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High tolerance of repeated heatwaves in Australian native plants

Kris French

University of Wollongong, kris@uow.edu.au

Isaac Jansens

University of Wollongong

Michael B. Ashcroft

University of Wollongong, ashcroft@uow.edu.au

Heath Ecroyd

University of Wollongong, heathe@uow.edu.au

Sharon A. Robinson

University of Wollongong, sharonr@uow.edu.au

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Abstract

Heatwaves, with increases in day and night time temperatures, are predicted to increase in frequency. We investigated the response of forbs, shrubs, grasses and non-grass monocotyledons from warm temperate environments in Australia to repeated heatwaves to determine if responses differed with growth form and whether the addition of hot night temperatures influenced the ability of species to grow and acclimate. Plants were subjected to 3, 3-day heatwaves comprising either hot days and nights or hot days and cool nights, with control plants maintained under cool days and nights. All species were thermotolerant to repeated heatwaves, although two species showed lower biomass under heat treatments, indicating repeated heatwaves influenced function in some, but not all species. While there was evidence of photosynthetic damage in some species, these recovered by the end of the experiment. While grasses and one herb showed some evidence of photosystem acclimation, increases in the threshold temperatures for membrane breakdown only occurred in one shrub. Leaf sacrifice in grasses was significantly increased after repeated heatwaves suggesting that fuel loads in grass communities will increase in the future. These results indicate high resilience for these Australian native warm temperate plants although lower growth rates in some species after heatwaves may result in changes to community composition.

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High tolerance of repeated heatwaves in Australian native plants

Kris French^{1*}, Isaac B. Jansens¹, Michael B. Ashcroft¹, Heath Ecroyd^{2,3}, Sharon A. Robinson¹

¹Centre for Sustainable Ecosystem Solutions, School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522

²Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW 2522

³School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522

Corresponding author: K. French. School of Biological Sciences, University of Wollongong. Northfields Avenue, Wollongong, NSW, 2522, Australia. kris@uow.edu.au. Phone 61 2 4221 3655

Running Head: Plant tolerance to repeated heat waves

21

22 **Abstract**

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40 **Keywords:** acclimation, chlorophyll fluorescence, climate change, growth forms, heatwaves,
41 relative electrical conductivity.

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45 **Introduction**

Climate change is likely to cause more frequent heatwaves, and night-time temperatures are predicted to increase in magnitude at twice that of day-time increases (IPCC 2014; BOM 2014). These predictions represent important thermal challenges for plants and understanding the capacity of native plants to acclimate will be important for predicting biodiversity losses. Detrimental effects of heatwaves include reduced photosynthetic capacity (Filewod and Thomas 2014), decreased photosynthetic nitrogen use efficiency (Wang et al. 2008), defoliation (Carnicer et al. 2011; Brouwers et al. 2013) and mortality (Allen et al. 2010). At a habitat scale, there is reduced primary productivity (Ciais et al. 2005), a decline in leaf area indices (Filewod and Thomas 2014) and a change in species composition (White et al. 2000). Many studies, however, are based on single heatwave events (Groom et al. 2004; Wang et al. 2008; Filewod and Thomas 2014 but see Brouwers et al 2015) or on a constant heating regime over a longer period of time (e.g. Bokhorst et al. 2013) which do not shed light on the potential adverse effects of the ecologically relevant repeated heatwaves intermixed with cooler days, expected under a warming climate.

High night temperatures potentially impact plant health (Prasad and Djanaguiraman 2011) reducing net carbon gain in plants (Peng et al. 2013). Plants typically undergo growth and repair at night, so warmer night temperatures might be beneficial, although they may hinder repair if temperatures are stressful. Previous studies have focused on plants of commercial interest; warm nights are positive for tomatoes (Willits and Peet, 1998) and rice (Mohammed and Tarpley 2010; Zhang et al. 2013) but negative for cowpeas (Warrag and Hall 1984) and sorghum (Prasad and Djanaguiraman 2011). As species' responses to increased night temperatures vary and be dependent on temperature, heatwaves associated with hotter night temperatures are likely to differentially affect species.

Stress can alter the way plants allocate resources to different organs (Iwasa and Roughgarden 1984). Under warmer conditions, carbohydrates may be allocated to root

production to increase water uptake (Domisch et al. 2001; Pumpanen et al. 2012). The ratio of root:shoot mass can be used to understand the effect of elevated temperatures on plant growth (Iwasa and Roughgarden 1984), however, as most research has focused on plants that receive very cold winters and warm summers (e.g. Domisch et al. 2001; Pumpanen et al. 2012), the effects of heatwaves on resource allocation of plants that experience cool winters and hot summers is less well understood.

Photosystem II (PSII) is extremely heat sensitive (Berry and Bjorkman 1980; Badger et al. 1982; Sage and Kubien 2007) and under severe heat stress, directs less energy towards photochemistry and emits more energy as heat and fluorescence (Genty et al. 1989; Maxwell and Johnson 2000). Chlorophyll fluorescence can be measured quickly and is thus a practical way to compare heat damage in multiple species and with appropriate data replication (Curtis et al. 2016). Maximum quantum yield (F_v/F_m) is thus a good indicator of stress experienced by PSII decreasing from approximately 0.83 (Maxwell and Johnson 2000). Under extreme stress, irreversible damage to PSII occurs, resulting in sustained reductions in F_v/F_m and an increase in minimum fluorescence (F_o) (Briantais et al. 1996). However, reductions in F_v/F_m can also indicate the presence of reversible photoprotective mechanisms, such as those mediated by the xanthophyll cycle (Demmig-Adams and Adams 1992; Cirocco et al. 2015). In this case quenching of fluorescence will also be manifest in a reduced F_o .

Exposure to heatwaves may induce an acclimatory response that would help plants to persist under repeated heatwaves and may occur seasonally (Larcher 2002; Baruffo and Tretiach 2007; Zaragoza-Castells et al. 2007; Reich et al. 2016). Electrolytic conductivity, which measures the amount of solutes in a solution, can be used to provide a measure of cell membrane breakdown under heat stress and thus determine lethal temperature thresholds for plants (Ismail and Hall 1999). Plants in warmer climates are predicted to acclimate through a range of cellular responses to the expected increase in frequency and intensity of heatwaves, although overall growth may decline and allocation to roots and shoots may differ.

Understanding how plants respond to an increase in repeated heatwave events will increase our ability to predict future changes in community structure. This study aims to better understand the capacity of native species from warmer climates, to cope with repeated heatwaves.

Different growth forms confer adaptive advantage to high light levels (Bonser and Geber 2005), and increased drought tolerance (Nagase and Dunnett 2010), however, growth forms may also confer advantages in a thermally challenging environment. Variation in the ability of different growth forms to acclimate is likely to result in changes in structural composition of plant communities if particular growth forms do better than others. As a result we investigated plant responses to an increase in heatwaves for a range of species with different growth forms.

Specifically, it was predicted that:

1. repeated heatwaves and hot night temperatures would reduce growth and alter root:shoot allocations in plants,
2. the effects of repeated heatwaves and hot night temperatures on PSII function, growth and acclimation would differ between growth forms.
3. temperature thresholds for PSII function and cellular membrane stability would be higher in plants exposed to repeated heatwaves, compared to a moderate temperature for day and night, and
4. temperature thresholds for PSII function and cellular membrane stability would be at higher temperatures in plants exposed to heatwaves with hot night-time temperatures compared to heatwaves with cool nights.

Methods

Study species

Two native species from each of four growth forms (grasses, non-grass monocotyledons, forbs and shrubs) were selected. Well established, similar sized stock plants (150 mm pots) were purchased from nurseries (Table 1). Pots contained slow release fertilizer pellets at standard rates (Osmocote ® Native; 15g per pot).

Growth conditions

Experiments were conducted at the University of Wollongong, Australia (34.40°S, 150.87°E). Plants were allowed to acclimate for 4 weeks on a shaded (80%) concrete slab. Plants were then transferred to a non-shaded concrete slab for 4 weeks prior and allowed 4 weeks adjustment to exposed conditions. Sides of pots were protected from direct solar irradiance to prevent abnormally high soil temperatures through using garden boxes made with wooden sides that were the height of the pots. Plants were watered using a sprinkler for 5 min at 0700 h each day for the whole experiment. For each species, plants were initially similar sized and were randomly assigned to heat treatments.

Heatwave simulation

Plants were exposed to three simulated heatwave events over 30 days from 4th November – 3rd December, 2013. The first heatwave was simulated on 9th – 11th Nov. (day 6 – 8), the second 18th – 20th Nov. (day 15 – 17), and the third 27th – 29th Nov. (day 24 – 26). Three heatwaves reflected a predicted increase in the mean number of days >35°C per year from 3.3 to 9 by 2070 in the Sydney region of Australia (Garnaut 2008). Six days of recovery was provided between each heatwave.

Four replicate plants of each species were allocated to one of three treatments: i) hot days and nights (39/25 °C) (HH), ii) hot days and cool nights (39/15 °C) (HC), and iii) cool days and nights (25/15°C) (CC) in three growth chambers (Model TPG-2400-TH-CO2,

Thermoline, Perth). Day-time maximum during heatwave treatments (39°C) was based on the BOM (2013) definition of a heatwave being 3 consecutive days over 39°C. Night-time minimum was based on the average of the previous four summers' highest monthly minimum temperatures (2009-2013) for the nearest weather station (Bellambi, 34.37°S, 150.93°E) (BOM 2013). Ramp rate for each treatment was calculated to ensure daily maximum was reached by midday (12:00 h): HH – 2 °C/hr, HC – 3.5 °C/hr, and CC – 1.5 °C/hr.

Illumination within growth chambers was ramped throughout the day with maximum irradiance of 850 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ between 1000-1500h. Plants were arranged randomly within growth chambers.

Measurements

Chlorophyll fluorescence was measured (Mini-PAM, Walz, Effeltrich) on d 1 to establish basal levels of photosynthetic efficiency, then after the three heatwaves on d 9, 18 and 27. The chlorophyll fluorescence parameters, F_v/F_m and F_o , were measured on healthy, mature leaves at 5:30 am (predawn or before the growth chamber light period), ensuring leaves were fully dark-adapted. At the end of each heatwave, plants were returned to the concrete slab for 6 d until the next simulated heatwave.

The effect of heatwaves on root and shoot biomass, and root:shoot ratio was determined using above-ground to below-ground dried biomass. Below ground material was washed under water to remove potting mix from the organic material in a sieve. For grasses which typically sacrifice leaves when stressed and maintain them on the plant, above-ground material was separated into living and non-living. Dead tissue was considered analogous to leaf abscission and, therefore, not contributing to functional biomass. Above- and below-ground samples were placed in a drying oven (Model 2400, Contherm, Wellington) at 60°C for 5 days to achieve constant weight.

Acclimation of PSII and membrane stability

For forbs, shrubs and grasses, chlorophyll fluorescence (F_v/F_m and F_o) and solute leakage from leaves (electrical conductivity) were measured on fresh leaves removed from 4 replicate plants of each species prior to any experimentation. To identify temperature thresholds, 6 test temperatures were used (35, 39, 43, 47, 51, 55°C) based on Larcher (2002). To determine changes in these parameters following a series of heatwaves, F_v/F_m , F_o and conductivity were measured following the 27-d experimental treatment for shrubs, forbs and grasses. Fresh leaves, removed from 4 replicate plants of each species from each temperature regime, were used for each test temperature ($n=24$).

Leaf material was placed in test tubes with distilled water at a ratio of 0.5 g: 15 mL to account for differences in leaf size between species. Initial conductivity was measured after 5 min at room temperature (25°C) using a conductivity meter (Model LF 320, Wissenschaftliche Technische Werkstätten, Oberbayern). Tubes were then placed in water baths (Model LWB-122D, Thermoline, Wetherill Park) for 30 min for each of the six test temperatures. Samples were removed from water baths and cooled rapidly before remeasuring conductivity. The same leaf samples were dark adapted for 20 min and F_v/F_m and F_o measured using a Mini-PAM (Heinz-Walz, Effeltrich). Finally samples were boiled at 95 °C for 30 min to determine maximum cellular solute leakage. Relative electrical conductivity (REC) was calculated:

$$REC = (EC_{30} - EC_0)/(EC_{MAX} - EC_0)$$

where, EC_0 and EC_{30} are electrical conductivity measured after 0 and 30 min heat treatment respectively, and EC_{MAX} is maximum electrical conductivity after 95 °C treatment.

Data analysis

To assess the photosynthetic effects of repeated heatwaves and hot nights, after each heatwave, F_v/F_m and F_o measurements were analysed using a 3-factor, repeated measures ANOVA with a hierarchical design. The Wilks-Lambda test statistic is reported. For each dependent variable,

treatment (HH, HC, CC) and growth form were fixed effects, and species was nested within growth form. Time i.e. Day 1, 9, 18, and 27, was a repeated factor. Two factor analyses for each growth form were used to tease apart significant changes in the dependent variables and then Tukey's multiple comparison tests were used to further investigate differences. A 3-factor, nested ANOVA was used to test for differences in above-ground, below-ground, root:shoot and total biomass between treatments and growth forms. Treatment and growth form were fixed effects and species was nested within growth form. To investigate leaf sacrifice in grasses we compared dead biomass and the percentage of dead biomass using a 2-factor ANOVA.

To determine whether PSII or membranes had become acclimated to heatwaves we compared threshold values for the control (CC) plants with those in the HC and HH treatments. Threshold values were determined using a Bayesian model to examine how the responses of each species and treatment varied according to temperature (based on Ashcroft et al. 2016; R2OpenBugs package for R; R Core Team 2014). For F_v/F_m and REC the Bayesian models were used to fit a generalised logistic function (e.g. Fig. S1 in Supporting Information), which is a generalised version of the sigmoid function that allows the data to determine the low and high temperature asymptotes between 0 and 1, the rate of change and the point of inflection. For F_v/F_m , the generalised logistic functions suggested there were low temperature asymptotes where the F_v/F_m was ~ 0.6 – 0.8 and high temperature asymptotes of ~ 0.2 – 0.4 (Fig. S1). We defined the temperature threshold based on where the F_v/F_m was predicted to be midway between the high and low temperature asymptotes. We also examined the high temperature asymptotes directly to test if acclimation at higher temperatures increased the minimum F_v/F_m (Fig. S1). For REC, a similar approach was used, but the high temperature asymptote was not approached (the temperature exceeded our maximum temperature of 55 °C) and the low temperature asymptote was at an REC of ~ 0 . Therefore we defined the temperature threshold based on a fixed REC of 20% (Fig. S3). For F_o , the curves had more complex shapes and so the Bayesian model was used to fit penalised splines instead of generalised logistic functions

(Fig. S2). The temperature threshold for F_o was reached when F_o exceeded both 150% of the minimum F_o and was higher than the midpoint between the minimum and maximum F_o .

One response curve was fitted for each treatment (CC, HC, HH), species and response variable (F_v/F_m , F_o , REC; Fig. S1–3). The Bayesian models estimate response curves by iteratively and stochastically fitting alternative curves that can explain the data and calculating the 95% CI for the parameters or attributes of the responses. At each iteration, the model determined if the threshold for the HH or HC treatment was higher than the corresponding CC treatment and over 20 000 iterations this became a probability that the thresholds for those treatments were higher than the control (Ashcroft et al. 2016).

Results

Effect of heatwaves on growth and chlorophyll parameters

The effects of repeated heatwaves and hot nights on total, above-ground and below-ground biomass was more dependent on species identity than growth form (species(growth form)*treatment; total - $F_{8, 71} = 3.24$, $p = 0.0034$, above- $F_{8, 71} = 2.17$, $p = 0.0404$, below - $F_{8, 71} = 5.33$, $p < 0.0001$). Total biomass of the grass, *P. labillardierei*, and the non-grass monocot, *D. caerulea*, were significantly lower under both heat treatments (HH and HC) compared to the control (CC) (Figure 1). All other species showed no effects of repeated heatwaves on above-ground, below-ground or total biomass.

Despite variation in root:shoot ratio at the species level ($F_{4, 70} = 3.62$, $p = 0.0097$), root:shoot ratio did not vary with treatment ($F_{2, 70} = 2.11$, $p = 0.1284$), however, it did vary with growth form ($F_{3, 70} = 8.99$, $p < 0.0001$). Grasses tended to put more resources into roots relative to shoots (average = 3.93 ± 7.59), while all other growth forms had more shoots relative to roots

(shrubs, 0.21 ± 0.05 ; forbs, 0.18 ± 0.12 ; non-grass monocots, 0.33 ± 0.12). However, grasses were highly variable (high variation, see Table S1).

Leaf sacrifice in grasses was significantly greater in plants exposed to HC heatwaves than the control with leaf sacrifice being intermediate in plants exposed to HH ($F_{2,18} = 7.26$, $p = 0.0049$). Responses did not differ between species ($F_{2,18} = 0.023$, $p = 0.8805$). Similarly, the percentage of biomass sacrificed was greater in the two grass species exposed to both heatwave conditions than control plants ($F_{2,18} = 14.57$, $p = 0.0002$). While 34% of leaf biomass was sacrificed in control plants, between 68-81% of biomass was sacrificed in heatwaves (Figure 2).

Effect of heatwaves on chlorophyll fluorescence parameters

The effect of heatwaves on F_v/F_m was dependent on both growth form and heatwave number (Table 2; Table S1). In addition, the effect of heatwaves on F_o was dependent on both species' identity and treatment day (Table 3; Table S1). Using separate 2 factor ANOVAs on each growth form, there was no treatment effect on F_v/F_m nor F_o for native shrubs but for grasses, the effect of heatwaves depended on treatment day (Figure 3a) and was significantly lower in plants exposed to a heatwave 2nd heatwave (Figure 3a). F_o did not vary significantly in grasses over days or between treatments (Table 2 and 3).

For forbs, F_v/F_m was lower in plants exposed to heatwaves compared to the control after the 1st and 2nd heatwave but did not differ after the 3rd heatwave (Figure 3b, Table 2). Furthermore, all measurements were above 0.8 suggesting the photosynthetic stress was relatively minor. The effect of heatwaves on F_o was dependent on the treatment day for the native forb, *C. glaucescens* (Figure 4, Table 2 and 3). After the 2nd heatwave, F_o was significantly higher in plants exposed to heatwaves compared to the control (Figure 4). For the remaining forb, *D. repens*, F_o did not vary (Table 2 and 3).

For non-grass monocots, F_v/F_m was significantly lower in plants exposed to HC heatwaves than the control on day 18 (Figure 3c). All F_v/F_m values were high suggesting these differences are not biologically relevant.

Acclimation

Following the three heatwaves most species showed some acclimation in one aspect of either PSII functionality or cell membrane temperature thresholds (Figure 5, Figures S1-3, Tables S2-5). No acclimation was evident in *C. glaucescens*. PSII acclimation was evident in the two grasses where the temperature at which F_v/F_m declined by 50% was higher in plants treated with hot days and cold nights than control plants (Fig 5a, *T. australis* 1.9 °C higher, 95% CI 0.7–3.3 °C, $p(\text{HC} > \text{CC}) = 99.8\%$; *P. labillardierei* 1.2 °C higher; 95% CI 0.0–2.7 °C, $p(\text{HC} > \text{CC}) = 96.3\%$). *T. australis* also acclimated in this way under hot days and hot nights (1.2 °C higher, 95% CI 0.0–2.5 °C, $p(\text{HH} > \text{CC}) = 95.8\%$) while *P. labillardierei* did not show significant acclimation but showed a similar trend (1.0 °C higher, 95% CI -0.4–3.0 °C, $p(\text{HH} > \text{CC}) = 88.0\%$; Fig S1, Table S2). *P. labillardierei* plants that had experienced the HH treatment also maintained higher F_v/F_m values at 55 °C suggesting some build up of resilience for plants in this treatment (Fig 5d, F_v/F_m 0.08 higher, 95% CI 0.01–0.14, $p(\text{HH} > \text{CC}) = 98.7\%$, Fig S1, Table S3), and a similar response was also seen in *W. fruticosa* (F_v/F_m 0.09 higher, 95% CI 0.00–0.26, $p(\text{HH} > \text{CC}) = 95.8\%$). No other species showed evidence of increased resilience of PSII following a month of heat treatment.

We found no evidence in any species of a change in the temperature at which F_o began to show signs of damage (increase) as a result of heat stress regime (Figure 5b). Both shrubs, *C. tetragona* and *W. fruticosa*, and the two herbs, *C. glaucescens* and *D. repens* (to a lesser extent), followed the typical response where F_o began to increase at around 45 °C, declining above 50 °C when permanent damage was experienced (Figure S2, Table S4). However, *P. labillardierei* and *T. australis* did not follow this pattern, remaining at low levels throughout

all trials, indicating strong resilience of the photosynthetic apparatus to permanent damage at all of these temperatures.

Acclimation of membranes, expressed as an increase in the temperature at which the conductivity reached 20%, was evident in one shrub, *C. tetragona*, and one herb, *D. repens* (Figure 5). In *C. tetragona*, membrane temperature thresholds increased under both heating regimes, although only HC was significant (HC REC 2.7 °C higher, 95% CI 1.6–3.5 °C, $p(\text{HC} > \text{CC}) > 99.9\%$; HH REC 1.0 °C higher, 95% CI -0.2–2.3 °C, $p(\text{HH} > \text{CC}) = 91.4\%$) while in *D. repens*, membrane temperature thresholds only increased in the HC treatments (REC 1.3 °C higher, 95% CI 0.8–2.0 °C, $p(\text{HC} > \text{CC}) > 99.9\%$; Figure S3, Table S4).

Discussion

Heatwaves had relatively few impacts on most plant species studied with only small changes in biomass and only short-term effects on photosynthesis. As a result, the study identified that these native species were generally resilient to repeated heatwaves and suggest that a future change in the frequency of heatwaves may have little impact provided plants have adequate water during these hot periods. Other growth forms apart from grasses changed very little in response to heatwaves.

Only two species showed lower overall biomass after repeated heatwaves; a C3 grass, *P. labillardierei*, and a non-grass monocot, *D. caerulea*. Both these species accumulated high biomass in control conditions and have the capacity, therefore, to grow very quickly, but heatwaves significantly slowed the ability of these two species to maintain high growth. Significant variation in plant responses at a species-level suggests that shifts in community composition may occur with predicted increases in frequency and duration of heatwaves where species with slower growth rates may be selected against over time (Kelly and Goulden 2008; Bremond et al. 2012; IPCC 2014).

While the plants used in this study varied in the time taken for PSII to develop resilience to heating, all species had F_v/F_m values that were the same as pretreatment and control values by the end of the experiment. Decreases in F_v/F_m , along with maintenance of F_o may be non-damaging and indicate a high capacity for photoprotection, however, if F_o increases and then begins to decline, this likely indicates irreversible damage to PSII and potential chlorophyll destruction. Grasses showed complete recovery by the 3rd heatwave. The lack of lasting damage to PSII was confirmed by F_o remaining unchanged for the duration of the study period. The only species that showed any evidence of a significant increase in F_o was the forb, *C. glaucescens*, and even this had recovered by the end of the experiment.

Furthermore both grass species showed acclimation of the photosynthetic apparatus and membrane temperature thresholds, with both the temperature at which F_v/F_m showed a 50% decline, and the absolute F_v/F_m at the highest temperature, significantly higher in response to one or other heat treatment. This resilience, coupled with the significant increase in leaf sacrifice response of grasses exposed to repeated heatwaves compared to the control suggests that new leaves produced post-heat stress are more thermotolerant than sacrificed leaves (Lovelock et al. 1994). Well over half of the biomass in grasses was sacrificed after heatwaves suggesting that grasses may rapidly increase fuel loads, increasing the risk of fire. Furthermore, while *T. australis* did not differ amongst treatments in biomass accumulation, its overall growth during heatwaves and in control conditions was equivalent or less than *P. labillardierei*, suggesting that this C4 will not have a particular competitive advantage in a future increase in heatwaves providing water is sufficient.

Future work needs to distinguish between the effects of heat stress and drought stress in order to comprehensively understand the capacity of plants to tolerate ecologically relevant heatwaves (Wahid et al. 2007; Curtis et al, 2016). Furthermore, as only two representatives of each growth form were included in this experiment, widespread generalisations would require further work with more species.

Hot night temperatures were found to have a minimal effect. In contrast to the negative effects of hot night temperatures on photosynthesis and pollen function for other species (Prasad and Djanaguiraman 2011), these non-crop species displayed thermotolerance to hot night temperatures suggesting that native species were already well-adapted. Resilience to hot night temperatures may have been due to high plasticity of photosynthetic and mitochondrial apparatuses (Ryan et al. 1997; Wataru et al. 2014) but further work is needed to enable predictions for many species to a future of increasing heatwaves.

Conclusions

This study has been one of the first to identify a set of naturally-occurring species which appear to suffer less under a scenario mimicking the higher temperatures predicted under future climate models. Using these species as case studies for understanding tolerance mechanisms will provide important predictive power for identifying plant communities and species that will be at risk in the future. The species in this study were able to adjust to repeated heatwaves within a 27-day period although some species had slower growth under the heatwave regimes. Short-term plasticity of photosynthetic apparatus is a key component of thermotolerance and is a favourable trait for species' survival (Wahid et al. 2007; Wataru et al. 2014). All growth forms assessed; forbs, grasses, shrubs and non-grass monocots, displayed resilience and plasticity of PSII under conditions where water was not restricted. Our results suggest that most of these Australian native species will persist, and potentially thrive, in a warming climate with an increased frequency of heatwaves and increased night temperatures, however, future research is needed to investigate thermotolerance capacity under water restrictions. The slower growth measured in some species may indicate long term changes in community assemblages with these species becoming rarer and we would expect more extreme effects if heat and drought occur together.

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 497 respiration? Insights from a Mediterranean tree with long-lived leaves. *Plant, Cell*
 498 *Environ* 30:820-8

499 **Table 1** Australian native species used in this study.

Growth Form	Family	Species	Description
Forb	Convolvulaceae	<i>Dichondra repens</i> J.R. Forst and G. Forst	Perennial herb with creeping stems.
	Aizoaceae	<i>Carpobrotus glaucescens</i> (Haw.) Schwantes	Prostrate perennial with thick, fleshy leaves.
Non-grass monocot	Liliaceae	<i>Dianella caerulea</i> Sims var. <i>caerulea</i>	Tufted, solitary perennial herb (50 cm high) with linear leaves.
	Liliaceae	<i>Thelionema umbellatum</i> (R.Br.) R.J.F.Hend.	Tufted, rhizomatous perennial.
Shrub	Myrtaceae	<i>Calytrix tetragona</i> Labill.	Shrub with linear leaves.
	Lamiaceae	<i>Westringia fruticosa</i> (Willd.) Druce	Shrub with narrow leaves.
Grass	Poaceae	<i>Themeda australis</i> (R.Br.) Stapf	Tufted perennial.
	Poaceae	<i>Poa labillardierei</i> Steud. var. <i>labillardierei</i>	Tufted perennial.

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Table 2 Summary of the significant interaction effects found in repeated measures ANOVAs for F_v/F_m and F_o for overall model and then each growth form. All other factors in the model were not significant or are encapsulated in these interaction terms. ANOVAs distinguished where treatment differences occurred within each time period. Significance levels (α) calculated using Bonferroni's correction (Zar, 1999). Bold font indicates significant values.

Parameter	Growth form/Species	Effect	D.F.	α	P-value
F_v/F_m		time*treatment* growth form	18, 199	.05	<.0001
Within interaction comparisons	Forbs	time*treatment	6, 38	.025	.0144
		treatment (day 1)	2, 23	.0167	.0896
		treatment (day 9)			.0093
		treatment (day 18)			.0011
		treatment (day 27)			.6882
	Non-grass. monocot	time*treatment	6, 38	.025	.0114
		treatment (day 1)	2, 23	.0167	.1746
		treatment (day 9)			.4784
		treatment (day 18)			.0128
		treatment (day 27)			.7974
	Shrubs	time*treatment	6, 38	.025	.1214
		time	3, 19		<.0001
	Grasses	time*treatment	6, 38	.025	.0161
		treatment (day 1)	2, 23	.0167	.9071
		treatment (day 9)			.0783
		treatment (day 18)			.0004
		treatment (day 27)			.6110
F_o		time*treatment* species(growth form)	24, 204	.05	.0139
Within interaction comparisons	Forbs				
	<i>D. repens</i>	time*treatment			.8633
	<i>C. glaucescens</i>	time*treatment			.0040
		treatment (day 1)	2, 11	.0167	.9010
		treatment (day 9)			.3966
		treatment (day 18)			.0064
		treatment (day 27)			.(2003
	Non-grass. monocot				
	<i>D. caerulea</i>	time*treatment			.4733
	<i>T. umbellatum</i>	time*treatment			.0282
	Shrub				
	<i>C. tetragona</i>	time*treatment			.3909
	<i>W. fruticosa</i>	time*treatment			.4754
	Grasses				
	<i>P. labillardierei</i>	time*treatment	6, 14	.025	.2956
	<i>T. australis</i>	time*treatment			.5248

510 **Table 3** Mean minimal fluorescence (F_0), for Australian native plants. Data were pooled across
 511 treatments and treatment days.

Growth form	Species	Mean $F_0 \pm$ standard error
Forb	<i>D. repens</i>	208 ± 5.7
	<i>C. glaucescens</i>	202 ± 13.1
Non-grass monocot	<i>D. caerulea</i>	231 ± 6.3
	<i>T. umbellatum</i>	325 ± 22.8
Shrubs	<i>C. tetragona</i>	260 ± 20.3
	<i>W. fruticosa</i>	198 ± 8.6
Grasses	<i>T. australis</i>	185 ± 9.3
	<i>P. labillardierei</i>	205 ± 17.2

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FIGURE LEGENDS

Fig. 1 Mean above- and below-ground biomass, ($n=4$, $\pm$ SE) for Australian native species after 3 consecutive heatwaves. HH = hot day/night, HC = hot day/cool night, and CC = cool day/night. Lower standard error bars are for above-ground biomass and upper standard error bars are for below-ground biomass means. Letters represent post-hoc tests between treatments for each species. Columns not connected by the same letter represent significant differences.

Fig. 2 Sacrificed leaf mass (dead leaves) as a percentage of total above ground shoot mass (alive and dead leaves) in Australian native grasses. Data were pooled from both grass species, *Poa labillardierei* and *Themeda australis* ($n=8$, $\pm$ SE). HH = hot day/night, HC = hot day/cool night, and CC = cool day/night. Columns not connected by the same letter represent significant differences. Whiskers represent 97.5% and 2.5% confidence intervals.

Fig. 3 Mean photosynthetic efficiency, F_v/F_m , for Australian native a) grasses, b) forbs, and c) non-grass monocots after 3 simulated heatwaves. Shrubs showed no significant effect and are therefore not included. Species data pooled ($n=8$, $\pm$ SE). Day 1 = first day of study period prior to heatwave exposure, Day 9 = immediately after 1st heatwave, day 18 = after 2nd heatwave and day 27 = after 3rd heatwave. HH = hot day/night, HC = hot day/cool night, and CC = cool day/night. Boxplots not connected by the same letters are significantly different. Whiskers represent 97.5% and 2.5% confidence intervals.

Fig. 4 Mean minimal fluorescence (F_o), for Australian native forb, *Carpobrotus glaucescens* ($n=4$, $\pm$ SE). Day 1 = first day of study period prior to heatwave exposure, Day 9 = immediately after 1st heatwave, day 18 = after 2nd heatwave and day 27 = after 3rd heatwave. HH = hot day/night, HC = hot day/cool night, and CC = cool day/night. Boxplots not connected by the same letters are significantly different. NOTE: high F_o values indicate stress on PSII. Whiskers represent 97.5% and 2.5% confidence intervals.

Fig. 5 The acclimation of species to heatwave treatments. The capacity to cope with high temperatures was estimated by the temperature at which F_v/F_m was midway between the maximum and minimum values (a), the temperature at which F_o exceeded both 150% of the minimum F_o and was higher than the midpoint between the minimum and maximum F_o (b), the temperature at which the relative electrical conductivity (REC) exceeded 20% (c), or the absolute high temperature F_v/F_m value (d). HH = hot day/night, HC = hot day/cool night, and CC = cool day/night. Asterisks indicate where the Bayesian models found there was >95% chance that the HC or HH treatments were higher than the CC control. Whiskers represent 97.5% and 2.5% confidence intervals.

Figure 1:

Figure 2:

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Figure 4:

Figure 5:

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SUPPLEMENTARY INFORMATION

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Appendix S1

583

Average values for F_v/F_m , F_o and Root:Shoot ratios for 4 replicate plants of 2 species from 4 different functional groups exposed to 3 different temperature regimes over 30 days. HH – Hot days and Hot nights, HC – Hot days and Cool nights, CC – Cool days and Cool nights. Values below averages in smaller font are standard deviations. Species experiencing heat waves (HH and HC) experienced 3 x 3 day heat waves over the 30 day period with 6 days recovery between each heat wave.

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			F_v/F_m				F_o				Leaf biomass sacrificed (g)	% leaf mass sacrificed	Root:shoot Day 30
			Day 1	Day 9	Day 18	Day 30	Day 1	Day 9	Day 18	Day 30			
<i>Forbs</i>	<i>Dichondra repens</i>	CC	0.828	0.846	0.843	0.830	205	201	207	221	-	-	0.234
			0.014	0.009	0.006	0.036	12	11	23	20			0.213
		HC	0.832	0.817	0.826	0.829	196	195	209	219	-	-	0.197
			0.008	0.026	0.017	0.019	6	54	10	13			0.075
		HH	0.829	0.823	0.815	0.848	209	211	213	211	-	-	0.326
			0.013	0.013	0.025	0.016	12	12	31	9			0.063
	<i>Carpobrotus glaucescens</i>	CC	0.826	0.854	0.853	0.863	226	165	138	166	-	-	0.093
			0.007	0.016	0.009	0.007	34	21	13	34			0.005
		HC	0.801	0.825	0.804	0.847	192	207	246	189	-	-	0.069
			0.015	0.025	0.028	0.018	17	28	44	19			0.030
		HH	0.838	0.829	0.806	0.844	254	177	260	207	-	-	0.152
			0.009	0.017	0.014	0.017	53	15	60	32			0.055
<i>Non grass Monocots</i>	<i>Dianella caerulea</i>	CC	0.775	0.814	0.819	0.789	231	238	221	239	-	-	0.425
			0.036	0.006	0.010	0.028	32	31	17	28			0.084
		HC	0.802	0.819	0.794	0.782	231	220	253	226	-	-	0.351
			0.016	0.008	0.007	0.045	37	9	18	10			0.039
		HH	0.804	0.821	0.814	0.784	239	212	228	232	-	-	0.479
			0.021	0.010	0.012	0.036	16	31	14	12			0.145
	<i>Thelionema umbellatum</i>	CC	0.823	0.854	0.852	0.839	163	355	355	327	-	-	0.221
			0.016	0.003	0.009	0.026	23	26	79	67			0.053
		HC	0.825	0.833	0.822	0.826	250	335	351	355	-	-	0.274
			0.019	0.006	0.012	0.011	15	46	46	7			0.045

		HH	0.851 0.003	0.829 0.014	0.828 0.007	0.821 0.029	350 25	358 27	328 128	420 30	-	-	0.202 0.034
Shrub	<i>Calytrix tetragona</i>	CC	0.816 0.020	0.834 0.010	0.844 0.001	0.842 0.012	219 68	222 86	269 44	276 52	-	-	0.165 0.015
		HC	0.813 0.016	0.821 0.005	0.787 0.014	0.819 0.012	172 48	270 20	330 86	280 57	-	-	0.177 0.031
		HH	0.825 0.008	0.833 0.002	0.778 0.052	0.816 0.008	237 59	290 36	251 94	311 119	-	-	0.238 0.046
		CC	0.797 0.006	0.855 0.007	0.841 0.016	0.817 0.023	197 9	(200 11	182 50	199 13	-	-	0.243 0.033
		HC	0.804 0.008	0.846 0.019	0.827 0.010	0.814 0.030	187 9	219 53	187 44	188 50	-	-	0.198 0.020
		HH	0.805	0.830	0.812	0.805	171	212	224	206	-	-	0.238
	<i>Westringia fruticosa</i>	CC	0.755 0.034	0.814 0.014	0.739 0.050	0.732 0.132	209 18	169 42	202 11	221 35	11.220 4.833	33.143 11.967	0.326 0.076
		HC	0.744 0.024	0.818 0.010	0.320 0.303	0.739 0.118	161 21	189 13	171 39	185 29	28.265 8.731	72.619 12.496	0.687 0.277
		HH	0.761 0.028	0.807 0.024	0.453 0.287	0.757 0.057	194 34	185 28	180 48	155 41	19.553 8.501	59.807 28.826	4.24 7.982
		CC	0.792 0.031	0.824 0.016	0.822 0.019	0.805 0.012	210 42	238 12	221 11	244 31	17.058 5.466	35.515 9.269	0.807 0.124
		HC	0.820 0.026	0.773 0.025	0.237 0.154	0.594 0.397	216 21	229 46	174 50	156 88	23.540 5.654	90.186 8.000	10.548 12.195
		HH	0.790 0.023 0.004	0.784 0.016 0.014	0.277 0.341 0.003	0.585 0.391 0.016	207 38 11	267 37 29	165 78 23	138 119 17	17.273 0.928	76.263 25.634	7.353 10.783 0.058
Grass	<i>Themeda australis</i>	CC	0.755 0.034	0.814 0.014	0.739 0.050	0.732 0.132	209 18	169 42	202 11	221 35	11.220 4.833	33.143 11.967	0.326 0.076
		HC	0.744 0.024	0.818 0.010	0.320 0.303	0.739 0.118	161 21	189 13	171 39	185 29	28.265 8.731	72.619 12.496	0.687 0.277
		HH	0.761 0.028	0.807 0.024	0.453 0.287	0.757 0.057	194 34	185 28	180 48	155 41	19.553 8.501	59.807 28.826	4.24 7.982
		CC	0.792 0.031	0.824 0.016	0.822 0.019	0.805 0.012	210 42	238 12	221 11	244 31	17.058 5.466	35.515 9.269	0.807 0.124
		HC	0.820 0.026	0.773 0.025	0.237 0.154	0.594 0.397	216 21	229 46	174 50	156 88	23.540 5.654	90.186 8.000	10.548 12.195
		HH	0.790 0.023 0.004	0.784 0.016 0.014	0.277 0.341 0.003	0.585 0.391 0.016	207 38 11	267 37 29	165 78 23	138 119 17	17.273 0.928	76.263 25.634	7.353 10.783 0.058
	<i>Poa labillardierei</i>	CC	0.792 0.031	0.824 0.016	0.822 0.019	0.805 0.012	210 42	238 12	221 11	244 31	17.058 5.466	35.515 9.269	0.807 0.124
		HC	0.820 0.026	0.773 0.025	0.237 0.154	0.594 0.397	216 21	229 46	174 50	156 88	23.540 5.654	90.186 8.000	10.548 12.195
		HH	0.790 0.023 0.004	0.784 0.016 0.014	0.277 0.341 0.003	0.585 0.391 0.016	207 38 11	267 37 29	165 78 23	138 119 17	17.273 0.928	76.263 25.634	7.353 10.783 0.058
		CC	0.792 0.031	0.824 0.016	0.822 0.019	0.805 0.012	210 42	238 12	221 11	244 31	17.058 5.466	35.515 9.269	0.807 0.124
		HC	0.820 0.026	0.773 0.025	0.237 0.154	0.594 0.397	216 21	229 46	174 50	156 88	23.540 5.654	90.186 8.000	10.548 12.195
		HH	0.790 0.023 0.004	0.784 0.016 0.014	0.277 0.341 0.003	0.585 0.391 0.016	207 38 11	267 37 29	165 78 23	138 119 17	17.273 0.928	76.263 25.634	7.353 10.783 0.058

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590 Appendix S2 – Expected relationships between F_v/F_m and temperature based on generalised logistic curves fitted using a Bayesian Monte Carlo
 591 iterative modelling approach. Error bars indicate: 1) the 95% CI for where the curves are 50% between the upper and lower asymptotes, and 2)
 592 the high temperature F_v/F_m asymptote.

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Growth form	Species	F _v /F _m 50% temperature (°C)			HC-CC comparison		HH-CC comparison	
		CC	HC	HH	Difference (°C)	P(HC > CC)	Difference	P(HH > CC)
Forbs	<i>D. repens</i>	44.2 (43.2/45.5)	44.1 (43.3/45.3)	44.3 (43.3/45.7)	0.0 (-1.6/1.5)	0.445	0.1 (-1.5/1.8)	0.525
	<i>C. glaucescens</i>	45.4 (43.7/46.7)	44.5 (43.4/45.8)	45.0 (43.6/46.3)	-0.8 (-2.6/1.2)	0.177	-0.3 (-2.4/1.8)	0.337
Shrubs	<i>C. tetragona</i>	45.4 (44.4/46.3)	45.6 (44.4/46.6)	44.2 (43.5/45.2)	0.2 (-1.3/1.7)	0.594	-1.1 (-2.4/0.3)	0.045
	<i>W. fruticosa</i>	44.7 (43.4/46.4)	42.3 (39.4/45.7)	44.6 (43.6/45.8)	-2.3 (-5.6/1.1)	0.073	0.0 (-2.0/1.8)	0.459
Grasses	<i>T. australis</i>	46.1 (44.9/47.0)	48.0 (47.3/48.7)	47.2 (46.4/48.0)	1.9 (0.7/3.3)	0.998 *	1.2 (0.0/2.5)	0.958 *
	<i>P. labillardierei</i>	43.1 (42.0/44.0)	44.3 (43.5/45.5)	44.1 (42.9/45.9)	1.2 (0.0/2.7)	0.963 *	1.0 (-0.4/3.0)	0.880

Appendix S3 – Expected midpoint F_v/F_m (and 95% CI) from models in Fig. S1. The probabilities that the midpoint F_v/F_m for the HC and HH curves are higher than those for the CC curves were estimated based on the proportion of 20 000 iterations in the Bayesian models where those conditions were met. Results where the HC or HH models were 95% likely to be higher than the CC models are marked with an asterisk.. Any 95% CI that include the upper limit of 55.2°C may underestimate the F_v/F_m 50%.

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Growth form	Species	High temperature F_v/F_m			HC-CC comparison		HH-CC comparison	
		CC	HC	HH	Difference	P(HC > CC)	Difference	P(HH > CC)
Forbs	<i>D. repens</i>	0.26 (0.19/0.33)	0.26 (0.20/0.33)	0.13 (0.06/0.20)	0.00 (-0.08/0.10)	0.504	-0.12 (-0.22/-0.02)	0.005
	<i>C. glaucescens</i>	0.27 (0.18/0.36)	0.19 (0.11/0.27)	0.26 (0.18/0.34)	-0.07 (-0.18/0.03)	0.086	0.00 (-0.12/0.11)	0.442
Shrubs	<i>C. tetragona</i>	0.13 (0.08/0.18)	0.06 (0.01/0.11)	0.07 (0.02/0.11)	-0.06 (-0.13/0.00)	0.022	-0.05 (-0.12/0.00)	0.027
	<i>W. fruticosa</i>	0.20 (0.04/0.29)	0.15 (0.02/0.27)	0.30 (0.24/0.36)	-0.05 (-0.21/0.14)	0.254	0.09 (0.00/0.26)	0.958 *
Grasses	<i>T. australis</i>	0.23 (0.16/0.28)	0.08 (0.01/0.14)	0.06 (0.00/0.12)	-0.14 (-0.22/-0.05)	0.001	-0.16 (-0.24/-0.07)	0.000
	<i>P. labillardierei</i>	0.38 (0.34/0.43)	0.35 (0.31/0.39)	0.46 (0.41/0.49)	-0.02 (-0.08/0.03)	0.163	0.08 (0.01/0.14)	0.987 *

601

602 Appendix S4 – Expected high temperature F_v/F_m asymptote (and 95% CI) from models in Fig. S1. The probabilities that the high temperature
603 F_v/F_m for the HC and HH curves are higher than those for the CC curves were estimated based on the proportion of 20 000 iterations in the
604 Bayesian models where those conditions were met. Results where the HC or HH models were 95% likely to be higher than the CC models are
605 marked with an asterisk.

606

607 Appendix S5 – Expected relationships between F_0 and temperature based on penalised splines fitted using a Bayesian Monte Carlo iterative
 608 modelling approach. Error bars indicate the 95% CI for where the curves are at least 50% above the minimum F_0 and higher than midway
 609 between the minimum and maximum values of the curves.

610

Growth form	Species	F _o 50% temperature (°C)			HC-CC comparison		HH-CC comparison	
		CC	HC	HH	Difference	P(HC > CC)	Difference	P(HH > CC)
Forbs	<i>D. repens</i>	47.7 (37.0/55.2)	43.1 (37.2/55.2)	44.2 (37.6/55.2)	-4.5 (-17.4/14.1)	0.257	-3.5 (-16.6/11.7)	0.330
	<i>C. glaucescens</i>	45.3 (41.3/51.2)	45.3 (43.1/46.3)	44.6 (42.3/45.7)	0.0 (-7.3/4.2)	0.649	-0.6 (-6.9/3.6)	0.337
Shrubs	<i>C. tetragona</i>	46.3 (44.7/48.5)	46.2 (41.7/55.2)	45.7 (44.3/46.6)	0.0 (-5.8/9.0)	0.327	-0.5 (-3.8/1.3)	0.231
	<i>W. fruticosa</i>	43.7 (34.8/55.2)	38.6 (35.4/41.8)	45.3 (44.3/46.1)	-5.0 (-18.1/4.0)	0.164	1.6 (-10.2/10.3)	0.786
Grasses	<i>T. australis</i>	55.1 (55.2/55.2)	51.5 (37.3/55.2)	52.7 (45.6/55.2)	-3.5 (-17.8/0.0)	0.004	-2.3 (-9.5/0.0)	0.004
	<i>P. labillardierei</i>	52.6 (44.1/55.2)	41.1 (37.0/55.2)	48.0 (42.3/55.2)	-11.4 (-18.0/4.7)	0.048	-4.5 (-12.1/5.9)	0.171

611

612 Appendix S6 – Expected F_o 50% values (and 95% CI) from models in Fig. S2. The probabilities that the F_o 50% for the HC and HH curves are
613 higher than those for the CC curves were estimated based on the proportion of 20 000 iterations in the Bayesian models where those conditions
614 were met.. Any 95% CI that include the upper limit of 55.2°C may underestimate the F_o 50%.

615

616 Appendix S7 – Expected relationships between REC and temperature based on generalised logistic curves fitted using a Bayesian Monte Carlo
 617 iterative modelling approach. Error bars indicate the 95% CI for where the curves increase above an REC of 20%.

618

Growth form	Species	REC 20% temperature (°C)			HC-CC comparison		HH-CC comparison	
		CC	HC	HH	Difference (°C)	P(HC > CC)	Difference (°C)	P(HH > CC)
Forbs	<i>D. repens</i>	53.9 (53.2/54.4)	55.2 (55.2/55.2)	53.9 (53.4/54.6)	1.3 (0.8/2.0)	1.000 *	0.0 (-0.7/0.9)	0.480
	<i>C. glaucescens</i>	55.0 (54.4/55.2)	54.2 (51.5/55.2)	55.2 (55.2/55.2)	-0.8 (-3.5/0.7)	0.220	0.1 (0/0.8)	0.468
Shrubs	<i>C. tetragona</i>	52.5 (51.7/53.6)	55.2 (55.0/55.2)	53.6 (52.7/54.5)	2.7 (1.6/3.5)	1.000 *	1.0 (-0.2/2.3)	0.914
	<i>W. fruticosa</i>	55.2 (55.2/55.2)	51.3 (47.6/53.5)	55.2 (55.2/55.2)	-3.7 (-7.5/-1.6)	0.002	0.0 (0.0/0.0)	0.004
Grasses	<i>T. australis</i>	50.0 (48.3/51.6)	50.5 (48.7/52.3)	50.1 (48.7/51.2)	0.5 (-1.9/2.9)	0.655	0.1 (-2.2/2.2)	0.523
	<i>P. labillardierei</i>	52.4 (51.3/53.9)	50.4 (49.6/50.8)	53.2 (51.6/54.5)	-2.0 (-3.5/-0.7)	0.000	0.8 (-1.3/2.7)	0.764

619

620 Appendix S8 – Expected REC20% values (and 95% CI) from models in Fig. S3. The probabilities that the REC20% for the HC and HH curves
621 are higher than those for the CC curves were estimated based on the proportion of 20 000 iterations in the Bayesian models were those
622 conditions were met. Results where the HC or HH models were 95% likely to be higher than the CC models are marked with an asterisk. Any
623 95% CI that include the upper limit of 55.2°C may underestimate the REC20%.

624