated in a 12:12 hour light cycle at 30 ± 5 µmol photons $m^{-2}s^{-1}$.

At 120 h after fertilisation, germlings growing on coverslips were removed from temperature 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 germling 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, germlings were scored into five ontogenetic stages: 0 = fertilisation through condensation of the chloroplasts; 1 = protrusion of germling cell wall to create a pear shape which later develops into the rhizoid; 2 = division of the germling germinating cell and elongation of a single rhizoid; 3 = elongation of the rhizoid coupled with secondary and tertiary rhizoid development; and 4 = paraphysis development (apical hairs) on top of the germinating cell. These equate to stages 1, 2, 3-5 and 6 (a-d), respectively, in Clarke and Womersley (1981).

A pulse amplitude modulated fluorometer (Microscope Imaging-PAM, Walz GmbH, Germany) was used to examine photophysiological traits of multiple germlings in one field of view while returning individual measurements from each individual. 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. Germlings attached to cover slips (120 h post fertilisation) were wet mounted and scanned under green light (non-stimulatory for photosynthesis). Selection of germlings for measurement involved maximising the number of germlings in a field of view, as coverslips were discarded after each fluorescence assay. To ensure we obtained high-quality data across all treatments and limit a potential diel effect in photophysiological assessments, we had to restrict our photophysiological measurements to one assay per coverslip. Therefore, the coverslip was briefly scanned to find a field of view where there were numerous germlings sufficiently close together for simultaneous assessment. Although coverslips had the same zygote density before being randomly allocated to temperature treatments, the germling density differed at the time of measurement (120 h), varying between two and eight. Gain settings were adjusted so that base fluorescence (F_t) was between 0.1 and 0.3 for each germling, 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 germlings. This was done by recording the base fluorescence (F_t) of 120 h old germlings determined using the full range of gain settings. The increase in F_t with gain was then fitted with a log linear

regression model ($R^2 > 0.90$) so that F_t could be estimated amongst all germlings, no matter what gain setting was used to perform the steady-state analysis (Kramer et al., 2004).

The measurement protocol involved dark adapting germlings 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 traits were only determined for 24, 28 and 32 °C (3 of the 6 temperature treatments) and represented the highest and stressful temperature for both populations with other two temperatures equally spanning the thermal gradient.

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). F_V/F_M is the measure of photosynthetic efficiency of PSII after dark acclimation of photosystems (Genty et al., 1989) with greater values (0-1) equating to greater number of photosystems available for light capture. 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., 2004). To estimate the capacity of germlings 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.

2.5. Statistical analyses

2.5.1. In situ temperature regimes

The difference in temperature variability between high and low shores at each location, temperature data obtained by HOBO® was tested using Levene's test of variance. SST recorded for each location (MODIS-Aqua satellite 2003-2014) was analysed with a two-factor ANOVA to test for differences between location and months.

2.5.2. 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). 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). For each measure of genetic variation, univariate analyses of variances (ANOVA) were conducted to test for differences between locations with between regions (warm-edge and non-edge) 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 0-0.05 indicate little differentiation, 0.05-0.25 indicate moderate genetic differentiation

and values over 0.25 represent pronounced levels of genetic differentiation (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., 2007) 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 within 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.

2.5.3. Effects of temperature on phenotypic traits

Physiological responses (variation in germling length and ontogenic development) were analysed with permutational ANOVA, with location (warm-edge and non-edge), 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 location. For significant location x temperature or shore height x temperature interactions, main effects were tested with a reduced two-factor ANOVA to compare each temperature. Tukey's HSD post-hoc comparisons were conducted on significant interactions to determine temperature effects. Thermal breadth was obtained by arbitrarily setting a threshold of 80% of maximum germling length for each height on the shore and location combination. 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-E (v6) and the proportion of variance explained by each factor calculated by least square estimates of variance components (Anderson et al., 2008). Data was visualised using package 'ggplot2' (Wickham, 2009) and post-hoc comparisons were conducted in R Studio (version 1.2.5019) using R (R Core Team, 2020, version 3.6.2 (2019-12-12)).

3. Results

3.1. Thermal exposure

Mean maximum monthly air temperatures recorded at local weather stations were similar at both locations, however minimum temperatures were lower at the non-edge location. The warm-edge location experienced a narrower temperature range, 9.7 to 26.7 °C compared to 4.8 to 27.6 °C at the non-edge location (Fig. S1a,b)—a difference between max and min air temperatures of 17.0 °C and 22.8 °C, at each location respectively (Fig. S1a, b). The warm-edge location experienced fewer days per year where air temperature exceeded 35 °C than the non-edge location (on average ~1 vs ~8 d y^{-1}, respectively; Fig. S1). Sea surface temperature recorded between 2003-2014 by satellite (MODIS-Aqua; https://oceancolor.gsfc.nasa.gov/data/aqua/), ranged from 19.4 to 26.7 °C at the warm-edge location versus 16.3 to 24.4 °C at the non-edge location and were significantly different between locations (F_1 =1619, P<0.001) and months (F_{11} = 325, P<0.001; Fig. S2c).

At the local scale, HOBO® sensors recorded similar mean temperatures on the low and high shore at the warm-edge location (21.9 ± 2.8 °C vs 21.5 ± 3.6 °C, respectively; Fig. S2) as well as the non-edge

location (16.0 ± 2.4 °C vs 15.3 ± 3.5 °C, respectively; Fig. S2). High shore temperatures were however more variable than low shore temperatures at both locations (warm-edge: $F_{1,2074}$= 55.89, P = 0.001; non-edge: $F_{1,1960}$, P = 0.001).

3.2. Genetic diversity and structure

Null alleles were found in warm-edge populations, Minnie Water (MW) and Angourie (ANG) at low and high tidal heights for locus HB3 but not at any other locations or loci. All analyses were run with and without HB3 and results were consistent, 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 S1). Amongst the 235 individuals collected, a total of 28 different alleles were genotyped across 10 loci. Total mean (± SD) number of alleles across all locations and shore heights sampled was 26.00 ± 1.18 where unique alleles were found at ANG high shore (2 alleles), MW low shore (1 allele) and BB low shore (2 alleles) populations (Table 1). The total number of alleles in any single population varied from 24 to 28 and was similar between regions ($F_{1,79}$ = 0.055, P = 0.820) amongst all locations ($F_{3,79}$ = 0.106, P = 0.967), and between heights on the shore (Table 1; $F_{1,79}$ = 0.053, P = 0.814).

Genetic diversity was determined by expected heterozygosity (H_E) which was lower in warm-edge populations (Table 1, Table S1). A trend for positive (but not significant) F_{IS} values indicated some selfing or inbreeding at both warm-edge and non-edge populations but a significant deviation from random mating (negative F_{IS} value) was only found in low shore populations at Pearl Beach, indicating an excess of heterozygotes (Table S2).

The overall F_{ST} estimate amongst all spatial scales tested was 0.256 indicating high genetic structure, but genetic structuring diminished from regional to local scales (Table 2). There was pronounced and significant levels of genetic structuring between pairs of non-edge (Bilgola and Pearl Beach) and warm-edge populations (Minnie Water and Angourie) with F_{ST} values ranging between 0.274 - 0.413 (Table 2). Among non-edge locations there was moderate and significant genetic structure with pairwise F_{ST} values ranging from 0.150 – 0.190 (Table 2). Similarly, there was low but significant genetic structure between locations within the warm-edge region (Minnie Water and Angourie; F_{ST} = 0.066 - 0.105 (Table 2)). Pairwise F_{ST} estimates between vertical shore heights were not significantly different at any location (Table 2).

There was a large (25.7 %) and significant amount of genetic variation explained by differences in regions, (warm-edge vs non-edge). Similarly, a large and significant amount of genetic variation (8.0%) occurred between locations within each region, with the largest amount of genetic variation (66.3%) amongst individuals within each location (F_{ST} = 0.337, P < 0.001). When shore heights were pooled within each location, a separate AMOVA revealed greater variation was explained at the scale of locations (28.0 %) and amongst individuals within each location (72.5%; F_{ST} = 0.275, P < 0.001). There were strong significant relationships between geographic and genetic distance across all locations (Mantel test: Z = 2620.41, r = 0.924, P = 0.002, Fig. S1) but not within non-edge (Mantel test: Z = 7.56, r = 0.976, P = 0.113, Fig. S3) or warm-edge regions (Mantel test: Z = 6.90, r = 0.831, P = 0.250, Fig. S3).

3.3. Effects of temperature on germling growth and ontogenesis

Temperature had a strong effect on germling growth, resulting in distinct thermal performance curves at each location and height on the shore (Fig. 2). Overall, there was a significant *location* × *temperature* interaction (15% of the total variation in germling length explained by this interaction) as well as a significant *shore height* × *temperature* interaction (3 % of total variation; Table 3) and the interaction between *male-female* × *temperature* contribution was 2%. Separate two-factor ANOVAs found

significant differences for the main effects of location and height on the shore for each temperature (Table S3) with *location* × *shore height* interactions found for germling length at 22, 24, 26, and 30 °C (Table S3, Fig 2). Among these interactions, Tukey's post-hoc comparisons were significant between all *location* × *shore height* combinations at each temperature except for some combinations at 22, 26 and 30 °C (see Table S4 for all pairwise results).

Thermal breadth, defined as the temperature range in which germling length reached 80% of maximum germling length, varied at each shore height nested in each location. At the warm-edge population (MW) germling lengths from both low and high shore demonstrated greatest growth in the cooler range of temperatures tested (24–26°C)). For overall length, warm-edge high shore germlings, were the longest at 24 and 26 °C compared to all other heights on the shore tested (mean length ± SD; 408.73 ± 46.03 µm and 399.49 ± 42.61 µm respectively) and declined significantly in temperatures beyond 26 °C (22 < 24 = 26 > 28 > 30 > 32 °C; $P<0.05$, Table S5; Fig 2). Similarly, warm-edge low shore germlings grew optimally at 22- 26°C, (274.03 ± 37.21 µm – 352.52 ± 66.11 µm) with a significant decline in growth after 26 °C (22 = 24 = 26 > 28 > 30 = 32 °C; $P<0.01$, Table S5; Fig 2). For the non-edge population, germling growth was greater in warmer range of temperatures tested. Non-edge high shore germling growth was sustained across a wider thermal breadth between 22–28°C and low shore between 24–28 °C (Fig. 2). Non-edge high shore germlings were the greatest between 24 - 28°C (356.48 ± 43.84 µm – 377.24 ± 33.14 µm) and were significantly longer than other temperatures tested (22 < 24 = 26 = 28 > 30 > 32 °C; $P <0.001$, Table S5, Fig 2). Non-edge low shore germlings growth peaked at 28 °C (204.78 ± 41.44 µm) and were significantly greater than germling length tested at other temperatures ((22 < 24 = 26 < 28 > 30 > 32 °C; $P <0.05$, Table S5, Fig 2). For all germlings despite location or height on the shore, temperatures beyond 28 °C showed significantly reduced growth of germlings with substantial inhibitory effects at 30 ºC for warm-edge germlings. At the most extreme temperature, 32 °C, germlings from the non-edge location grew 3-4 fold slower than at their maxima and those from the warm-edge location grew 4-5 fold slower.

At the level of the individual, the effect of temperature varied significantly with male identity for germling length for shore height and location (*temperature* × *male* interaction; Table 3), which provides evidence of heritable genetic variation in thermal tolerance in different populations of *Hormosira banksii*. The effect of temperature also varied with parental identity (a significant *temperature x male x female* interaction, Table 3) with ~2% of the variance in growth of all germlings attributed to the variation in temperature effects among male/female combinations.

The effect of temperature on the ontogenic development of germlings varied among locations, heights on the shore and among genotypes (Fig. 3). Overall, germlings from the warm-edge location developed more rapidly than those from the non-edge location (significant *location* × *temperature* interaction for stages 0, 3 and 4, Table 4), with up to 60% of warm-edge germlings reaching stage 3 or 4 after 5 days, in contrast to only 20% from non-edge (Fig. 3a, c). The proportion of warm-edge germlings with delayed development (i.e. stage 0) increased steadily in temperatures that surpassed the temperatures optimal for growth (24–26 °C) reaching ~85% at 32 °C. At the non-edge location, between 5 and 15% of germlings had delayed development across all temperatures except for high rates of ~40% at 32 °C (Fig. 3b, d). Development at 32 °C was characterised by enlargement of the germinating cell rather than through cell differentiation and rhizoid development.

The proportion of germlings at any stage, or with delayed development in stage 0, did not vary with temperature and height on the shore in either location (non-significant *temperature* × *height* on shore interactions, Table 4). However, the effect of temperature varied significantly with male identity for

the proportion of germlings in stage 2- 4 (genotype by environment interaction; Table 4) indicating that there is heritable genetic variation in the effects of temperature on rates of ontogenic development.

3.4. 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), respectively) amongst all germlings, but there were no significant interactions with location or heights on the shore (Table 5; Fig. 4). Germlings all showed similar photophysiological responses 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. 4). Maximum quantum yield (F_V/F_M) was generally greater in warm-edge germlings compared to non-edge germlings but did not differ among heights on the shore or temperature (Fig. 4, Table 5).

High and low shore germlings used regulated nonphotochemical quenching (Y(NPQ)) as a means of photoprotection, with Y(NPQ) remaining similar under ambient and high light intensity (Fig 4, Table 5). However, germlings from the warm-edge location diverted more energy proportionally to Y(NPQ) under high light intensities than those from the non-edge location (Fig. 4; Table 5). This was also evident with the ratio of regulated nonphotochemical quenching under ambient and high light (HL: LL Y(NPQ), Fig. 4, Table 5) where the germlings from the warm-edge location had ratios above 1 (indicating increased regulated quenching of energy at saturating light intensity) compared to germlings from the non-edge location 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 higher in the germlings from the non-edge location and those from high on the shore (Table 5). For all photophysiological traits, there were no significant interactions between temperature and location or height on the shore, indicating that germling responses to temperature did not vary at regional and local scales (Table 5).

4. Discussion

Populations inhabiting the warm range edge of distributions are suggested to be at the forefront of climate change. The increasing prevalence of extreme climate events such as heatwaves may challenge marine macroalgal dominated populations that have limited physiological plasticity to tolerate prolonged, elevated temperatures or reduced capacity to adapt (Wernberg et al., 2018; Gurgel et al., 2020). In this study, germlings of the dominant intertidal macroalga, *Hormosira banksii*, demonstrated contrasting thermal performance curves which was governed by the thermal environment from where they originated rather than their relative distributional origin. Warm-edge germlings had greater growth rate and development and a narrower thermal breadth, but were sensitive to higher temperatures compared to non-edge germlings, which grew optimally across a wider range of temperatures. Relative position on the vertical shore, had a greater influence on thermal physiology and breadth illustrated by wider thermal breadth for non-edge, high shore germlings. Warm-edge germlings, however, had greater capacity to regulate excess energy as nonphotochemical quenching (Y(NPQ)) when exposed to greater temperature and light, suggesting they are less photophysiologically sensitive than non-edge population germlings. Evidence of heritable genetic variation (significant genotype by environment interaction) found for growth and development indicate that there is potential for adaptation in thermal tolerance traits. These physiological responses coincided with lower genetic diversity, restricted gene flow and evidence of inbreeding at warm-edge populations. This suggests that warm-edge germlings utilise physiological plasticity to tolerate short-term exposure (hours-days) to environmental stressors but over longer time scales (years) may potentially be less thermally buffered and at greater risk to global warming.

4.1. Thermal effects of physiology

Thermal history at study locations played an important role in governing thermal tolerances in *H. banksii* germlings. Germlings grew and developed faster in the warm-edge population but in the cooler, narrower range of temperatures tested, compared to non-edge germlings. Although previous research found that 90% of seaweeds displayed population level variation in upper thermal limits (King et al., 2017), which agrees with our findings, the result of increased thermal sensitivity to high temperatures for warm-edge germling growth is contrary to previous research. Many studies on marine macrophytes and invertebrates in lower latitudes were found to be more tolerant of higher temperatures, as they generally experience greater temperatures throughout their life history (Gerard and Du Bois, 1988; Stillman and Somero, 2000; Kelly et al., 2012; Sunday et al., 2012; Mota et al., 2018). Air temperature data collected from local meteorological stations demonstrated that the warm-edge population (Minnie Water) experience similar annual maximum monthly temperatures as the non-edge population (Pearl Beach), but warmer minimum monthly temperatures, less seasonal variation and fewer days over 35 °C. This would suggest that the warm-edge population should also be more thermally tolerant. However, previous research on the effects of desiccation stress on adult thalli of *H. banksii* from the same warm-edge site (Minnie Water), adults were also more thermally sensitive to higher temperatures (Clark et al 2018). Further, previous research also found a warm-edge macroalgal population was not more thermally tolerant than cooler populations and proposed that the warm-edge population was thermally maladapted (Pearson et al., 2009). The low gene flow and low genetic diversity found in the warm edge populations of this study may support local adaptation to conditions (discussed in section 4.2), however physiological adaptation and local site effects may also play an important role in shaping thermal performance (discussed further in this section).

Our result of narrower thermal breadth of warm-edge population is consistent with the climate variability hypothesis of narrower thermal breadth towards lower latitudes (Stevens 1989). However, a recent study of a non-edge population of a subtidal macroalgal species (*Scythothalia dorycarpa*) had similar thermal safety margins (defined as the the temperature buffer between an organisms upper thermal-tolerance limit and the maximum ambient temperatures it experiences') to warm-edge populations but different absolute temperature tolerances, which demonstrated that not all species at distributional limits have a narrower thermal breadth (Bennett et al., 2015). One explanation for our result of different thermal breadths for both populations is that intertidal species are exposed to dynamic environmental stress imposed by the terrestrial and marine environment, opposed to constantly being submerged, therefore differences such as emersion and air temperature variation may be more important in shaping thermal niche. The significant difference in thermal breadth in non-edge high and low shore germlings illustrates how local scale effects in the intertidal can influence thermal performance.

Despite locational differences in thermal regimes among locations, germlings of *H. banksii* demonstrated similar photophysiological responses to elevated temperatures. Divergence among locations in the ability to tolerate greater light intensity, however, was found for dissipation of excess energy. The lack of any interactions between temperature and location for photophysiological parameters in *H. banksii* suggests that germlings have a high degree of plasticity, and can adjust their photosystems to tolerate differences in light and temperature regimes. Despite significant reductions of growth at 28 °C for warm-edge germlings and 30 °C for non-edge germlings, *H. banksii* was still able to maintain a high level of PSII efficiency in ambient and high light intensities across 24 and 28 °C, suggesting acclimation of photosystems (Major and Davison 1998). This result is consistent with previous studies which also found no significant temperature interactions in photosynthetic response of macroalgae (Clark et al., 2013; McCoy and Widdicombe, 2019). 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 with wave action and tidal cycles. Furthermore, in locations closer to the equator, light intensity is greater seasonally, therefore warm-edge germlings may be able to tolerate higher light intensity through phenotypic plasticity indicated by more energy being dissipated via Y(NPQ) rather than Y(NO), whereas non-edge germlings are more light sensitive indicated by the greater proportion of Y(NO). This may also explain the greater growth reduction at higher temperatures for germlings in the warm-edge location as more energy is being diverted towards photoprotection rather than to photochemistry. 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.

Growth of germlings from low and high on the shore was also affected by differences in local temperatures, indicated by significant interactions between height on the shore and temperature. Temperatures recorded by HOBO pendants in the high shore at both locations were significantly more variable than low shore temperatures. 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 et al., 2002, 2006; Helmuth, 2009). 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 (Helmuth and Hofmann, 2001; Harley, 2008). 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 hot and coldspots. In this study, the warm-edge location is characterised by large boulders 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 at the non-edge location. The shore topography at the warm-edge location could thus modify the thermal exposure of individuals and lead to similar growth rates of germlings from low and high on the shore as found in this study.

Maintaining thermal tolerance across broader temperatures can be physiologically costly, therefore germlings may not grow optimally across all temperatures (Huey et al., 2012). This is demonstrated by differences in optimal temperatures for germling growth and may reflect increased energy dissipation (i.e., non-photochemical quenching) and decreased photochemistry (YII) with increased temperatures and light intensities likely experienced for longer periods during low tide high on the shore (Davison and Pearson, 1996). In addition, the reduced growth and narrow thermal optima amongst low shore germlings in both populations may reflect light limited photosynthesis of adults as they experience longer periods spent submerged compared to those on the high shore, while optimising growth within a narrow range of temperature that they most commonly experience (Huey et al., 2012). There were no significant 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 dynamic variation in light and temperature during daily tidal cycles, it suggests that photosystems need to be able to rapidly acclimatise to different light and temperature regimes (Hanelt et al., 1993). 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 (Hanelt et al., 1993; Al-Janabi et al., 2019).

The potential for adaptation in temperature tolerance traits amongst *H. banksii* germlings is indicated by a significant male x temperature interaction for germling length and ontogenic development. This

is consistent with earlier investigations of this species (Clark et al., 2013) and suggests that as temperatures increase with global warming, genotypes that are better able to tolerate higher temperatures will be favoured (Deutsch et al., 2008; Sunday et al., 2012; Fusi et al., 2015). This will be particularly important for populations that have limited gene flow such as the warm-edge population. A significant interaction between female identity and temperature was also found for the proportion of germlings 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. bryozoans, Marshall, 2008; terrestrial plants, Galloway et al., 2009; sea urchins, Foo et al., 2012; fish, Chambers and Leggett, 2015) and are potentially relevant in *H. banksii* where egg size differs among different females (Clark, 2016). Maternal environment may impact the resources available for reproduction which can affect egg size and growth trajectory of offspring (Wolf and Wade, 2009). The significant interaction between temperature and parental identity (i.e., *male* × *female* × *temperature*) 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).

4.2. Genetic diversity and structure

Consistent with previous studies (Coleman et al., 2011a, 2019; Miller et al., 2019) we found strong genetic structure between the warm-edge and non-edge regions (~ 500 km apart) as well as isolation by distance suggesting that dispersal capacity is limited across long distances as well as between neighbouring populations (> 50 km). Moreover, trends for lower estimates of genetic diversity towards distributional edges found in this study is in accordance with previous studies of *H. banksii* across a longitudinal gradient (Miller et al., 2019) and other macroalgal species (Faugeron et al., 2004; Teixeira et al., 2016; King et al., 2017; Wernberg et al., 2018). The observed patterns of lower genetic diversity at warm-edge populations is suggested to be the result of reduced gene flow and connectivity which can create isolation among populations and reduce within population genetic diversity (Hampe and Petit, 2005). In addition, as distributional limits often represent the physiological limits of a species, environmental conditions can impose strong selection pressure resulting in decreased diversity as environmental conditions and habitat become suboptimal with only tolerant genotypes and phenotypes persisting at range edges. While we cannot tease apart these mechanisms with the neutral markers used here, the early life stages of *H. banksii* from warm-edge populations had a narrower range of thermal performance compared to populations found within the center of its distribution suggesting that reduced genetic diversity may constrain responses. With lower genetic diversity, the warm-edge population may not have the range of the functional responses such as greater tolerance for higher temperature, however, greater regulated nonphotochemical protection in warm-edge germlings, suggests that this population may have greater phenotypic plasticity to tolerate dynamic light conditions.

Moderate gene flow is evident between neighbouring *H. banksii* populations within each warm-edge and non-edge region separated by < 50 km. Dispersal of gametes or zygotes is not a likely method of long-distance dispersal as fertilised zygotes sink to the substrate and adhere within hours of fertilisation (Dimartino et al., 2015). 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 (Muhlin et al., 2008; Valero et al., 2011; Bussolini and Waters, 2015; Coleman et al., 2019). There is limited empirical evidence that supports whether floating thalli contribute to long distance gene flow in *H. banksii*,

674 however a recent study suggests that floating thalli can end up in estuaries where they can grow and
675 survive (Coleman et al., 2019). The moderate but significant levels of genetic structure between
676 neighbouring populations within each non-edge and warm-edge region may show restriction in gene
677 flow possibly due to the existence of physical barriers such as sandy beaches, and mouths of estuaries
678 which may serve as barriers to gene flow in other macroalgae (Billot et al., 2003; Coleman, 2013).

679
680 Within smaller scales (within 5-10 m), gene flow of *H. banksii* was not restricted between vertical
681 heights on the intertidal shore which agrees with other studies on macroalgae (Engel et al., 2004;
682 Tatarenkov et al., 2005; Teixeira et al., 2016; Bellgrove et al., 2017). The intertidal is characterised by
683 steep environmental gradients suggesting selection for stress-tolerant genotypes on high shores may be
684 an important driver of genetic structure. This has been demonstrated amongst barnacles (Schmidt and
685 Rand, 2001), gastropods (Johannesson et al., 1995) as well as amongst hybrids of the macroalgae *Fucus*
686 *vesiculosus* and *Fucus spiralis* (Billard et al., 2010; Zardi et al., 2011). Nonetheless, the lack of small-
687 scale genetic structure between shore heights found in this study, suggests that gene flow is
688 unobstructed and that *H. banksii* zygotes and gametes may be readily dispersed across these smaller
689 distances (Dudgeon et al., 2001). Studies on the attachment strength of *H. banksii* zygotes have found
690 that adhesion to the substrate is not at maximum strength until 24 h after fertilisation suggesting that
691 zygotes could potentially be dislodged and recruit elsewhere (Dimartino et al., 2015). Specific habitat
692 types related to strong environmental gradients within the intertidal have been found to influence
693 phenotypic divergence independently of genetic structure (Engel et al., 2004; Zardi et al., 2013) Zardi
694 *et al.* 2013). Lack of differences at small scales suggest that *H. banksii* may survive living in different
695 environmental gradients through phenotypic plasticity rather than genetic differentiation as
696 documented in *F. vesiculosus* (Zardi et al., 2013). This suggests that thermal exposure within the
697 intertidal may not necessarily select for different genotypes but perhaps genotypes that are highly
698 plastic. An alternative explanation is that adaptive genetic differentiation between tidal heights may
699 exist, but is not apparent in our neutral markers (which only show variation due to dispersal and
700 connectivity, not selection). Testing this idea would require use of markers such as SNPs which
701 examine portions of the genome under selection.
702
703 Genetic diversity was found to be lower at warm-edge populations. This is not surprising as these
704 populations are at the edge of their equatorward distribution, where populations are more fragmented,
705 conditions are not optimal and macroalgal populations are therefore at their physiological threshold.
706 The lower genetic diversity found at these populations suggests that these populations may lack the
707 potential to adapt to future warming and be particularly vulnerable to extreme climate events (i.e. heat
708 waves). Previous studies have already shown local extinction in warm-edge populations of macroalgal
709 populations with extreme climate events which may be a consequence of a smaller gene pool (Araújo
710 and Williams, 2001; Smale and Wernberg, 2013; Wernberg et al., 2018).
711
712
713 **5. Conclusions**
714 The results of this study provide evidence that germlings of *H. banksii* inhabiting populations within
715 the warm-edge of its distribution may at risk to increases in temperatures associated with global
716 warming (Fig. 5). The sensitivity to higher temperatures, narrower thermal breadth as well as relatively
717 low genetic diversity and limited gene flow are all indications of populations that are vulnerable to
718 warming (Pearson et al., 2009; Mota et al., 2018; King et al., 2019). Significant genotype by
719 environment interactions found for growth and ontogenic development suggests that there is heritable
720 genetic variation in growth and development under different temperatures, which could be important
721 particularly for the warm-edge populations with lower genetic diversity and gene flow. Our

722 experimental data show that these warm-edge populations may also be surviving through phenotypic
723 plasticity by obtaining similar levels of photochemistry through greater levels of regulated non-
724 photochemical quenching (photoprotection) at higher light and temperature than non-edge population,
725 however over the long-term the genetic impoverishment and reduced gene flow may be problematic as
726 global warming and extreme climate events continue to push species past their physiological limits.
727 The prevalence of greater number of hot days (days over 35 °C) in non-edge populations, suggests that
728 the non-edge population may be at risk to habitat fragmentation. Greater tolerance to higher
729 temperatures as well as the significant genotype x environment interactions suggest that non-edge
730 populations may be locally adapted to local environmental conditions and have heritable genetic
731 variation in thermal tolerance traits. Further greater genetic diversity and gene flow suggests that the
732 non-edge population have greater connectivity and therefore available for genetic rescue from
733 surrounding populations.
734
735 Contrasting the relative magnitude of within-population variation to variation in thermal responses on
736 larger spatial scales, this study shows that the interaction between temperature and location comprised
737 an effect size of 15% of the total variation in growth, the interaction between temperature and heights
738 on the shore had an effect size of 3%, and the interaction between temperature and male-female
739 combination had an effect size of 2%. This within-population variation in thermal tolerance will be
740 particularly important under a changing climate as populations with greater diversity will have a
741 broader suite of tolerant genotypes for selection to act upon (Reusch, 2014; Wernberg et al., 2018).
742 The results of this study and previous research on genetic diversity of *H. banksii* across its species
743 distribution (Miller et al 2019) has helped improve predictions of how this species will respond to
744 ongoing warming and identified potentially sensitive populations. Conservation efforts such as
745 transplanting tolerant individuals or reseeding to increase genetic diversity in genetically impoverished
746 populations may aid in providing greater functional resilience to warming climates (Campbell et al.,
747 2014; Wood et al., 2019; Fredriksen et al., 2020).

748

Data availability

Data is available upon request

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

JC conceived the idea for the manuscript, collected the data, curated and formally analysed the data and wrote the original manuscript. MD, AP, MC supervised the project, reviewed, wrote and edited the manuscript, MD collected some of the data and provided resources for the project.

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Figure legends

Figure 1: Map of locations from which *H. banksii* populations were collected from southeastern coast of Australia. Sea surface Temperature (SST) data reflect average SST taken from 2003 - 2015 from the Integrated Marine Observing System (IMOS) and used to illustrate coastal temperatures.

Figure 2: Mean (± SD) germling length after 5 days at 6 different temperatures (22, 24, 26, 28, 30 and 32 ° C) from the warm-edge and non-edge populations. Data from all crosses are pooled. (n = 54).

Figure 3: Percentage of *H. banksii* germlings reaching stage 1, 2, 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. Germlings are from warm-edge (A, C) and non-edge (B, D) populations. White columns represent ontogenic stage 0 (fertilisation through condensation of the chloroplasts); dark grey is stage 1 (protrusion of germling cell wall to create a pear shape which later develops into the rhizoid); medium grey is stage 2 (division of the germling germinating cell and elongation of a single rhizoid); light grey is stage 3 (elongation of the rhizoid coupled with secondary and tertiary rhizoid development); and black is stage 4 = paraphysis development on top of the germinating cell). Data represent pooled crosses (n= 40) amongst high shore (HS; A, B). low shore (LS; C, D).

Figure 4: Mean proportion of light energy dissipated by *H. banksii* germlings amongst three complementary PSII pathways: photochemistry (white bars, Y(II); unregulated nonphotochemical quenching (grey bars, Y(NO) or regulated nonphotochemical quenching (black bars, Y(NPQ)) and potential photodamage, in germlings from warm-edge (A, C, E, G) and non-edge (B, D, F, H) populations from high on the shore (A, B, E, F) and low on the shore (C, D, G, H) after incubation for 120 h at three temperatures: 24, 28 and 32 °C. Photophysiological measurements were made under two irradiances - low light: 32 µmol photons m^{-2} s^{-1} (A-D) and high light 113µmol photons m^{-2} s^{-1} (E-H).

Figure 5: Conceptual model of summarized data indicating how physiological traits differed amongst high and low on the shore as well as between warm-edge and non-edge locations. Thermal breadth was determined through arbitrarily setting a threshold of 80% of maximum germling length. Optimal temperature for growth was determined through pairwise comparisons. Ontogenic staged reached was the furthest developmental stage reached within 120 h. Photosynthetic efficiency stated are the overall changes in the proportion of energy dissipated in to unregulated nonphotochemical quenching (Y(NO) or regulated nonphotochemical quenching (Y(NPQ)) at both light intensities. Temperature data (air and SST) are presented for each location only.

Table 1: The 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 *Hormosira banksii* for each height on the shore within each of the four locations.

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 2: Pairwise F_{ST} estimates between all pairs of heights on the shore (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 non-edge and warm-edge regions.

		Warm-edge				Non-edge			
		ANGL	ANGH	MWL	MWH	PBL	PBH	BBL	BBH
Warm-edge	ANGL								
	ANGH	0.002							
	MWL	**0.066**	**0.105**						
	MWH	0.032	**0.075**	0.000					
Non-edge	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	

Table 3: Results of analysis of variance of *Hormosira banksii* germling length 5 days (120 h) post fertilisation. Germlings from non-edge and warm-edge populations at low and high shore heights in 9 different genotypes were grown at 6 temperatures (22, 24, 26, 28, 30 and 32 ºC). Location, height on the shore, and temperature are fixed factors, with male and female 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	**Germling Length**		
	df	*F*	*P*
Location	1	0.556	0.749
Height	1	25.701	**0.001**
Temperature	5	159.160	**0.001**
Location x Height	1	3.871	**0.029**
Location x Temperature	5	22.746	**0.001**
Height x Temperature	5	5.501	**0.001**
Male (Location x Height)	8	6.524	**0.004**
Female (Location x Height)	8	1.840	0.163
Location x Height x Temperature	5	1.976	**0.024**
Temperature x Male (Location x Height)	40	1.680	**0.027**
Temperature x Female (Location x Height)	40	1.168	0.281
Male (Location x Height) x Female (Location x Height)	16	3.148	**0.001**
Temperature x Male (Location x Height) x Female (Location x Height)	76	2.927	**0.001**
Residuals	1080		

Bold denotes significance at P < 0.05

Table 4: Results of analysis of variance of the percent of *Hormosira banksii* germlings in each developmental stages, (stage 0-4) at 120 h after fertilisation. Germlings from non-edge and warm-edge populations, and 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, height on the shore, and temperature are fixed factors, with male and female 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.

	Ontogenic Development									
ANOVA Source	Stage 0			Stage 1		Stage 2		Stage 3		Stage 4	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Location	1	0.712	0.596	3.838	**0.026**	1.754	0.178	8.523	**0.003**	8.079	**0.002**
Height	1	0.320	0.950	0.321	0.944	0.338	0.912	0.286	0.971	0.382	0.895
Temperature	5	10.595	**0.001**	10.354	**0.001**	22.597	**0.001**	10.430	**0.001**	9.302	**0.001**
Location x Height	1	0.502	0.784	3.528	**0.031**	3.276	**0.046**	0.584	0.714	0.605	0.73
Location x Temperature	5	2.232	**0.009**	1.222	0.226	0.829	0.653	4.438	**0.001**	4.055	**0.001**
Height x Temperature	5	1.091	0.341	1.336	0.189	0.484	0.986	1.064	0.388	1.110	0.338
Male (Location x Height)	8	2.803	**0.042**	1.638	0.192	0.667	0.730	3.932	**0.012**	4.512	**0.004**
Female (Location x Height)	8	2.301	0.060	2.395	0.055	2.709	**0.048**	1.470	0.239	1.537	0.218
Location x Height x Temperature	5	0.766	0.720	0.500	0.974	1.475	0.102	1.003	0.475	1.117	0.337
Temperature x Male (Location x Height)	40	0.850	0.718	0.977	0.537	1.567	**0.050**	1.799	**0.010**	1.952	**0.006**
Temperature x Female (Location x Height)	40	1.836	**0.013**	1.326	0.127	1.394	0.104	1.072	0.385	1.284	0.162
Male (Location x Height) x Female (Location x Height)	16	1.382	0.148	1.001	0.452	1.791	**0.042**	1.105	0.358	1.019	0.439
Residuals	80										

Bold denotes significance P < 0.05

Table 5: Results of analysis of variance of the temperature effects on chlorophyll-a fluorescence F, maximum quantum yield (F_V/F_M), complementary photosynthetic pathways of photosynthesis Y(II), nonregulated nonphotochemical quenching Y(NO), regulated nonphotochemical quenching Y(NPQ) and high light (HL) to low light (LL) ratio of Y(NPQ). Location, height on the shore and temperature 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*
Location	1	12.17	**0.003**	4.39	**0.050**	0.09	0.790
Height	1	4.83	**0.038**	1.91	0.178	0.74	0.430
Temperature	2	1.61	0.222	5.40	**0.011**	9.35	**< 0.001**
Location x Height	1	0.69	0.417	0.09	0.771	0.84	0.397
Location x Temperature	2	0.82	0.446	0.89	0.433	2.18	0.123
Height x Temperature	2	0.06	0.938	0.02	0.981	0.46	0.671
Location x Height x Temperature	2	0.10	0.902	0.86	0.4373	0.18	0.872
Residuals	23						
Source	**LY(II)**			**LY(NO)**		**LY(NPQ)**	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Location	1	0.46	0.500	3.20	0.091	5.44	**0.028**
Height	1	0.56	0.468	0.20	0.666	0.01	0.922
Temperature	2	8.76	**0.001**	6.50	**0.006**	0.89	0.433
Location x Height	1	1.37	0.255	0.73	0.405	0.01	0.927
Location x Temperature	2	0.62	0.562	0.40	0.681	0.09	0.918
Height x Temperature	2	0.26	0.783	0.05	0.947	0.04	0.964
Location x Height x Temperature	2	0.55	0.578	0.10	0.913	0.29	0.755
Residuals	23						
Source	**HY(II)**			**HY(NO)**		**HY(NPQ)**	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Location	1	0.52	0.477	6.66	**0.016**	8.03	**0.009**
Height	1	1.47	0.252	0.001	0.982	0.58	0.444
Temperature	2	25.29	**< 0.001**	1.70	0.207	3.68	**0.044**
Location x Height	1	1.81	0.190	2.68	0.112	0.54	0.467
Location x Temperature	2	0.44	0.659	0.24	0.783	0.28	0.757
Height x Temperature	2	1.18	0.327	0.19	0.828	0.74	0.482
Location x Height x Temperature	2	1.11	0.346	0.21	0.809	0.19	0.831
Residuals	23						

Bold denotes significance at P < 0.05

Figure 1.JPEG

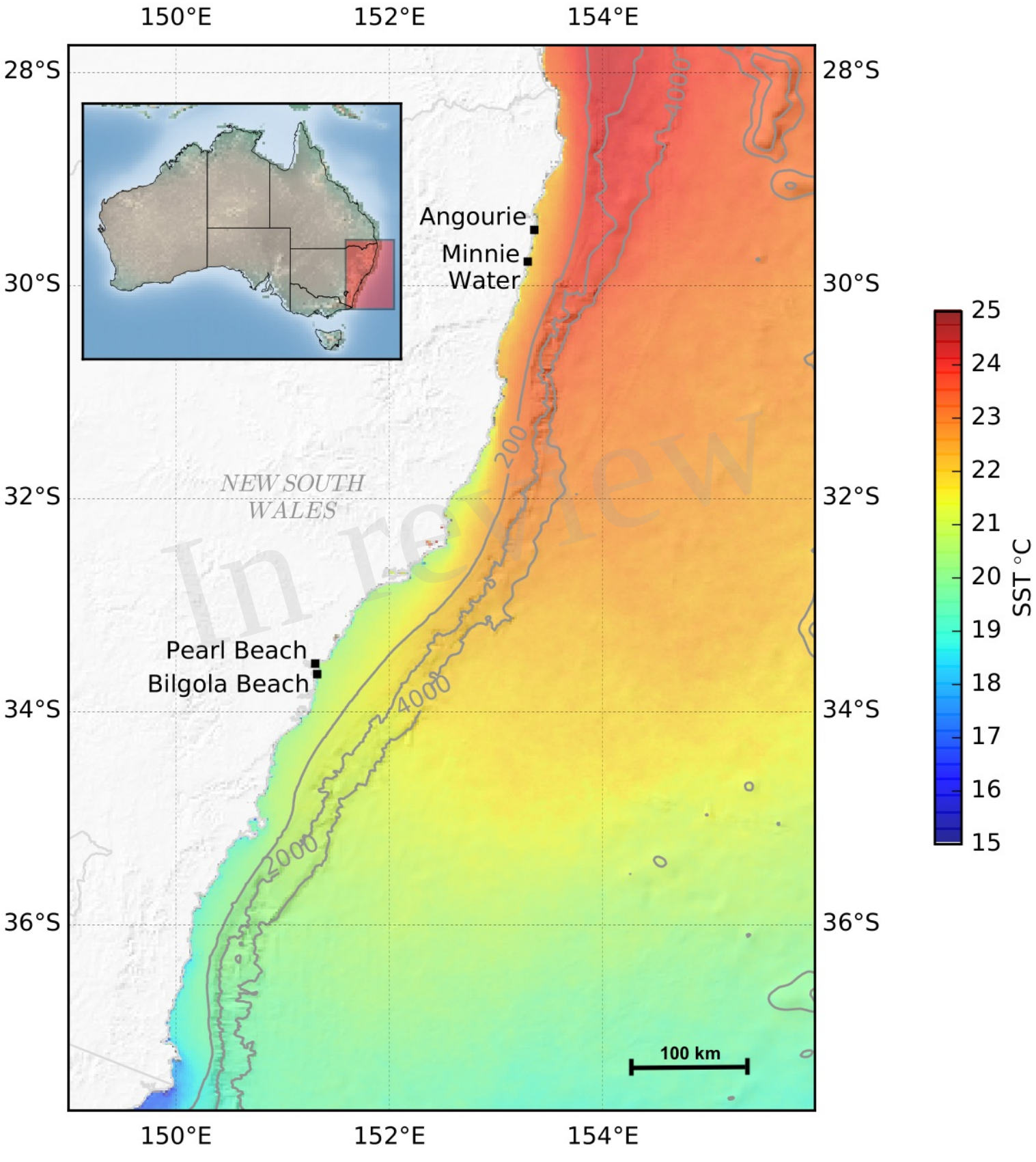
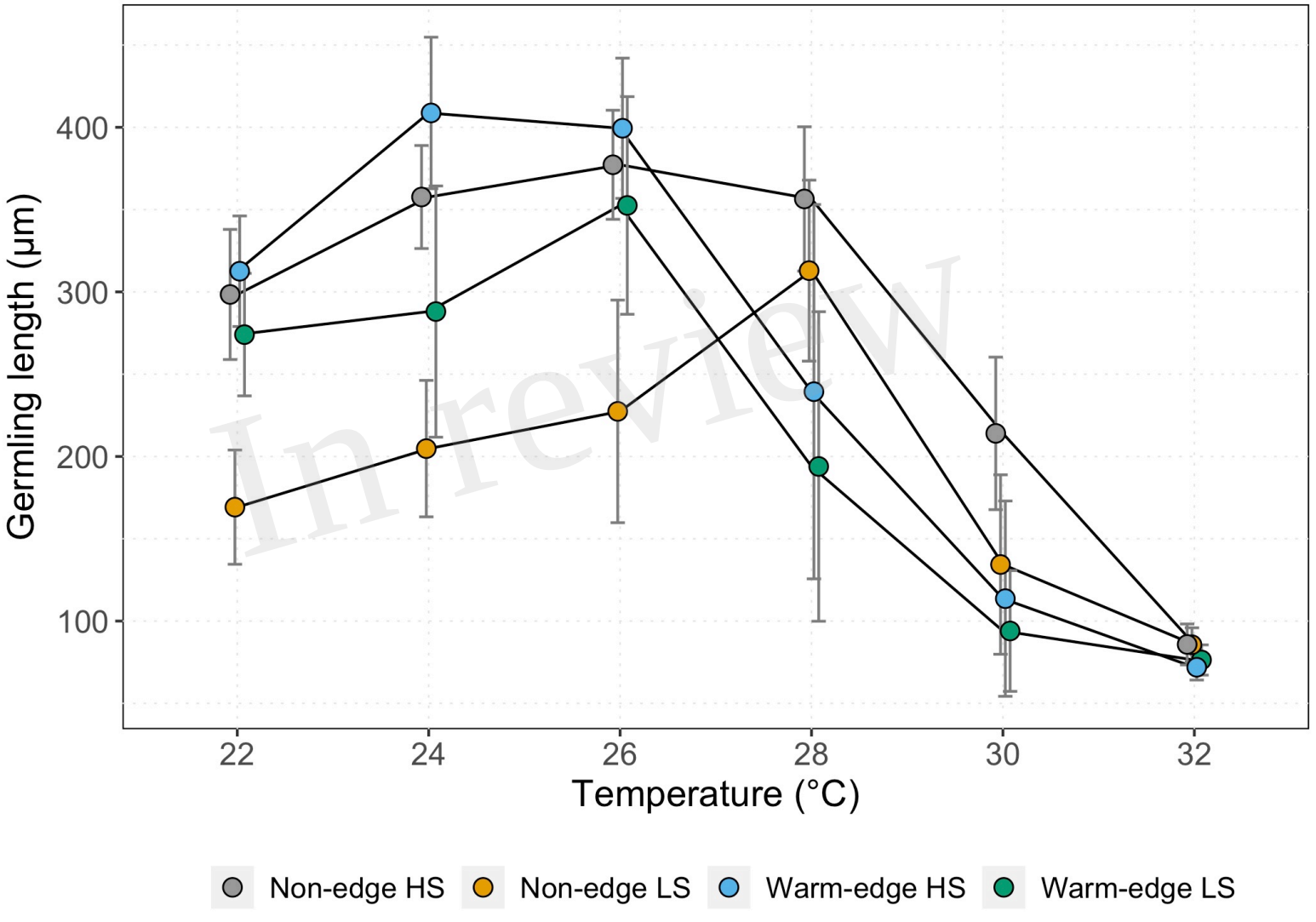


Figure 2.JPEG



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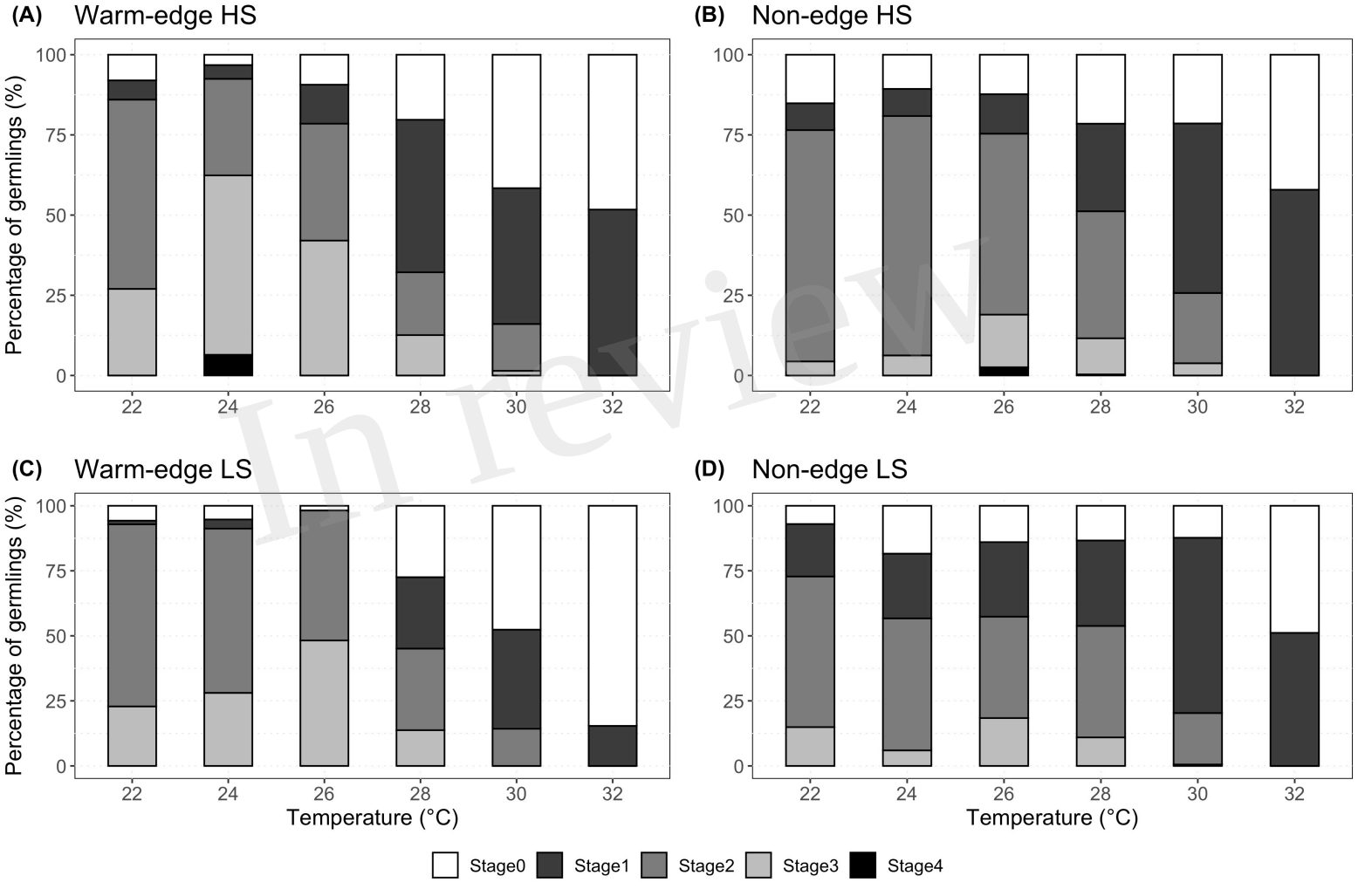

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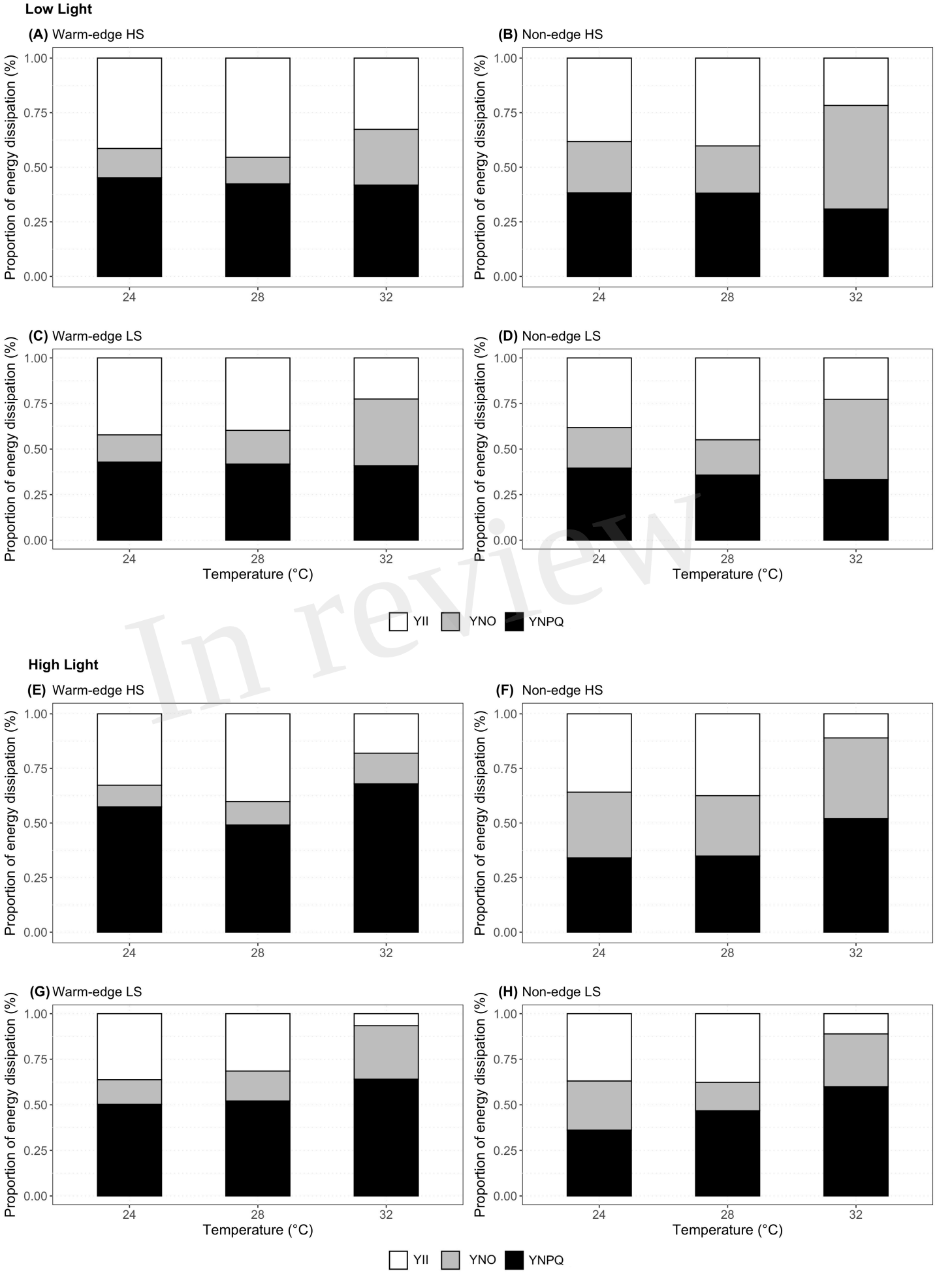

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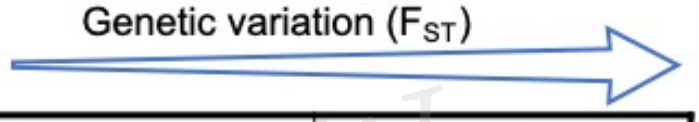

		Warm-edge	Non-edge
High	Thermal breadth (°C)	Narrow (24–26)	Wide (22–28)
	Optimal temperature for growth (°C)	24–26	24–28
	Ontogenic stage reached	Stage 4 (paraphysis)	Stage 3 (>2 rhizoids)
	Photosynthetic efficiency	↑ YNPQ ↓ YNO	↓YNPQ ↑ YNO
Low	Thermal breadth (°C)	Narrow 24–26	Wide 24–28
	Optimal temperature for growth (°C)	24–26	24–28
	Ontogenic stage reached	Stage 4 (paraphysis)	Stage 3 (>2 rhizoids)
	Photosynthetic efficiency	↑ YNPQ ↓ YNO	↓YNPQ ↑ YNO
(°C)	Air temperature	9.7–26.7	4.8–27.6
	SST	19.4–26.7	16.3–24.4