POTENTIAL FOR ADAPTATION IN RESPONSE TO THERMAL STRESS IN AN INTERTIDAL MACROALGA

Jennifer S. Clark

Plant Functional Biology and Climate Change Cluster, University of Technology, Sydney, PO Box 123 Broadway, New South Wales 2007, Australia

Alistair G. B. Poore

Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, New South Wales 2052, Australia

Peter J. Ralph and Martina A. Doblin[1]

Plant Functional Biology and Climate Change Cluster, University of Technology, Sydney, PO Box 123 Broadway, New South Wales 2007, Australia

[1]Author for correspondence: email Martina.Doblin@uts.edu.au, phone: +61 2 9514 8307, fax: +61 2 9514 4079

Running head: Potential for adaptation to thermal stress

ABSTRACT

Understanding responses of marine algae to changing ocean temperatures requires knowledge of the impacts of elevated temperatures and the likelihood of adaptation to thermal stress. The potential for rapid evolution of thermal tolerance is dependent on the levels of heritable genetic variation in response to thermal stress within a population. Here we use a quantitative genetic breeding design to establish whether there is heritable variation in thermal sensitivity in two populations of a habitat-forming intertidal macroalga, *Hormosira banksii* (Turner) Descaisne. Gametes from multiple parents were mixed and growth and photosynthetic performance measured in the resulting embryos incubated under control and elevated temperature (20 and 28 °C). Embryo growth was reduced at 28 °C, but significant interactions between male genotype and temperature in one population indicated the presence of genetic variation in thermal sensitivity. Selection for more tolerant genotypes thus has the ability to result in the evolution of increased thermal tolerance. Furthermore, genetic correlations between embryos grown in the two temperatures were positive, indicating that those genotypes that performed well in elevated temperature also performed well in control temperature. Chlorophyll-a fluorescence measurements showed a marked decrease in maximum quantum yield of photosystem II (PSII) under elevated temperature. There was an increase in the proportion of energy directed to photoinhibition (non-regulated non photochemical quenching – Y(NO)) and a concomitant decrease in energy used to drive photochemistry (Y(II)) and xanthophyll cycling (regulated non photochemical quenching – Y(NPQ)). However, PSII performance between genotypes was similar, suggesting that thermal sensitivity is related to processes other than photosynthesis.

Keywords: macroalgae, thermal tolerance, *Hormosira banksii*, quantitative genetics, genotype x environment interactions, adaptation, photosynthesis

Abbreviations:

PSII - photosystem II; Y(PSII) - photochemistry; Y(NO) – non-regulated non-photochemical quenching; Y(NPQ) – regulated non-photochemical quenching.

INTRODUCTION

Intertidal macroalgae live at the interface of marine and terrestrial habitats and are subjected to environmental challenges posed by both aquatic and atmospheric climatic regimes (Helmuth et al. 2006). They experience large temporal changes in temperature, irradiance and nutrient availability, and are faced with frequent desiccation and osmotic stresses (Li and Brawley 2004). By virtue of their habitat, these algae are exposed to significant climatic and anthropogenic stresses, and are being increasingly used as an early warning system for climate change impacts (Harley et al. 2006; Lima et al. 2007). With relatively short life-spans, they may respond more rapidly to climatic change than terrestrial plants (Southward et al. 2004). In addition, many intertidal species live close to their thermal tolerances (Helmuth et al. 2002; Williams et al. 2008; Somero, 2010) and show reductions in fitness to sub-lethal temperatures, making them sensitive to even slight changes in climate (Davison and Pearson1996).

While intertidal macroalgae are threatened by a wide range of anthropogenic stressors including eutrophication and habitat fragmentation (Worm and Lotze 2006, Coleman and Kelaher 2009,Coleman et al. 2011), increasing and potentially more variable ocean temperatures are likely to be of fundamental importance to marine primary producers such as macroalgae (Harley et al. 2012). Temperature is a major factor controlling the rate of

photosynthesis in all plants and algae (Davison et al.1991) and the growth, photosynthesis and reproduction of intertidal macroalgae are frequently limited by elevated air and water temperatures.

The effects of increased ocean temperatures on algae depend not only on measuring the impact of elevated temperature on algal fitness, but also the likelihood of adaptation in response to thermal stress. The responses to thermal stress can involve localised extinction, shifts in distribution, adaptation via phenotypic plasticity (i.e., acclimatization) or evolutionary change (Bijlsma and Loeschcke 2005, Gienapp et al. 2008, Hoffmann and Sgrò 2011). Acclimatization may enable stressed populations to persist in the short term, but over longer time scales, evolution favouring more tolerant genotypes will likely be required to enable population persistence. The likelihood of evolutionary adaptation to thermal stress can be assessed by measuring changes in tolerance through time, or differences among populations that vary in climatic regimes, each of which provide evidence that past selection has resulted in evolutionary responses. Alternatively, the potential for adaptation within populations can be assessed by artificial selection experiments, or quantitative genetic experiments that provide estimates of heritable variation in stress tolerance (Hoffmann and Sgrò 2011). These approaches have provided evidence of rapid evolution in response to thermal stress in terrestrial environments, but very few data exist that address the potential of marine organisms to genetically adapt to climate-driven change in ocean conditions (Császár et al. 2010, Pandolfi et al. 2011, Pistevos et al. 2011, Sunday et al. 2011, Foo et al, 2012).

Given the need to assess potential adaptation to increased thermal stress in marine organisms, we use a quantitative genetic breeding design to determine whether there is genetic variation in response to a thermal stress in an ecologically important intertidal alga, *Hormosira banksii* (Turner) Descaisne (Phaeophyta: Fucales). By rearing known genotypes in control and stressful conditions, we test for genotype by environment (G x E) interactions,

which indicate the presence of heritable (additive genetic) variation in thermal sensitivity (Lynch and Walsh 1998). Previous laboratory experiments with embryos of fucoid macroalgae have demonstrated that increased thermal tolerance can arise when parents have been previously exposed to high temperatures (Li and Brawley 2004), a maternal influence on offspring performance that could be due to female genotype or non-genetic maternal effects (e.g., egg provisioning). We are not aware of any previous studies with macroalgae that have the quantitative genetic designs required to partition variation in thermal sensitivity among paternal, maternal effects and environmental effects. More generally, we are aware of only two studies that have used quantitative genetic designs to test for heritable variation in thermal sensitivity in marine primary producers (e.g. among clonal lineages of coral symbionts, Császár et al. 2010; among clones of the seagrass *Zostera marina*, Ehlers et al. 2008).

Hormosira banksii is an abundant, habitat-forming brown alga occurring on intertidal rocky shores in southern Australia and New Zealand (Lindauer 1947; Clarke and Womersley 1981; Ralph et al. 1998; Macinnis-Ng et al. 2006, Harper et al. 2012). Using a North Carolina II breeding design (Lynch and Walsh 1998), we test for heritable genetic variation in responses to elevated temperature in the early life history stages of *H. banksii*. We measured embryo performance under control and elevated temperatures by estimating their size after five days, and by chlorophyll-*a* fluorescence parameters that measure photosynthetic performance. We calculated genetic correlations between embryo growth at control and elevated temperatures to test whether increased tolerance to stressful conditions is associated with reduced performance in ambient conditions. Selection by stressful conditions will only result in adaptation if tolerance is both heritable and unconstrained by negative genetic correlations with other fitness traits (Blows and Hoffmann 2005).

MATERIALS AND METHODS

Study organism and sample collection. Adult *H. banksii* were collected during June to September 2010 (austral winter-spring) from two wave-exposed, rocky intertidal sites: Bilgola Beach (33º38'54.57"S, 151º19'39.65"E) and Pearl Beach (33º32'47.09"S, 151º18'36.10"E), north of Sydney, Australia. Fronds (spaced at least 5 m apart) were haphazardly collected from the mid-intertidal area while still submerged, placed in plastic bags on ice, transported back to the laboratory and kept overnight at 4 ºC before use in experiments. Extraction of gametes involved washing the fronds briefly in freshwater and drying them under room temperature for 10 min. Individual male and female fronds were placed into separate beakers containing 0.7 µm filtered and aerated seawater from the collection site, and gametes released by gentle swirling (Doblin and Clayton 1995). Sperm and egg solutions were combined in a ratio of approximately 50:1 to prevent polyspermy (Brawley 1992). The mean (± SE) diameter of the collected eggs from Bilgola Beach females was 56.0 ± 1.95 µm, slightly larger than those collected from Pearl Beach females (54.5 ± 1.04 µm).

Effects of elevated temperature on embryo growth. The performance of *H. banksii* embryos among genotypes and temperatures was quantified using a North Carolina II breeding design (Lynch and Walsh 1998). In each of three experimental blocks conducted on separate days for each population (with different individuals used for each block), the sperm from three males (M1, M2, M3) was crossed with the eggs of three females (F1, F2, F3), such that there were nine parent combinations (M1F1, M1F2, M1F3, M2F1 etc) and the resultant embryos allocated to each of two temperatures. Sperm and egg solutions were added to petri dishes partly filled with 0.7 µm filtered seawater and lined with glass coverslips. Petri dishes were left for one hour to allow the negatively phototactic zygotes to settle to the bottom and

adhere to coverslips, before transferring the dishes (n = 3 per cross per treatment) to incubators at 20 ºC (control temperature) and an elevated, sub-lethal temperature of 28 ºC. The size of eggs (diameter) collected from Pearl Beach did not differ among female parents ($F_{2, 36} = 0.67$, $P = 0.52$), but did vary among female parents collected from Bilgola Beach ($F_{2, 36} = 11.53$, $P < 0.001$).

Embryos were grown under ~30 µmol photons·m^{-2}·s^{-1} light intensity as in other *H. banksii* studies (Kevekordes and Clayton 1996; Bellgrove et al. 1997; McKenzie and Bellgrove 2006) in a 12:12 hour light and dark cycle. Seawater in each petri dish was exchanged with fresh filtered seawater after 72 h, and their position was randomized in the incubator every second day.

The growth of embryos was determined by measuring the developing (pear-shaped) embryos using a compound microscope (Olympus BX50, Center Valley, Pennsylvania, USA) equipped with a digital camera (Leica DP320, Solms, Germany). Images of developing embryos from each cross (n = 6) were taken 24, 72 and 120 hours after fertilisation and measured using calibrated Image software (Leica IM500).

Effects of elevated temperature on embryo photosynthesis. The capacity to perform photochemistry is defined by the number of functional Photosystem II (PSII) reaction centres and their efficiency of energy transfer. Solar energy that reaches PSII has one of three fates: photochemistry, regulated non-photochemical quenching (related to photoprotection), and non-regulated non-photochemical quenching (often related to photoinhibition and damage). Information about all three energy pathways can be gathered from patterns of chlorophyll fluorescence (for details, see Kramer et al. 2004, Schreiber 2004).

When assessing photochemistry, the measurable parameters of interest include maximum fluorescence (F_M = fluorescence when PSII reaction centers are fully reduced), variable fluorescence (F_V, which is the difference between F_M and the minimum fluorescence

measured in the dark (F_O)), and maximum quantum yield of PSII (F_V/F_M), which is a measure of photosynthetic efficiency. Under illumination, the measure of photosynthetic health is called the effective quantum yield of PSII, ($\Delta F/F_M$'), which is calculated using (F_M'-F)/F_M', where F is the minimum fluorescence in the light and $F_{M'}$ is the light-adapted maximum fluorescence. Effective quantum yield is always less than maximum quantum yield as some of the energy is being dissipated as heat to protect the photosynthetic apparatus.

Regulated non-photochemical quenching of light energy (Y(NPQ)), is a proxy of xanthophyll pigment cycling, which responds to protect photosystems from over-excitation by dissipating the excess irradiance as heat. Non-regulated non-photochemical quenching (Y(NO)) is also an energy dissipation pathway, so any changes to PSII antenna complex or PSII damage or thylakoid impacts will increase Y(NO). Importantly, the total energy is conserved whereby Y(PSII) + Y(NPQ) + Y(NO) = 1. If Y(NPQ) increases relative to Y(NO) and Y(PSII) then photoprotection is occurring, however if Y(NO) increases at the cost of Y(PSII) and Y(NPQ), this suggests photoinhibition or irreversible damage to the photosynthetic apparatus (Klughammer and Schreiber 2008).

Thermal stress affects numerous components of the photosynthetic apparatus, but PSII is one of the most temperature sensitive (Yamamoto et al. 2008). Elevated temperature leads to over-excitation of PSII, which results in higher levels of photoinhibition and a greater potential for photodamage (Takahashi and Murata 2008). In this study, it was therefore expected that embryos would have lower maximum quantum yield (F_V/F_M) if they were not thermally adapted. Embryos were also expected to show greater Y(NPQ) and Y(NO) at the expense of photochemistry (Y(PSII)) under elevated temperature, as a means of dissipating excess energy.

To optimise fertilisation success, photosynthetic health of male and female gametes was checked using a Water-Pulse Amplitude Modulated (PAM) fluorometer (Walz GmbH,

Effeltrich, Germany) before gametes were mixed. Developing embryos adhered to coverslips were dark-adapted for 5 min before their F_O was recorded with a Microscope-PAM fluorometer (Walz GmbH, Effeltrich, Germany). Upon application of a saturating pulse of light (pulse duration = 0.6 s) maximum fluorescence (F_M) was determined. To test the capacity of developing embryos to function under light, they were then illuminated for 2 min at 182 µmol photons m^{-2} s^{-1}, before recording their minimum fluorescence (F) and exposing them to a saturating pulse of light to determine maximum fluorescence (F_M'). Maximum quantum yield (F_V/F_M) was measured after 24, 72 and 120 hours and effective quantum yield Y(PSII), Y(NPQ), and Y(NO) were measured after 24 and 120 hours. The chlorophyll-a fluorescence measurements in the light were only completed for the Pearl Beach population due to time restrictions within the season of our study.

The test temperatures were chosen following a pilot study that revealed maximum quantum yield of PSII was reduced by up to 53% when embryos were grown at 28 ºC compared to 20 ºC and that 30 ºC was lethal. Additionally, temperature regularly exceeds 28 ºC in the study region between September and January (Australian Bureau of Meteorology website, accessed 6 August 2012) and we have recorded temperatures > 28 ºC amongst *H. banskii* at Bilgola in June (J. Clark, unpublished data).

Statistical analyses. For each population, growth rate and photosynthetic parameters were contrasted among males, females and temperatures using analysis of variance with temperature as a fixed factor, experimental block as a random factor, and male and females as random factors nested within blocks. Separate analyses were conducted for growth and photosynthetic parameters at each time point. Data were log transformed after the assumptions of normality and homogeneity of variances were tested by frequency histograms or residuals and scatterplots of residuals versus means respectively. The interactions between male genotype and temperature were visualized using reaction norms of mean performance

per male across temperatures (i.e., the slope reflects thermal sensitivity of a genotype). Analyses of variance were conducted in the PERMANOVA routine in Primer V.6 (Plymouth, UK).

Genetic correlations of embryo size across temperatures were used to quantify the G x E interactions using variance components derived from restricted error maximum likelihood (REML) estimates calculated in SYSTAT V. 10 (Chicago, Illinois, USA). Genetic correlations, r_g, were calculated using the causal variance components associated with the sire effects (Vs) and the interaction effect between sires and temperature (Vs_{*T}) using the formulae in Astles et al. (2006) where $r_g = Vs/(Vs + Vs_{*T})$.

RESULTS

Effects of elevated temperature on embryo growth. Elevated temperatures affected both the growth rates and morphology of developing embryos of *H. banksii* (Figs. 1, 2). Fertilized eggs at 20 °C formed the primary rhizoid (making the zygote pear-shaped) within the first 24 to 48 hours. Over the next 48 hours, the embryo divided further, with growth directed towards elongation of the first rhizoid and formation of secondary rhizoids. At 120 hours, embryos were approximately 300–400 µm long (Fig. 1). In contrast, thermally stressed embryos grown at 28 °C had stunted growth, with embryos becoming highly deformed and producing irregularly shaped secondary rhizoids. After 120 h, embryos in sub-lethal temperatures were only 100 – 250 µm long. The mean (± SE) growth rate of Bilgola Beach *H. banksii* embryos was 49.2 ± 0.97 µm d^{-1} and 16.6 ± 1.23 µm d^{-1} for embryos grown at 20 and 28 ºC, respectively. Embryos from Pearl Beach grew faster, averaging 69.1 ± 1.08 µm d^{-1} and 31.9 ± 3.51µm d^{-1} at 20 ºC and 28 ºC, respectively.

There was a significant interaction between male parent and temperature, indicative of genetic variation in thermal sensitivity, in the embryos from Pearl Beach at 72 and 120 hours (Fig 2c,e, Table 1), and for the embryos from Bilgola at 72 h (Fig 2d, Table 1). Female identity interacted with temperature only for Pearl Beach embryos at the 72 hour stage, and for Bilgola embryos at 24 h. Male and female identity interacted strongly to determine embryo length in both populations (Table 1). Temperature also interacted with male and female identity (Table 1) indicating that the magnitude of the temperature x male interaction varies amongst females. Together, these data show that the impacts of elevated temperature on growth are highly variable within a population of embryos.

There were positive genetic correlations between the length of embryos at 20 ºC and 28 ºC for Pearl Beach (24 h, r_g, = 0.99, 72 h, r_g = 0.24; 120 h, r_g = 0.23) and Bilgola (24 h, r_g = 0.99; 72 h, r_g = 0.99; 120 h, r_g = 0.32), indicating that the individual genotypes that performed well in ambient temperatures also performed well at the elevated temperatures and vice versa.

Effects of elevated temperature on embryo photosynthesis. The maximum quantum yield of PSII (F_V/F_M) was lower in embryos reared at 28 °C compared to 20 °C at some times in the experiments (Fig. 3, Pearl Beach, 72 h, $F_{1,2}$ = 41.4, P = 0.02; Bilgola Beach, 24 h, $F_{1,2}$ = 18.9, P = 0.049), but did not differ significantly at all remaining times (full ANOVA tables in Table S1). In addition, embryos with different male and female parentage showed inconsistent differences in F_V/F_M amongst temperatures (i.e., significant temperate x male x female interaction).

Pearl Beach embryos incubated at 20 ºC initially showed variable energy distribution amongst the complementary energy dissipation pathways: photochemistry Y(PSII), non-regulated non-photochemical quenching Y(NO) and regulated non-photochemical quenching Y(NPQ). Energy allocation was also variable in embryos at 28 ºC after the first 24 h.

However, by 120 hours it was evident that at elevated temperature, Pearl Beach embryos demonstrated greater photoinhibition (non-regulated non photochemical quenching) than their 20 ºC counterparts (Fig. 4). Thus, after 5 days, temperature increased non-regulated non-photochemical quenching (Y(NO)) ($P < 0.01$; Table S1), indicating embryos had damaged photosystems and less capacity for photochemistry.

Effective quantum yield of PSII (photosynthetic efficiency in the light) was, on average, unaffected by temperature, but varied among male genotypes in the Pearl Beach population (Fig. 4, Table S2). Non-regulated non-photochemical quenching (Y(NO)) in embryos from Pearl Beach was increased in the elevated temperatures at 120 hours, but did not vary significantly among male genotypes (Table S2). There were no significant interactions between male genotype and temperature for either effective quantum yield or non-regulated non-photochemical quenching (Table S2).

DISCUSSION

Elevated temperatures reduced the growth of embryos of an ecologically important intertidal macroalga, but the effects of stressful temperatures were dependent on algal genotype and variable within and among populations. The presence of significant genotype x environment interactions for embryo growth highlights the potential for selection to result in the evolution of more thermally tolerant genotypes, and possible persistence of future populations in warming climates (IPCC 2007). Although no genotype x environment interactions were found for photosynthetic parameters, adjustment of photosystems was evident through increases in the proportion of non-regulated non-photochemical quenching (Y(NO)) suggesting that populations may be responding to their local environmental conditions.

Our experiments demonstrated that elevated temperature had serious consequences for the likely success of *H. banksii* embryos, with a sub-lethal temperature of 28 °C resulting in a 2–3 fold decrease in growth, and approximately 20% reduction in photosynthetic efficiency during the first five days post-fertilisation. The reduction in effective quantum yield Y(PSII) indicates down-regulation of photosynthesis as means to protect the macroalga from high temperature, also observed in coral symbionts by Czászár *et al.* (2010). In the 28 ºC treatment, the majority of the light energy captured was being directed towards photoinhibition (Y(NO)) instead of photochemistry or xanthophyll cycling (Y(NPQ)) (Fig. 4). Our findings of reduced growth rate at the cost of photosynthesis are consistent with previous research on temperature effects on macroalgae (e.g., Davison et al. 1991, Bell 1993, Kübler and Davison 1993, Major and Davison 1998, Keser et al. 2005).

Decreased growth rates under elevated temperature in combination with changes in ontogeny (Fig. 1), could lead to lower rates of recruitment due to less effective physical attachment to the rock substrate and hence greater risk of dislodgment (Dring 1982), or reduced ability to outgrow other recruits that may be competing for space (Doblin and Clayton 1995). Given the very low rates of survival of fucoid embryos to juvenile stages in the field (estimated by Dudgeon and Petraitis (2005) to be 0.3-3.8% over 400 days across various treatments), further reductions in the recruitment of early life history stages due to thermal stress may have consequences for population persistence. Algal embryos are already susceptible to desiccation and suffer large grazing losses from intertidal molluscs (Lubchenco 1982, Worm and Chapman, 1998, Brawley and Johnson 2004). However, if embryo performance declines strongly with increasing density, as found in other fucoids (Vadas et al. 1992, Worm and Chapman 1998, Bellgrove et al. 2004), then reduced embryo densities at high temperatures may increase the success of individual embryos, with higher temperatures thus having less of an impact on the total number of embryos that survive to reach subsequent

life history stages. Further research is required to establish whether the observed effects of thermal stress on growth rates of individual embryos will affect the population size of this species.

Given the limited dispersal of fertilised eggs of *H. banskii* (up to 10 m; McKenzie and Bellgrove 2006), the persistence of populations under increased thermal stress will be partly dependent on the levels of heritable variation in response to thermal stress within a population (in addition to other factors including possible immigration of tolerant genotypes, rates of temperature change, population demography, and the likelihood of physiological acclimatization). We found significant interactions between male identity and temperature and this varied amongst females (temperature x male x female) for embryo length, indicating that some genotypes are susceptible to the elevated temperatures while others are relatively unaffected (Fig. 2). Selection by thermal stress thus has the potential to result in the evolution of populations with more tolerant genotypes. To our knowledge, this is the first study to use quantitative genetic breeding designs to detect variation in thermal sensitivity in macroalgae. The few studies that have used this approach to assess the potential adaptation of marine organisms to biotic or abiotic stressors have routinely found heritable, within-population, variation for traits that predict population persistence (e.g., photosynthesis, Császár *et al.* 2010; defensive secondary metabolites, Wright et al. 2004; tolerance to metal contamination, Galletely et al. 2007, Pease et al. 2010; larval development, Sunday et al. 2011, Foo et al. 2012; growth and reproductive investment, Pistevos et al. 2011). Predicting the long term impacts of environmental stressors is thus dependent on measuring both average effects of a given stress, and the variation in responses to that stress. Even with heritable variation in thermal sensitivity, adaptation may be constrained by negative genetic correlations (Blows and Hoffmann 2005) between performance at varying temperatures (i.e., trade-offs where high performing genotypes at one temperature do poorly at other temperatures), among

responses to different stressors (e.g., predation, pCO2, and temperature), or between traits associated with thermal sensitivity and those associated with other determinants of fitness (growth vs. reproduction vs. survival). We found no evidence for negative genetic correlations between performances at two temperatures, with the genotypes having the largest embryos at elevated temperature also having the largest embryos at 20 ºC. There is thus no evidence for a trade-off, in which genotypes that perform best at one temperature perform poorly at another temperature, and selection for growth at one temperature is unlikely to counter selection at another. Further research is required to examine possible genetic correlations between thermal responses and other traits that may constrain adaptation. Few studies consider multiple stressors for macroalgae and there are none to our knowledge that have the breeding designs required to quantify genetic correlations.

There was no significant variation among the progeny of male parents for F_V/F_M (Supplementary Table S1) or any of the other measured photosynthetic parameters (Supplementary Table S2), suggesting that photosynthesis is highly regulated in this alga. This contrasts with a previous study involving marine primary producers that established F_V/F_M, Y(PSII) and Y(NO), but not Y(NPQ), were highly heritable for the photosynthetic symbionts of the coral *Acropora millepora* (Császár et al. 2010). It is expected that intertidal macroalgae are routinely subjected to a wide temperature range which would favour high plasticity in physiological traits. Our findings support this, with evidence of high plasticity in thermal tolerance of both *H. banksii* populations in maximum quantum yield of PSII (F_V/F_M). In the absence of thermal acclimation, F_V/F_M would be lower in embryos exposed to elevated temperatures. However, we found that embryos grown in 20 ºC and 28 ºC achieved similar F_V/F_M (Fig. 3). Increasing non-photochemical quenching can be seen as plasticity of photosystems, as it dissipates energy to optimize photosynthesis at the cost of lowered effective quantum yield of PSII (Long et al. 1994). These data provide evidence of an

uncoupling of growth and photosynthesis during early embryonic stage that allows this macroalga to tolerate elevated temperatures (Major and Davison, 1993).

There were significant differences in thermal tolerance among the progeny of female parents (i.e., female x temperature, female x male, female x male x temperature interactions) at some times, indicating that differences in female genotype or non-genetic maternal effects can contribute to within population variation in growth rates. Maternal effects frequently influence the success of embryos of marine invertebrates, but have been rarely considered for marine algae (Marshall et al. 2008). Exposure of adult fronds to elevated temperature has been shown previously to contribute to thermal tolerance in embryos of fucoid macroalgae (Li and Brawley 2004), with the offspring of female parents held in warmer temperatures being more tolerant to high temperatures. The mechanism is currently not understood but may involve production of heat shock proteins (Li and Brawley, 2004). While larger eggs are often assumed to have higher growth rates, we found that the smaller eggs produced at Bilgola resulted in larger embryos than those collected from Pearl Beach. Given that fecundity of females may vary with egg size, further experiments are required to establish how variation in egg provisioning or fecundity contributes to variation in thermal tolerance.

While our experiments were not aimed to explain possible differences between our two study populations, our results indicate that the performance of *H. banksii* embryos will vary at large spatial scales, in addition to the documented within population variation. In addition to possible genetic differences between the populations, differences in thermal sensitivity could arise due to algal acclimatization to different local environmental conditions (e.g., rock platform elevation and habitat complexity) or the morphology of individuals which may influence thermal exposure. In the red alga *Mastocarpus papillatus* for example, the degree of thallus branching affects heat transfer and rates of dessication (Bell 1995). It is

possible that *H. banskii* vesicles of different shapes, wall thickness and moisture content would have differing thermal (and desiccation) properties, but this has yet to be tested.

The persistence of *H. banksii* populations under thermal stress is enhanced by the presence of tolerant genotypes (as shown by this study) and possible dispersal of those genotypes among populations. Fucoids have large eggs that are negatively buoyant and rapidly sink and adhere to the substratum (Forbes 1979). This results in high rates of fertilisation (Brawley 1992, Pearson and Brawley 1996) but limited dispersal (Bellgrove et al. 2004) which can limit gene flow and potentially result in inbred, fragmented populations. Although *H. banksii* is capable of dispersing genetic material via detached floating thalli, the likelihood of novel genetic material contributing to gene flow is not well understood. McKenzie and Bellgrove (2008) found that many detached fronds were washed up on sandy beaches, an unsuitable substratum for zygote attachment and successful recruitment, but were not seen on adjacent rocky shores. Furthermore, the likelihood of warm water genotypes being introduced into southerly populations also seems low, as the dominant poleward flowing oceanographic current is >15 km offshore and the prevailing nearshore currents flow in the opposite direction (Roughan et al. 2011). Empirical evidence shows a significant positive relationship between genetic differentiation and geographic distance in *H. banksii* along the east Australian coast, and migrants (50-75% of individuals) were more often sourced from adjacent shores as opposed to shores in more distant latitudes (Coleman et al. 2011). Despite the genetic distances among populations, "evolutionary rescue" of threatened populations by migration of more heat tolerant genotypes remains possible if source populations vary in thermal sensitivity.

With best estimates of global mean sea surface temperatures projected to rise between 1.8 and 4.0 ºC by 2099 based on SRES emission scenario (B1 and A1FI, respectively), and predictions of more frequent extreme temperatures (IPCC 2007), an understanding of how

tolerance to thermal stress varies among and within populations is critical for predicting the effects of climate change. Changes to ocean temperatures have already resulted in shifts in algal species distributions (Lima et al. 2007), changes in ecological function (see Harley *et al.* 2006 for review) and potential local extinction of some species such as the subtidal kelp, *Macrocystis pyrifera* (Edgar et al.2005). Our finding of genetic variation in thermal tolerance of *H. banksii* embryos suggests resilience to thermal stresses, but the persistence of populations will also depend on tolerance to other environmental stressors, and anthropogenic disturbance (e.g., trampling by visitors to the rocky shore, Keough and Quinn 1998; sewage discharge, Doblin and Clayton 1995). *Hormosira banksii* has a broad geographic range (Macinnis-Ng *et al.* 2005) but potentially limited dispersal. If localised extinction occurs in a population that lacks genetic variation in tolerance to environmental stressors, then recolonisation of tolerant genotypes from distant populations may be unlikely. Given that *H. banksii* is the dominant habitat-forming macroalga on intertidal rocky shores in southern Australia and New Zealand, and facilitates a diverse community of associated invertebrates (Lilley and Schiel 2005), further understanding of genetic variation in stress tolerance, dispersal and genetic connectivity amongst populations is needed on multiple geographic scales.

Acknowledgments. We thank Charlotte Robinson for help in the field as well as technical assistance, the APG group for their feedback, knowledge and support, operational funds from School for the Environment, UTS and a Plant Functional Biology and Climate Change Cluster (C3) scholarship to JSC. Collection was permitted through the NSW government Industry and Investment (permit no. P10/0057-1.0). This is SIMS contribution number XXX.

445 REFERENCES

Astles, P.A., Moore, A.J. & Preziosi, R.F. 2006. A comparison of methods to estimate cross-environment genetic correlations. *J. Evol. Biol.*19:114-122.

450 Bell, E.C. 1993. Photosynthetic response to temperature and desiccation of the intertidal alga *Mastocarpus papillatus*. *Mar. Biol.* 117:337-346.

Bell, E.C. 1995. Environmental and morphological influences on thallus temperature and desiccation of the intertidal alga *Mastocarpus papillatus* Kützing. *J. Exp. Mar. Biol. Ecol.*.
455 191:29-55.

Bellgrove, A., Clayton, M.N. & Quinn, G.P. 1997. Effects of secondarily treated sewage effluent on the intertidal macroalgal recruitment processes. *Mar. Freshwater Res.* 48: 137-146.

460

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. *J. Exp. Mar. Biol. Ecol.* 310:207-225.

465

Bijlsma, R. & Loeschcke, V. 2005. Environmental stress, adaptation and evolution: an overview. *J. Evol. Biol.* 18:744-749.

Blows, M.W. & Hoffmann, A.A. 2005. A reassessment of genetic limits to evolutionary change. *Ecology* 86:1371-1384.

Brawley, S.H. 1992. Fertilization in natural populations of the dioecious brown alga *Fucus ceranoides L.* and the importance of the polyspermy block. *Mar. Biol*. 113:145-157.

Brawley, S.H. & Johnson, L. 2004. Survival of fucoid embryos in the intertidal zone depends upon developmental stage and microhabitat. *J. Phycol.* 27:179-186.

Coleman, M.A. & Kelaher, B.P. 2009. Connectivity among fragmented populations of habitat-forming alga, *Phyllospora comosa* (Phaeophyceae, Fucales) on an urbanised coast. *Mar. Ecol. Prog. Ser.* 381:63-70.

Coleman, M.A., Chamber, J., Knott, N.A., Malcolm, H.A., Harasti, D., Jordan, A. & Kelaher, B.P. 2011. Connectivity within and among a network of temperate marine reserves. *PLoS ONE.* 6: e20168.

Clarke, S.M. & Womersley, H.B.S. 1981. Cross-fertilization and hybrid development of forms of brown alga *Hormosira banksii* (Turner) Descaisne. *Aust. J. Bot.* 29:497-505.

Császár, N.B.M., Ralph, P.J., Frankham, R., Berkelmans, R. & van Oppen, M.J.H. 2010. Estimating the potential for adaptation of corals to climate warming. *Plos One.* 5:1-8.

Davison, I.R., Greene, R.M. & Podolak, E.J. 1991. Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina*. *Mar. Biol.* 110:449-454.

Davison, I.R. & Pearson, G.A. 1996. Stress tolerance in intertidal seaweeds. *J. Phycol.* 32:197-211.

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*. *Mar. Biol.* 122:689-698.

Dring, M.J. 1982. *The biology of marine plants*. Edward Arnold, London.

Dudgeon, S.R. & Petraitis, P.S. 2005. First year demography of the foundation species, *Ascophyllum nodosum*, and its community implications. *Oikos*. 109:405-415.

Edgar, G.J., Samson, C.R. & Barrett, N.S. 2005. Species extinction in the marine environment: Tasmania as a regional example of overlooked losses in biodiversity. *Conserv. Biol.* 1294-1300.

Ehlers, A., Worm, B. & Reusch, T.B.H. 2008. Importance of genetic diversity in eelgrass *Zostera marina* for its resilience to global warming. *Mar. Ecol. Prog. Ser.* 355:1-7.

Foo, S.A., Dworjanyn, S.A, Poore, A.G.B., & 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* 7: e42497.

Forbes, M.A. & Hallam, N.D. 1979. Embryogenesis and substratum adhesion in the brown

alga *Hormosira banksii* (Turner) Decaisne. *Br. Phycol. J.* 14:69-81.

Galletly, B.C., Blows, M.W. & Marshall, D.J. 2007. Genetic mechanisms of pollution resistance in a marine invertebrate. *Ecol. Appl.* 17:2290-2297.

Gienapp, P., Teplitsky, C., Alho, J.S., Mills, A. & Merila, A. 2008. Climate change and evolution: disentangling environmental and genetic responses. *Mol. Ecol.* 17:167-178.

Harley, C.D.G., Hughes, A.R., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F., Tomanek, L. & Williams, S.L. 2006. The impacts of climate change in coastal marine systems. *Ecol. Lett.* 9: 228-241.

Harley, C.D.G., Anderson, K.M., Demes, K.W., Jorve, J.P., Kordas, R.L. & Coyle, T.A. 2012. Effects of climate change on global seaweed communities. *J. Phycol*. 48:1064-1078.

Harper, M.A., Cassie Cooper, V., Chang, F.H., Nelson, W.A. & Broady, P.A. 2012. Phylum Ochrophyta: brown and golden-brown algae, diatoms, silicioflagellates, and kin. In: *New Zealand inventory of biodiversity. Volume Three. Kingdoms Bacteria, Protozoa, Chromista, Plantae, Fungi*. (Gordon, D.P. Eds), pp. 114-163. Christchurch: Canterbury University Press.

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* 298:1015-1017.

Helmuth, B., Mieszkowska, N., Moore, P. & Hawkins, S.J. 2006. Living on the edge of two

changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. *Annu. Rev. Ecol. Evol. Syst.* 37:373-404.

Hoffmann, A.A. & Sgrò, C.M. 2011. Climate change and evolutionary adaptation. *Nature* 470:479-485.

Intergovernmental Panel on Climate Change. 2007. Climate Change 2007: Synthesis Report. Contribution of workings Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. [Core Writing Team, Pachauri RB, Reisinger A (eds.)] IPCC, Geneva, Switzerland.

Keough, M.J. & Quinn, G.P. 1998. Effects of periodic disturbances from trampling on rocky intertidal algal beds. *Ecol. Appl.* 8:141–161.

Keser, M., Swenarton, J.T. & Foertch, J.F. 2005. Effects of thermal input and climate change on growth of *Ascophyllum nodosum* (Fucales, Phaeophyceae) in eastern Long Island Sound (USA). *J. Sea Res.* 54:211-220.

Kevekordes, K. & Clayton, M.N. (1996) Using developing embryos of *Hormosira banksii* (Phaeophyta) as a marine bioassay system. *Int. J. Plant Sci.* 157(5): 582-585.

Klughammer, C. & Schreiber, U. 2008. Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the saturation pulse method. *PAM Application Notes*. 1: 27-35.

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

Kübler, J.E. & Davison, I.R. 1993. High-temperature tolerance of photosynthesis in the red alga *Chondrus crispus*. *Mar. Biol.* 117:327-335.

Li, R. & Brawley, S.H. 2004. Improved survival under heat stress in intertidal embryos (*Fucus* spp.) simultaneously exposed to hypersalinity and the effect of parental thermal history. *Mar. Biol.* 144:205-213.

Lilley, S.A. & Schiel, D.R. 2005. Community effects following the deletion of a habitat-forming alga from rocky marine shores. *Oecologia* 148:672-681.

Lima, F. P. & Ribeiro, P. A. 2007. Do distributional shifts of northern and southern species of algae match the warming pattern? *Glob. Change Biol.* 13: 2592-2604.

Lindauer, V.W. (1947) An annotated list of the brown seaweeds, Phaeophyceae, of New Zealand. *Trans. Royal Soc. N.Z.* 76(4): 542-566.

Long, SP, Humphries S, Falkowski PG, (1994) Photoinhibition of photosynthesis in nature. *Annu Rev Plant Physiol Plant Mol Biol* 45: 633-662.

Lubchenco, J. 1982. Effects of grazers and algal competitors on fucoid colonization in tide pools. *J. Phycol.* 18:544- 550.

Lynch, M. & Walsh, B. 1998. Genetics and the analysis of quantitative genetics. Sinauer Associates, Massachusetts.

Macinnis-Ng, C.M.O., Morrison, D.A. & Ralph PJ. 2005. Temporal and spatial variation in the morphology of the brown macroalga *Hormosira banksii* (*Fucales, Phaeophyta*). *Bot. Mar.* 48:198-207.

Major, K.M. & Davison, I.R. 1998. Influence of temperature and light on growth and photosynthetic physiology of *Fucus evanescens* (Phaeophyta) embryos. *Euro. J. Phycol.* 33: 129-138.

Marshall, D.J., Allen, R.M. & Crean, A.J. 2008. The ecological and evolutionary importance of maternal effects in the sea. Oceanography and Marine Biology: An Annual Review. 46: 203-250.

McKenzie, P.F. & Bellgrove, A. 2006. No outbreeding depression at a regional scale for a habitat forming intertidal alga with limited dispersal. *Mar. Fres. Res.* 57:655-663.

McKenzie, P.F. & Bellgrove , A. 2008. Dispersal of *Hormosira banksii* (Phaeophyceae) via detached fragments: reproductive viability and longevity. *J. Phycol*. 44: 1108-1115.

Pandolfi, J.M., Connolly, S.R., Marshall, D.J. & Cohen, A.L. 2011. Projecting coral reef futures under global warming and ocean acidification. *Science* 333:418-422.

Pearson, G.A. & Brawley, S.H. 1996. Reproductive ecology of *Fucus distichus* (Phaeophyceae): an intertidal alga with successful external fertilization. *Mar. Ecol. Prog. Ser.* 143:211–223.

Pease, C.J., Johnston, E.L. & Poore, A.G.B. 2010. Genetic variability in tolerance to copper contamination in a herbivorous marine invertebrate. *Aquat. Toxicol.* 99:10-16.

Pistevos, J. C. A., P. Calosi, S. Widdicombe, & Bishop, J.D.D. 2011. Will variation among genetic individuals influence species responses to global climate change? *Oikos*. 120:675-689.

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. *J. Exp. Mar. Biol. Ecol.* 225:285-300.

Roughan, M., Macdonald, H.S., Baird, M.E., Glasby, T.M. 2011. Modelling coastal connectivity in a Western Boundary Current: Seasonal and inter-annual variability. *Deep-Sea Res Pt II:* 58(5): 628-644.

Schreiber, U. 2004. *Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview.* In: Papageorgiou GC, Govindjee (eds) Chlorophyll fluorescence: a signature of photosynthesis. Kluwer Academic Publishers, Dordrecht, pp 279-319.

Somero, G.N. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* 213:912-920.

Southward, A.J., Langmead, O. & Hardman-Mountford, N.J., et al. 2004. Long-term oceanographic and ecological research in the western English Channel. *Adv. Mar. Biol.* 47:1-105.

Sunday, J.M., Ryan N.C., C.D.G. Harley & M.W. Hart. 2011. Quantifying rates of evolutionary adaptation in response to ocean acidification? *PLoS ONE*. 6(8): 1-8.

Takahashi, S. & Murata, N. (2008) How do environmental stresses accelerate photoinhibition? *Trends in Plant Science*, 13, 178-182.

Vadas, R.L., Johnson, S. & Norton, T.A. 1992. Recruitment and mortality of early post-settlement stages of benthic algae. *Brit. Phycol. J.*, 27:331-351.

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 One* 6:2621-2626.

Worm, B. & Chapman, A.R.O. 1998. Relative effects of elevated grazing pressure and competition by a red algal turf on two post-settlement stages of *Fucus evanescens. J. Exp. Mar. Biol. Ecol.* 220:247–268.

Worm, B. & Lotze, H.K. 2006. Effects of eutrophication, grazing, and algal blooms on rocky shores. *Limnol. Oceanogr*. 51: 569–579.

Wright, J.T., de Nys, R., Poore, A.G.B. & Steinberg, P.D. 2004. Chemical defence in a marine alga: heritability and the potential for selection by herbivores. *Ecology* 85:2946-2959.

Yamamoto, Y., Aminaka, R., Yoshioka, M., Khatoon, M., Komayama, K., Takenaka, D., Yamashita, A., Nijo, N., Inagawa, K., Morita, N., Sasaki, T., Yamamoto, Y. 2008. Quality control of photosystem II: impact of light and heat stresses. *Photosynth. Res.*, 98:589–608.

TABLE 1: Analysis of variance on the length of *H. banksii* embryos from Pearl Beach and Bilgola grown at 20 °C and 28 °C. Block is a random factor representing experiments done on 3 different dates, temperature is a fixed factor, with male and female random factors nested in each block. * denotes significant effects ($P < 0.05$).

		Pearl Beach						**Bilgola**					
		24 hours		**72 hours**		**120 hours**		**24 hours**		**72 hours**		**120 hours**	
Source	**df**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Temperature	1	37.16	0.026*	54.99	0.02*	11.61	0.08	315.6	0.003*	709.6	0.001*	59.48	0.02*
Block	2	36.99	<0.001*	1.82	0.21	15.64	0.001*	3.03	0.09	1.06	0.38	0.68	0.62
Male (Bl)	6	2.309	0.10	5.57	0.006*	6.31	0.003*	5.21	0.007*	4.72	0.01*	1.11	0.41
Female(Bl)	6	0.74	0.63	6.77	0.003*	1.88	0.17	6.41	0.003*	6.73	0.003*	3.14	0.04*
Block x Temp	2	3.29	0.08	1.11	0.36	5.03	0.03*	0.59	0.68	0.33	0.92	1.80	0.21
Temp x Male(Bl)	6	0.97	0.48	5.52	0.006*	5.67	0.005*	1.56	0.24	3.16	0.04*	0.96	0.49
Temp x Female(Bl)	6	1.04	0.45	4.77	0.01*	2.14	0.12	3.74	0.03*	2.64	0.07	2.48	0.09
Male (Bl) x Female (Bl)	12	8.53	<0.001*	2.98	0.001*	3.31	<0.001*	1.52	0.12	4.15	<0.001*	6.55	<0.001*
Temp x Male(Bl) x Female(Bl)	12	9.02	<0.001*	2.34	0.007*	2.52	<0.004*	1.19	0.29	2.67	0.002*	5.98	<0.001*
Error	270												

680

FIGURE LEGENDS

FIG. 1. Representative embryo morphology at 20 °C and 28 °C after 24 and 120 hours.

FIG. 2. Reaction norms showing the length of *H. banksii* embryos across temperatures after 24, 72 and 120 hours in the Bilgola Beach (b, d, f) and Pearl Beach (a, c, e) populations. Lines represent the contrast between the mean length of paternal half-siblings derived from each of the nine males (n = 18 embryos per male). The average standard error for each mean is presented above the X axis.

FIG. 3. The effect of elevated temperature on maximum quantum yield (F_V/F_M) of *H. banksii* embryos from a) Pearl Beach and b) Bilgola populations. Data are means ± SE of F_V/F_M at each of 24, 72 and 120 hours n = 162. * denotes a significant effect of temperature. Note that the error bars are based on replicate embryos and do not reflect the error terms in the analysis of variance (Table S1).

FIG. 4. Complementary energy dissipation pathways in Pearl Beach embryos after 120 h. Y(PSII) = photochemistry (black bars); Y(NO) = heat dissipation (non-regulated non photochemical quenching; light gray bars); Y(NPQ) = xanthophyll cycling (regulated non photochemical quenching; dark gray bars); (average of 6 embryos for each of 27 crosses). Data are means with error bars across all embryos (± SD) presented to the right of each plot.

SUPPLEMENTARY TABLES

TABLE S1: Analysis of variance on the maximum quantum yield (F_V/F_M) of *H. banksii* embryos from Pearl Beach and Bilgola grown at 20 °C and 28 °C. Block is a random factor representing experiments done on 3 different dates, temperature is a fixed factor, with male and female random factors nested in each block. * denotes significant effects ($P < 0.05$).

		Pearl Beach						**Bilgola**					
		24 hours		**72 hours**		**120 hours**		**24 hours**		**72 hours**		**120 hours**	
Source	**df**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Temperature	1	2.76	0.24	41.41	0.02*	15.51	0.06	18.86	0.049*	7.19	0.12	13.75	0.07
Block	2	2.16	0.17	0.78	0.53	0.69	0.67	5.57	0.04*	2.24	0.15	0.96	0.54
Male (Bl)	6	1.72	0.20	3.18	0.04*	0.56	0.76	0.41	0.86	1.83	0.18	0.66	0.68
Female(Bl)	6	4.75	0.01*	2.16	0.12	2.34	0.10	5.53	0.006*	2.63	0.07	0.48	0.81
Block x Temp	2	1.23	0.34	0.98	0.48	1.60	0.25	1.55	0.28	2.15	0.16	1.94	0.17
Temp x Male(Bl)	6	2.07	0.13	1.27	0.34	1.68	0.21	0.35	0.90	1.61	0.23	0.82	0.58
Temp x Female(Bl)	6	2.02	0.14	0.83	0.57	1.78	0.19	1.407	0.29	2.21	0.11	0.55	0.76
Male (Bl) x Female (Bl)	12	2.25	0.01*	3.76	<0.001*	2.25	0.01*	2.70	0.002*	1.87	0.04*	15.60	<0.001*
Temp x Male(Bl) x Female(Bl)	12	3.38	<0.001*	4.56	<0.001*	2.44	0.01*	2.65	0.002*	2.06	0.02*	16.02	<0.001*
Error	270												

TABLE S2. Analysis of variance on the effective quantum yield (Y(PSII)) and non-regulated non-photochemical quenching (Y(NO)) of *H. banksii* embryos from Pearl Beach grown at 20 °C and 28 °C. Block is a random factor representing experiments done on 3 different dates, temperature is a fixed factor, with male and female random factors nested in each block. * denotes significant effects ($P < 0.05$).

715

		Y(PSII)				**Y(NO)**			
		24 hours		**120 hours**		**24 hours**		**120 hours**	
Source	**df**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Temperature	1	6.47	0.13	4.99	0.16	2.14	0.25	282.4	0.004*
Block	2	0.49	0.70	0.69	0.58	0.82	0.58	2.43	0.10
Male (Bl)	6	5.76	0.005*	3.90	0.02*	2.93	0.05	0.51	0.79
Female(Bl)	6	4.02	0.02*	1.70	0.20	5.62	0.007*	0.37	0.89
Block x Temp	2	0.94	0.46	5.22	0.03*	0.77	0.63	0.44	0.91
Temp x Male(Bl)	6	1.21	0.37	1.07	0.43	1.94	0.15	2.05	0.14
Temp x Female(Bl)	6	3.03	0.048*	1.40	0.29	2.46	0.08	0.77	0.61
Male (Bl) x Female (Bl)	12	1.17	0.31	0.84	0.61	1.25	0.25	1.47	0.15
Temp x Male(Bl) x Female(Bl)	12	1.76	0.06	1.21	0.29	1.53	0.12	0.62	0.82
Error	108								