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Physiological plasticity of metabolic rates in the invasive honey bee and an endemic Australian bee species

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Abstract Seasonal variation in metabolic rate and evaporative water loss as a function of ambient temperature were compared in two species of bees. The endemic blue-banded bee, *Amegilla chlorocyanea,* is a solitary species that is an important pollinator in the south-west Australian biodiversity hotspot. Responses were compared with the European honeybee, *Apis mellifera,* naturalised in Western Australia almost 200 years ago. Metabolic rate increased exponentially with temperature to a peak in both species, and then declined rapidly, with unique scaling exponents and peaks for all species-by-season comparisons. Early in the austral summer, *Apis* was less thermally tolerant than *Amegilla*, but the positions reversed later in the foraging season. There were also significant exponential increases in evaporative water loss with increasing temperature, and both season and species contributed to significantly different responses. *Apis* maintained relatively consistent thermal performance of metabolic rate between seasons, but at the expense of increased rates of evaporative water loss later in summer. In contrast, *Amegilla* had dramatically increased metabolic requirements later in summer, but maintained consistent thermal performance of evaporative water loss. Although both species acclimated to higher thermal tolerance, the physiological strategies underpinning the acclimation differed. These findings may have important implications for understanding the responses of these and other pollinators to changing environments and for their conservation management.

Keywords Thermal tolerance · Standard metabolic rate · Evaporative water loss · Physiological plasticity · *Apis mellifera* · *Amegilla chlorocyanea*

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Introduction

Ecological resilience and sustainability are increasingly becoming goals of conservation management and ecological restoration (SERI Science and Policy Working Group 2004), requiring better understanding and attention to ecosystem services and plant/animal interactions (Dennis et al. 2007; Dixon 2009; Dunstan et al. 2013; Gómez 2003; Menz et al. 2011). Among these interactions has been an increasing effort to understand the energetics of pollination (Abrol 2005; McCallum et al. 2013; Tomlinson et al. 2014), particularly that of insects as they are the predominant pollinator group of angiosperms (Kevan et al. 1993; Machado and Lopes, 2004; Petanidou and Vokou 1990). The energetics and water loss patterns of ectothermic animals such as insects are highly dependent upon thermal performance,

and changes in the thermal environment may have powerful impacts upon pollination energetics.

In the south-west of Western Australia, a biodiversity hotspot threatened by habitat loss (Myers et al. 2000), there are two bee species that dominate many of the ecological systems, and are thought to be key pollinators. One is an invasive bee with a global distribution, the European honeybee *Apis mellifera* Linnaeus, 1758 (hereafter referred to as *Apis*). *Apis* is perennially active in the south-west of Western Australia. The other is the endemic Australian blue-banded bee, *Amegilla (Notomegilla) chlorocyanea* Cockerell, 1914 (hereafter referred to as *Amegilla*), which is an important pollinator in native ecosystems (Houston 2000). In the south-west, *Amegilla* is a summer-active species, first emerging as adults in late November, and persisting through to March (Didham et al. unpublished). Questions have been raised as to the effectiveness of *Apis* as a pollination vector (Aizen and Feinsinger 1994; Dick et al. 2003; Gross 2001; Gross and Mackay 1998; Mendes do Carmo and Franceschinelli, 2004; Paton 1993) and also whether its presence in a landscape displaces and disrupts native pollination associations (Kearns et al. 1998). Both species also have potentially high agricultural and horticultural value for crop pollination (Hogendoorn et al. 2006, 2007; Winfree et al. 2011).

Given broad geographical and habitat tolerances, we expect *Apis* to be an effective physiological generalist, capable of tolerating a broad range of temperatures, compared to perhaps more specialised responses of an Australian endemic insect that exhibits specific, strong seasonal patterns of behaviour. If physiological plasticity can compensate for seasonal variation (Glanville and Seebacher 2006; Seebacher 2005; Seebacher and Franklin 2012), then an innately more flexible species may be at lower risk when changing conditions disrupt the species' thermal tolerances or thermo-energetic requirements.

Our aims were to construct thermal performance curves characterised by biologically relevant parameters, rather than arithmetic models that are difficult to interpret (Angilletta 2006; Kovac et al. 2007), and to compare these curves between species and season. Such comparisons should be informative of how flexible our target species are to seasonal shifts. We constructed thermal performance curves for *Apis* and *Amegilla*, by measuring the responses of wild-captured bees to acute temperatures at the start and the end of the austral summer, to assess the plasticity of each species following seasonal thermal acclimation.

Materials and methods

Experimental design

To test for seasonal changes in thermal physiology, bees were captured in two distinct flight periods: early in the main forage season (October for *Apis* and early January for *Amegilla*) and late in the main forage season (late February to early March for both species). Although these periods differ between the species, this reflects their predominant active forage season in the field (Didham et al. unpublished data), and should result in a test of seasonal acclimatisation to increasingly hot and dry conditions in the transition into the hottest period of summer (February/March). Temperatures in the Perth region average 20.3 ± 0.19 °C in September, 29.1 ± 0.40 °C in December and 31.7 ± 0.30 °C in February (Australian Bureau of Meteorology), and February also averages 18 days warmer than 30 °C, compared to 12 in December and 0 in September. Initially, three nominal experimental temperatures (T_a) were selected (15, 25 and 35 °C) to establish respiratory responses to T_a. However, it became apparent that *Amegilla* could not survive at $T_a \leq 25$ °C, and all initial T_a treatments were below the upper tolerance thresholds of either *Amegilla* or *Apis* in late summer. Therefore, the experimental T_a range was extended within logistical constraints of the availability of respirometry equipment. The resulting T_a treatments for *Apis* were 15, 25, 30 and 32 °C during early summer, and 15, 25, 35, 40, 42 and 45 °C during late summer. For *Amegilla*, the T_a treatments were 25, 30, 32, and 35 °C during early summer, and 25, 35, 40 and 42 °C during late summer. These temperatures encompass the mean annual range for the Perth region (15.7–21.1 °C in July to 25.7–37.5 °C in February), and also capture the extreme temperatures on record for our study period (15.8 °C in October to 44.5 °C in February) according to the Australian Bureau of Meteorology online database. Temperature treatments were administered at one T_a treatment, selected at random, per day.

Naïve bees were individually wild-caught in the hour preceding respirometry trials, and each bee was only tested once (then frozen at the completion of measurement to ensure that there was no chance of repeated measurement). Six *Apis* workers were allocated to each ambient temperature treatment, resulting in a final pool of 30 independent measures in early summer, and 39 independent measures in late summer (i.e. 69 bees in total). Due to the difficulty and unpredictability in obtaining *Amegilla*, sample sizes were restricted to only four to six individuals per treatment, resulting in 19 independent measures in early summer, and 14 independent measures in late summer (i.e. 33 bees in total).

Flow-through respirometry

A three-channel flow-through respirometry system was constructed following the guidelines of Withers (2001). Dry (~4 % humidity), compressed air was passed through a mass flow controller (Aarlborg DFC-17, USA) at a rate of 50 mL min^{-1} (ATPD). In each season, the same on-site compressor was used, which has previously been shown to have minimal to

no season fluctuations in excurrent humidity. The incurrent air was passed into a cylindrical, 5 mL glass chamber containing a bed of soda lime (CaOH, Sigma chemicals, Australia) separated from the bee by a plug of cotton wool. The excurrent airstream was dried with Drierite (anhydrous calcium sulphate, W.A. Hammond Company Ltd, USA) and passed through a Qubit S151 infra-red CO_2 analyser (Qubit systems Inc, USA). Although Drierite has an established affinity for CO_2, and has been suggested to increase washout times, White et al. (2006) demonstrated that Drierite is an appropriate desiccant to use for steady-state metabolic measurements such as those undertaken in this study. Each Qubit was calibrated and checked for linearity using a three-point calibration by linear regression with N_2 gas (0 ppm CO_2), compressed air (350 ppm CO_2) and a calibration gas mix (1500 ppm CO_2; BOC Gases, Australia). Each respirometry system was maintained at constant T_a as detailed above in a custom-built incubator accurate to 0.1 °C. The interior of the incubator was unlit, and the headspace within the metabolic chambers was small (approximately 1 mL), both of which served to relax and restrict movement of the bees quite rapidly. The real-time data acquisition software allowed observation of the bees' activity accurate to the last 30-s washout period of the respirometry system. Since activity was generally low and stable (see below), we infer that we measured resting metabolic rate (RMR). We assume that body temperature (T_b) in the metabolic chamber is nearly equal to T_a of exposure. Analogue data signals from all equipment were interfaced to a computer via a DataQ 710 data acquisition board (DataQ Instruments, USA) collected every 10 s using a custom-written Visual Basic version 6.0 data acquisition program. Each bee was weighed to 0.1 mg using a Metler Toledo AG245 balance immediately before and after the respirometry trials, and was exposed to a randomly selected T_a for one hour. A minimum additional 30-minute baseline was always run before and after the respirometry trials. This typically resulted in eight individual measurements taken per day, and a full range of T_a treatments being completed in five days for each species in each season, during which period local conditions remained relatively stable during both spring and summer.

Statistical analyses

Estimation of RMR was made using custom-written Visual Basic v6.0 software that calculated VCO_2 according to Withers (2001), by averaging the lowest and most stable 20-min period. Discontinuous breathing patterns were not observed at any time during the study. Potential confounding variation in the pre-treatment body mass of individual bees between species, seasons and T_a treatment categories was tested using a linear model (LM) with Gaussian error distribution. For this analysis, T_a was treated as a categorical (not continuous) variable because we were more interested in any inadvertent differences in the body mass of experimental subjects among treatment categories than in the pattern of mass variation with T_a. We tested for patterns of normality and heteroscedasticity by plotting the residuals of the mass model against the fitted values. Where no pattern in the residual distribution was found, no transformation was applied to normalise the mass data prior to analysis. A model averaging approach was carried out in the 'MuMIn' package (Barton 2013) in R 3.0.3 (R Core Team 2014) using Akaike Information Criterion values (AICc, corrected for small sample bias) to find the most parsimonious model of variation in body mass (Burnham and Anderson 2002). If mass was found to differ significantly between treatment categories, then all metabolic measurements (VCO_2) were allometrically corrected using mass 0.75 [after Chown et al. (2007)], and evaporative water loss (EWL) measurements were allometrically corrected using mass 0.67 [after Chown et al. (1998) and Edney (1977)], prior to statistical analysis.

There is a strong expectation that the thermal response of VCO_2 should be unimodal and asymmetrical (Angilletta 2006), with an exponential increase to a maximal threshold followed by rapid decline representing the thermal inhibition of metabolism (Chown and Gaston 2008; Chown and Nicholson 2004; Huey and Kingsolver 1993; Withers 1992). Although traditional analytical approaches generally attempt to linearise the relationship, there is debate over the potential pitfalls of this approach (Gurka and Edwards 2011; Hayes and Shonkwiler 2006), as opposed to simply fitting a non-linear function directly. Consequently, we estimated unimodal model fits to our data using a three-parameter bi-exponential function (Fig. 1):

$$VCO_2 = y_0 \times (e^{k \times T_a} - e^{T_a - T_d}) \qquad (1)$$

where y_0 is the intercept of the curve at $T_a = 0$ °C, k is the metabolic scaling exponent, and T_d the temperature where performance begins to diverge (negatively) from the exponential pattern of increase expected in the absence of thermal inhibition (Fig. 1). The theoretical concept of T_d in Eq. 1 is consistent with empirical evidence that 'preferred' T_a in poikilotherms is generally lower than the T_a at which metabolism reaches its numerical peak (i.e. T_{MMR} in Fig. 1) (Martin and Huey 2008). T_{MMR} was calculated from the first-order derivative of Eq. 1 [following Fornberg and Sloan (1994)] obtained using the 'numDeriv' package (Gilbert and Varadhan 2012) in R 3.0.3, solving for the maximum T_a where the instantaneous rate of change in VCO_2 was zero using the 'uniroot' function.

Following respirometry measurements, evaporative water loss was calculated gravimetrically as final body mass minus initial body mass. Although advances in technology have made it possible to measure changes in partial pressures of H_2O vapour using dew-point hygrometers, such equipment was not available in this study. Gravimetric techniques have
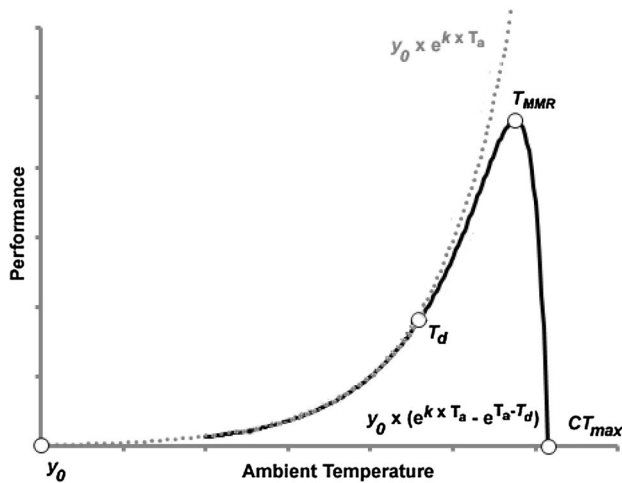
Fig. 1 Our theoretical left-skewed thermal performance curve (*solid curve*) was fitted using a two-phase bi-exponential function incorporating an initial exponential increase in VCO_2 with T_a, combined with an exponential penalty above a hypothetical temperature of divergence T_d. The T_a at which maximum VCO_2 is observed (T_{MMR}) can be calculated empirically from the function where the instantaneous rate of change is equal to zero

a long heritage of use (Ahearn and Hadley 1969; Brodie 2005; Mason et al. 2013; Seymour 1974; Smigel and Gibbs, 2008; Thurman 1998; Tomlinson and Phillips 2012), and are likely to be only slightly less accurate than values calculated from in-line respirometry measurement in the sense that they are an average over the entire period of respirometry, rather than an average of the lowest period of EWL. Evaporative water loss in insects also tends to increase exponentially with increasing T_a (Hadley 1994), both below as well as above the point of metabolic inhibition (Tomlinson and Phillips 2012)

and the effects of species, season and T_a on EWL were modelled using a simple exponential model:

$$EWL = y_0 \times e^{k \times T_a} \tag{2}$$

Thermal performance curves for both VCO_2 and EWL were fitted by an iterative non-linear regression algorithm in the 'stats' package in R v3.0.3 (R Core Team 2014). The *nls* function uses a convergence criterion that seeks to reduce the imprecision of the residual sum-of-squares by refining the parameters of the regression function in a similar manner to conventional reduced-squares linear regression (Bates and Watts 1988; Ritz and Streibig 2008). Once a convergent fit was resolved, unique values were fitted for the function parameters on the basis of species and season, resulting in a number of permutations of the basic form (Table 1). The explanatory power of each of these permutations was compared between species and seasons with the Akaike Information Criterion (Burnham and Anderson 2002) using the AICcmodavg package (Mazerolle 2013). Statistical effects of species, season and species-by-season interactions were quantified by comparing the most parsimonious curve of each treatment to the convergent common curve using the anova.nls function described by (Ritz and Streibig 2008).

Results

Variation in body mass

On average, wild-caught *Apis* weighed 101.4 ± 2.76 mg in early summer and 90.6 ± 2.54 mg in late summer, while *Amegilla* weighed 69.4 ± 4.76 mg in early summer and

Table 1 Akaike information criterion comparison (AICc) of the unique parameter permutations of the thermal performance curve of (A) metabolic rate and (B) evaporative water loss

Model	k	AICc	ΔAICc	w_i	Log likelihood
(A)					
$y_0 \times (e^{k[fac] \times T_a} - e^{T_a - T_d[fac]})$	10	399.6	0.00	0.66	−188.5
$y_0[fac] \times (e^{k \times T_a} - e^{T_a - T_d[fac]}$	10	401.0	1.46	0.32	−189.2
$y_0[fac] \times (e^{k[fac] \times T_a} - e^{T_a - T_d[fac]})$	13	407.7	7.39	0.02	−188.2
$y_0[fac] \times (e^{k[fac] \times T_a} - e^{T_a - T_d})$	10	419.2	19.6	0.00	−198.3
$y_0[fac] \times (e^{k \times T_a} - e^{T_a - T_d})$	7	422.0	22.4	0.00	−203.3
$y_0 \times (e^{k[fac] \times T_a} - e^{T_a - T_d})$	7	422.0	22.5	0.00	−203.4
$y_0 \times (e^{k \times T_a} - e^{T_a - T_d})$	4	439.7	40.1	0.00	−215.6
(B)					
$y_0[fac] \times e^{k[fac] \times T_a}$	9	−502.2	0.00	0.92	261.2
$y_0 \times e^{k[fac] \times T_a}$	6	−496.9	5.35	0.06	254.9
$y_0[fac] \times e^{k \times T_a}$	6	−494.3	7.97	0.02	253.6
$y_0 \times e^{k \times T_a}$	3	−473.4	28.8	0.00	239.9

Unique parameters are denoted in each permutation of the function by [fac]

62.2 ± 4.61 mg in late summer. Model averaging indicated strong support for these values representing significant differences in mean body mass between species ($F_{1,86} = 75.7$, $p = 1.98 \times 10^{-13}$), but only equivocal support for the observed differences between seasons being statistically significant (in one of the two most parsimonious models; Table S1). In addition, there was significant variation in the average mass of individuals allocated to different temperature treatments ($F_{7,86} = 7.20$, $p = 8.99 \times 10^{-7}$), with bees randomly allocated to the 15, 30 and 35 °C temperature treatments being significantly heavier than bees allocated to other temperature treatments.

Metabolic rate

After accounting for variation in individual bee mass, the response of CO_2 production (VCO_2) to temperature (T_a) was hump-shaped (Fig. 2a, b), with VCO_2 increasing exponentially up to a peak, and then declining rapidly. The hump-shaped thermal performance profiles differed
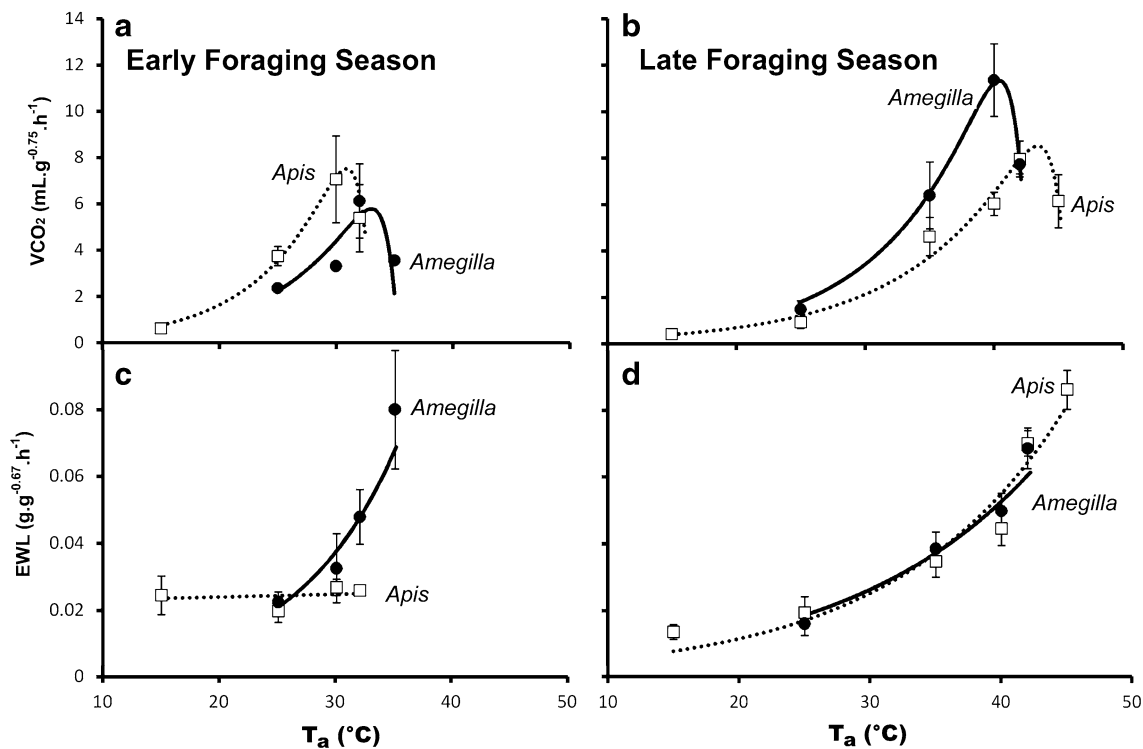

Fig. 2 Physiological responses of *Apis mellifera* (*square*, *dashed lines*) and *Amegilla chlorocyanea* (*dark circle*, *solid lines*) to varying experimental temperatures early in the foraging season in October (*Apis*) and January (*Amegilla*) and late in the foraging season in March (both species): **a**, **b** metabolic rate (VCO_2), and **c**, **d** evaporative water loss (EWL). Data are presented as means ± 1 SEM of values standardised by allometric scaling of individual body mass (see "Methods" for details)

Table 2 Coefficients (± 1 SEM in parentheses) for the most parsimonious model of effects of species and season on thermal responses of metabolic rate (VCO_2) and evaporative water loss (EWL), where y_0 is the y-intercept, k is the scaling exponent, and T_d the temperature of divergence from the base pattern of exponential increase

	VCO_2				EWL	
	y_0	k	T_d	T_{MMR}	y_0	k
Early summer						
Amegilla	0.073 (0.045)	0.137 (0.020)	30.5 (0.76)	33.0 (0.05)	0.0003 (0.0003)	0.155 (0.029)
Apis		0.155 (0.022)	28.0 (0.91)	30.9 (0.08)	0.021 (0.011)	0.006 (0.019)
Late summer						
Amegilla	0.073 (0.045)	0.128 (0.016)	37.3 (0.79)	40.4 (0.06)	0.002 (0.002)	0.082 (0.024)
Apis		0.112 (0.015)	40.7 (0.79)	43.4 (0.05)	0.002 (0.001)	0.086 (0.013)

For VCO_2, T_{MMR} is estimated from the first derivative of the fitted function, and errors represent the precision of estimation

between the four species-by-season treatment groupings, as compared to a fitted model with a common thermal performance curve for all individuals (ΔAICc = 40.1, w_i = 0.66; Table 1). The best non-linear model incorporating species was not significantly different to the common performance curve (anova.nls F = 1.77, df = 3, p = 0.158), but a model incorporating season was (anova.nls F = 14.6, df = 2, p = 3.27 × 10^{-6}). However, the most parsimonious model resolved a common y_0 (intercept point), but unique k (the scaling exponent) and T_d (point of divergence from the exponential) for each species-by-season comparison (anova.nls F = 11.1, df = 6, p = 4.47 × 10^{-9}, Tables 1, 2; Fig. 2a, b). In early summer, VCO_2 was higher in *Apis* than in *Amegilla* at a given T_a, but reached T_d sooner (Fig. 2a), whereas in late summer the reverse pattern was shown, with *Amegilla* having substantially higher VCO_2 but reaching T_d at a lower T_a than *Apis* (Fig. 2b). These differences in the pattern of thermal performance had substantial impacts on VCO_2, which was 1.3-fold greater near to T_{MMR} for *Apis* compared to *Amegilla* earlier in their flying seasons. The reversed pattern later in their flying seasons generated a 1.3-fold greater VCO_2 near to T_{MMR} in *Amegilla* compared to *Apis*.

The resulting thermal performance curves (Fig. 2a, b) had estimated T_{MMR} (homologous with M_TR of Tomlinson and Phillips (2012)) values that reflected the same species-by-season interaction effect (Table 2), with *Apis* having a much wider range of apparent acclimatisation response between seasons, despite the much lower amplitude of season variation observed in VCO_2, compared with *Amegilla* (Fig. 2a, b).

Evaporative water loss

There were exponential increases in evaporative water loss (EWL) as a function of T_a for most species-by-season combinations tested, except for *Apis* in early summer (Fig. 2c). Thermal performance models incorporating species (anova.nls F = 5.52, df = 1, p = 0.021) and season (anova.nls F = 16.8, df = 1, p = 9.04 × 10^{-5}) were significantly different to the common performance curve, but the most parsimonious model (AICc = 502.2, w_i = 0.92; Table 1) resolved a unique y_0 and k for each of the species-by-season comparisons (anova.nls F = 8.27, df = 6, p = 4.81 × 10^{-7}). As might be expected, *Apis* exhibited a substantially higher and more rapidly increasing EWL during late summer than during early summer (Fig. 2c, d). Surprisingly, the reverse was true for *Amegilla*, in which EWL increased more rapidly and over a lower T_a range in early summer than in late summer (Fig. 2c, d).

Discussion

Study species

Apis mellifera is thought to have evolved in tropical Africa and differentiated into geographic races as they spread from Africa into Europe and Asia (Whitfield et al. 2006). *Apis* is a eusocial species, building hives of related individuals that become a repository of resources, as well as a thermal shelter, since the hive is thermally and hygrically regulated (Fahrenholz et al. 1989; Human et al. 2006; Kronenberg and Heller 1982). *Amegilla chlorocyanea* is an Australian endemic bee belonging to an afro-asiatic node of the Anthophorini, which probably arose in oriental Asia (India to south-east China) and expanded outwards (Dubitzky 2007). Most species of *Amegilla* build solitary nests (Cardale 1968), although this has not been specifically studied for western Australian taxa. As well as presumably fulfilling similar generalist pollination roles as *Apis*, *Amegilla* also has a number of specialist associations with "buzz pollinated" flowers (Buchmann 1983; Duncan et al. 2004; Houston and Ladd 2002), probably enhancing its value as a pollinator in natural systems.

Curve fitting

Our first aim was to develop a process of fitting non-linear thermal performance curves that could be readily compared across a range of factors, including species and season. The bi-exponential non-linear regression that we have developed and employed here displays the correct theoretical form of exponential increase, followed by rapid decline above a critical temperature (Angilletta 2006; Schmidt-Nielsen 1983; Withers 1992). We have two reasons to believe this approach has greater utility than other curve-fitting approaches. First, the nature of the non-linear equation is that it fits the number of parameters that define the equation and no more. Unlike a generalised additive modelling (GAM) approach, there is no danger of over-fitting to the data by adding increasingly complex splines (Wood 2008). This non-linear scaling (regression) approach is, however, dependent upon assumptions concerning the shape of the thermal performance curve. As long as there is a good theoretical basis for the shape of the curve, the non-linear scaling (regression) approach is a more objective and parsimonious approach than a GAM.

The main advantage of our curve-fitting approach is that each of the parameters of the curve can be readily translated into a factor that has biological meaning, and these parameters can be compared directly to gain insight into patterns of variation. Hence, we are able to conclude that there is a common minimum metabolic rate for all species-by-season

combinations where the intercept y_0 does not differ between groups, nor is in fact different from zero. Furthermore, we can speculate on different physiological mechanisms at play in response to temperature as a result of different metabolic scaling exponents, k. Finally, at least two measures of critical temperatures can be derived from the equation. The point of maximal metabolic rate, T_{MMR}, is analogous to M_TR of Tomlinson and Phillips (2012), and would also represent a classical optimal temperature, where performance reaches its peak. Recent discussion has explored the incongruity between physiological performance optima, and the preference in ectotherms for a body temperature below this point (Huey and Kingsolver 1989; Martin and Huey 2008), and has suggested that T_{opt} may need more accurate definition (Martin and Huey 2008). The temperature of divergence, T_d, which we find as the point where the thermal performance curve departs from a pure exponential pattern, is congruent with these alternative interpretations of T_{opt}. We also suggest that, with a broader range of temperature treatments, it may be possible to solve the equation for the temperature where VCO_2 is equal to zero, as an estimate of CT_{max}, similar to the extrapolation inherent in the curve fit reported by (Kovac et al. 2007). This approach is, however, dependent upon assumptions concerning the shape of the thermal performance curve. While these assumptions are theoretically sound, application to a wider range of taxa is required to understand how different parameters respond ecologically and evolutionarily (Felsenstein 1985; Garland and Adolph 1994).

Our second aim was to quantify the physiological plasticity of two species of hymenopteran pollinators that are common in the native ecosystems of south-western Western Australia. We acknowledge that we cannot infer adaptiveness for the seasonal changes that we report, since this requires a phylogenetically informed context of more than just two species (Felsenstein 1985; Garland and Adolph 1994). However, the data have value in establishing that there are substantial differences in the thermal performance curves of the two species, which will have ecological implications for their foraging distances, food requirements and pollination capability. Further, substantial seasonal shifts occur in thermal performance of both metabolic rate and susceptibility to water loss. Both these aspects of the thermal physiology of an ectotherm have substantial implications for the ecology and ecosystem services provided by the species, both spatially and seasonally.

Comparative physiology

Both species had high metabolic rates for their body size during the early austral summer, roughly 146 ± 1.8 % of allometric expectations for *Apis* and 142 ± 0.8 % for *Amegilla*, compared with the relationship established by

Chown et al. (2007) for metabolic responses at 25 °C. Later in the season, values were closer to allometric expectations, at 119 ± 4.8 % for *Apis* and 131 ± 6.2 % for *Amegilla*. Despite the increase above allometric expectations, the metabolic rates that we report in the late summer are congruent with those reported by Kovac et al. (2007, 2014), and earlier reports by Lighton and Lovegrove (1990) that Chown et al. (2007) used in their allometry. Therefore, early in the foraging season, when the bees are acclimated to cooler temperatures, it might be expected that 25 °C represents thermal elevation above the average season conditions to which the bees are acclimated. This therefore represented a moderate heat stress, and resulted in metabolic rates that greatly exceeded allometric expectations.

The intraspecific differences between seasons appear to reflect real patterns in acclimatisation of thermal metabolic responses in these bees, but the quantitative magnitude of this acclimatisation is equivalent to the level of interspecific differences frequently observed among species (Chown et al. 2007). Previously, *Apis* has not shown convincing evidence of physiological acclimation, even after greater seasonal differences than those that we compared here (Kovac et al. 2007). Physiological plasticity has long been identified as an important component of variation enabling species to respond to environmental variability (Feder 1987; Glanville and Seebacher 2006; Lewontin 1969; Seebacher 2005; Nespolo et al. 2001), and the seasonal patterns that we describe conform to the seasonal patterns established by Cloudsley-Thompson (1991). Of the two, *Apis* shows very little change in the magnitude of metabolic rate in response to T_a, but is capable of shifting its thermal tolerance to match environmental conditions. When compared with *Amegilla*, which shows tolerance to higher temperatures later in the forage season, but also substantially higher metabolic rates at T_{MMR} later in the forage season, it suggests that *Apis* is buffered in some way from the energetic implications of changing climate. From our two-species comparison, it is impossible to define the mechanism of this buffering, which may result from their sociality and the reserves stored in the hive (Fewell and Winston 1992, 1996), or may result from phylogenetic differences in their physiology. Future studies would benefit from consideration of the role that intraspecific plasticity plays in the physiological responses to environmental change (Tomlinson et al. 2014).

The patterns of EWL that we found remain difficult to interpret. In the late summer, there appears to be no difference in the thermal performance of EWL between *Apis* and *Amegilla*. Further, there appears to be little seasonal acclimation in the EWL of *Amegilla*. Hence, the statistical differences that we found all appear to relate to the very low, flat performance curve for *Apis* early in the season ($k = 0.006$; Fig. 2c). This would suggest that *Apis* loses

less water in response to changing temperatures early in the season, which is out of step with expectations (Cloudsley-Thompson 1991). Although there are few comparable studies of seasonal effects on EWL, we remain unconvinced that these data are realistic. Our difficulty in resolving this statistically may result from our gravimetric calculations of EWL, which incorporate greater variance than measuring atmospheric relative humidity in the excurrent airstream. Given the general conservative patterns in the rest of the data, the response of *Apis* early in the season is unusual and needs further study.

At both ends of the season, T_{MMR} was similar between *Apis* and *Amegilla*, but the patterns of acclimation differed between the species, where *Apis* was the less thermally tolerant species early in the summer, but became the more tolerant species later in the summer. Assuming that VCO_2 represents a suitable analogue of caloric requirement (Schmidt-Nielsen 1983; Withers 1992), *Apis* seems able to shift its metabolic response to remain active, with relatively consistent peak metabolic rate despite seasonal variation in abiotic conditions (and presumably also variation in resources such as nectar quantity and quality acting as energetic constraints). Speculation by Brooks (1988) suggests that *Amegilla* as a genus might be biogeographically restricted by weak tolerance for lower temperatures, and indeed we found that *Amegilla* entered chill coma and often died following our 15 °C and even 25 °C exposures. This indicates that they can only forage during times when ambient temperature rarely falls below this lower limit.

The mechanism by which either species can produce such pronounced shifts in their metabolic responses from early to late forage times remains unclear from this study, but may relate to their short life cycle, which is just six weeks in *Apis* (Sammataro and Avitabile 2011). Thus, through the duration of our study, new cohorts of bees may have been generated that were pre-acclimated to higher temperatures, essentially supporting a developmental acclimation interpretation (Angilletta 2009). While such a pathway of acclimation potentially matches the energetics and foraging capacity of the bees to their climatic conditions (Berrigan 1997; Berrigan and Partridge 1997; Booth and Kiddell 2007), the mechanisms supporting this pathway remain a highly productive area of research (Angilletta 2009).

The mechanism notwithstanding, however, the seasonal shift in thermal tolerance, while similar for both species, implies much higher energetic requirements for *Amegilla* later in the flying season, both intra- and interspecifically. This interpretation is consistent with the 'beneficial acclimation' hypothesis (Angilletta 2009; Chown and Nicholson 2004), in that *Apis* has a broader tolerance range and a longer active season than *Amegilla*, but with an additional insight that its energetic requirements are more consistent throughout the year.

Conclusions

Our study provides evidence of substantial seasonal shifts in the thermal performance of both metabolic rates and susceptibility to water loss in a social and a solitary bee. *Amegilla* is relatively flexible to changing thermal conditions, and is tolerant to higher T_a following seasonal acclimation, but at the expense of a remarkably high energetic requirement. This potentially constrains *Amegilla* to thermo-energetic envelopes where the influence of environmental temperatures on their metabolic requirements can be met ecologically (i.e. through resource acquisition), regardless of warm climatic conditions. *Apis*, on the other hand, is an invasive species and its broadly adaptable physiology would appear to contribute greatly to invasive capacity (Baker 1965; Strayer 1999; Wolff 2000) with the capacity for hive provisioning enabling honey bees to physiologically withstand periods of resource limitation. While both bees represent dominant hymenopteran pollination vectors in the south-west of Western Australia, *Amegilla* is probably the most valuable, and appears to be the more ecologically fragile species, with narrower thermal tolerance, and higher energetic requirements.

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