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7 **Differences in metabolic rate and evaporative**
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9 **water loss associated with sexual dimorphism in**
10 **thynnine wasps**
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51 Running Head: Sexual Dimorphism and Thermal Physiology of Thynnine
52 wasps.
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Abstract

30 Species with sexual dimorphism provide powerful study systems for understanding
adaptation to different lifestyles as it removes the potentially confounding effects of
phylogeny. Thynnine wasps have a stark sexual dimorphism where males fly patrols in
search of the flightless, predominantly fossorial females with which to mate. Using flow-
through respirometry, we tested the prediction that the highly active males of the
thynnine wasp *Zaspilothynnus nigripes* would have high metabolic rates (VCO_2) relative
to females. Further, the females, which spend more time underground, were predicted to
exhibit lower evaporative water loss (EWL) than males. Metabolic rate of both sexes
increased exponentially between 12 and 28 °C. As predicted, males had higher mass-
corrected VCO_2 at identical temperatures than females. Alternatively, there were no
40 differences in the EWL at identical temperatures between sexes, suggesting that
experiencing the same environmental conditions during mating may favour similar EWL.
Interestingly, *Z. nigripes* were estimated to undergo a decrease in metabolism at
approximately 30 °C. It is proposed that *Z. nigripes* persist despite sensitivity to high
temperatures using a combination of behavioural strategies and emergence during a
period of relatively benign climate that ameliorates the impacts of high temperatures.

Keywords: pollinator, wasp, metabolic rate, evaporative water loss, sexual dimorphism,
temperature

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Introduction

Interspecific variation in the standard metabolic rates (SMR) of insects is correlated with broad differences in ecology (Schmidt-Nielsen, 1983; Withers, 1992). In particular, after accounting for the pervasive effects of mass, taxa with lifestyles that have high energy demands are expected to have higher basal energy requirements at any given temperature to fuel these lifestyles (Reinhold, 1999). Since maximal metabolic rate (MMR) reflects a proportional increase above resting levels (Clarke and Fraser, 2004), higher MMR may impart a detectable signal of increased SMR by the increase in maintenance requirements of highly metabolically-active tissues (Addo-Bediako et al., 2002; Daan et al., 1990; Reinhold, 1999). The profile of the metabolic response curve across a range of temperatures may also be associated with differences in ecology. The decline in metabolic rate above some critical threshold, which is associated with thermal inhibition of metabolism by enzyme inactivation or denaturation {Withers, 1992 #105}, may be indicative of the thermal tolerance of the species and is expected to occur at higher temperatures in species from hot climates (Tomlinson and Phillips, 2012; Angilletta, 2006).

Differences in lifestyle activity may not exhibit a relationship with the evaporative water loss (EWL) of a species, though increasing endogenous metabolic water production may lead to increased water use efficiency (WUE; (Hinds and MacMillen, 1986; Klok and Chown, 1998; MacMillen and Hinds, 1983; Zachariassen et al., 1987)). However, other aspects of a species' biology do inform predictions concerning EWL. For example, there is a tendency for lower EWL in species that inhabit arid rather than mesic environments (Addo-Bediako et al., 2001; Klok and Chown, 1998; Mason et al., 2013; Zachariassen, 1996; Zachariassen et al., 1987). In an even more extreme contrast, fossorial taxa typically exhibit higher water losses than related epigeal taxa (Ahearn and Howarth, 1982; Hadley et al., 1981; Humphreys and Collis, 1990), as there is reduced selection pressure on water conservation capabilities due to the reduced ambient temperature (T_a) fluctuations and higher relative humidity underground (Gray, 1968; Hadley, 1970; Mason et al., 2013; Nevo, 1999). On average, more active taxa in

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4 drier environments are expected to have low EWL and high WUE compared to less
5 active taxa in high humidity environments.
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10 Theoretically, in a species where males and females have different lifestyles, there
11 may also be differences in physiological traits between the sexes. Associated with these
12 different lifestyles is often a level of sexual dimorphism, the morphological differences
13 between males and females of a species beyond the reproductive structures (Kardong,
14 2012). Species with sexual dimorphism, and contrasting lifestyles between the sexes,
15 provide powerful study systems for understanding adaptations, as it removes the
16 90 potentially confounding effects of phylogeny. Studies of differences in resting metabolic
17 rates between sexes in invertebrates have typically recorded higher resting metabolic
18 rates (RMRs) in males than females (Rogowitz and Chappell, 2000; Shillington, 2005;
19 Shillington and Peterson, 2002a; Tanaka and Ito, 1982; Watson and Lighton, 1994),
20 although some research reports no differences in sexually dimorphic species
21 (Humphreys, 1977; Penteado and Hebling-Beraldo, 1991). Higher metabolic rates in
22 males have usually been ascribed to higher energy lifestyles of males actively searching
23 for, or defending access to mates (Rogowitz and Chappell, 2000; Shillington, 2005;
24 Shillington and Peterson, 2002b; Watson and Lighton, 1994). For example, studies of
25 100 flight energetics in the Hymenoptera have suggested that this trend is likely to arise
26 through higher energy requirements of the tissues associated with flight (Suarez, 2000;
27 Suarez et al., 2005) and structural dimorphism in the mass of flight muscles between
28 males and females (Darveau et al., 2014; Radloff et al., 2003; Skandalis and Darveau,
29 2012). Alternatively, differences in EWL between the sexes has received little attention,
30 despite its importance in understanding adaptations to the physical environment (Chown
31 and Nicholson, 2004; Withers, 1992). However, in most cases minimal variation would
32 be expected as both sexes typically inhabit environments with similar water vapour
33 potentials, and similar evaporative gradients.
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55 110 The Thynnidae (Hymenoptera) is a diverse family of strongly sexually dimorphic
56 solitary wasps (Figure 1), with over 1600 species in Australia (Brown, 2009) and a lesser
57 diversity in South America (Austin et al., 2004). Males fly rapidly, patrolling for the
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4 flightless females, which typically crawl to a prominent perch and release a pheromone
5 plume to attract mates (Alcock and Gwynne, 1987; Bohman et al., 2014; Schiestl et al.,
6 2003). Pairs copulate in flight, with males carrying the female to food sources (Alcock
7 and Gwynne, 1987), typically nectar or the sugary secretion of scale insects or lerps
8 (Brown and Phillips, 2014). When not *in copula* females remain underground, while
9 during the day males continue to feed or search for mates (Ridsdill Smith, 1970). As the
10 flight of the males should require much higher energy expenditure than the terrestrial
11 locomotion of females, this pronounced difference in locomotion between the sexes is
12 more extreme than other species where energetic differences between the RMR of males
13 and females have been demonstrated (Rogowitz and Chappell, 2000; Shillington, 2005;
14 Shillington and Peterson, 2002b; Watson and Lighton, 1994).
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26 Here we present the first study comparing the metabolic physiology and thermal
27 tolerance of volitant and fossorial sexes of the same species. We compared SMR (VCO_2)
28 and EWL between sexes of *Zaspilothynnus nigripes* (Hymenoptera:Tiphiidae:Thynninae,
29 Guér 1842) at a range of temperatures. Specifically, we aimed to test whether males have
30 higher standard metabolic rates than females, resulting from maintenance costs of highly
31 metabolically active tissues such as flight muscles. Males were also expected to have
32 lower EWL than females as a result of adaptation to environments with low atmospheric
33 relative humidity, compared to the subterranean environments which females typically
34 encounter. Further, males should have higher WUE than females as a result of both their
35 volitant locomotion and aerial lifestyle. By investigating metabolic rates near the high
36 end of the thermal tolerance of *Z. nigripes*, we also tested if females show greater
37 sensitivity to high temperatures.
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50 **Method**

51 *Study species*

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57 *Zaspilothynnus nigripes* is distributed throughout the higher rainfall parts of
58 south-western Australian from Eneabba to Esperance (Phillips et al., 2014), favouring
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4 areas with sandy soil. Males generally only fly at temperatures above 18 °C (Peakall,
5 1990). The flying season extends between August and November, though at any one site
6 they typically fly for approximately two months at the beginning of spring (R. Phillips,
7 unpublished data).
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15 Thirty-seven pairs of *Z. nigripes* were collected *in copula* (eight naïve pairs per
16 experimental temperature, with the exception of five pairs at the highest temperature
17 treatment) by sweep netting pairs feeding on *Pericalymma ellipticum* (Myrtaceae) from a
18 single site (Ambergate Reserve, Busselton, Western Australia; 33.7 °S, 115.3 °E) and
19 transported from the field in vials held at 4 °C in a portable refrigerator. Respirometry
20 trials began early in the day. Wasps were assumed to be post-absorptive because they did
21 not feed overnight, and were given no opportunity to feed prior to introduction to the
22 respirometer.
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28 29 30 *Respirometry trials* 31

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34 160 The standard metabolic rate (SMR) of both sexes was measured using flow-
35 through respirometry after protocols described by (Withers, 2001) and consistent with
36 (Tomlinson and Phillips, 2012). Due to the limitations of small body size, only CO₂
37 production (VCO₂) was considered a reliable and sensitive enough measure of SMR.
38 Body temperature (T_b) in the metabolic chamber is assumed to be nearly equal to T_a of
39 exposure. Ambient temperatures for testing were nominally 12, 25, 28, 30 and 32 °C. A
40 pilot study (Tomlinson and Phillips, unpublished) found that *Z. nigripes* lost co-
41 ordination in the chamber in some individuals at 30 °C and suffered frequent mortality
42 following acute exposure at T_a = 32 °C. We used these observations to define our upper
43 experimental temperature. The rationale for selecting this range of temperatures was to
44 provide a low T_a reference point for minimal metabolism below the T_a that elicits activity
45 in the field (approximately 18 °C), but to focus attention at the upper limits, to more
46 closely refine the T_a where metabolism is compromised. The 25 °C temperature was
47 chosen to facilitate direct comparison with the data presented by Chown et al (2007),
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4 where this was the most commonly measured experimental temperature in studies of
5 insect metabolism.
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10 Experimental T_a was maintained to within ± 0.1 °C in a custom-built temperature
11 controlled cabinet. A dual-channel flow-through respirometry system was constructed
12 where compressed air was passed through a mass flow controller (Aarlborg DFC-17,
13 USA) at a rate of 50 mL/min (ATPD). The incurrent air was passed into a cylindrical,
14 5mL glass chamber containing a bed of soda lime (CaOH, Sigma-aldrich Chemicals,
15 180 USA) separated from the wasps by a plug of cotton wool. The excurrent airstream
16 was dried with Drierite (anhydrous calcium sulphate, W.A. Hammond Company Ltd,
17 USA) and passed through infra-red CO₂ analysers (Qubit S151, Qubit systems Inc, USA).
18 The CO₂ analysers were calibrated and checked for linearity using a three-point
19 calibration with N₂ gas (0 ppm), air (350 ppm) and a calibration gas mix (1500 ppm;
20 BOC Gases, Australia). Analogue data signals from all equipment were interfaced to a
21 computer via a DataQ 710 data acquisition board (DataQ Instruments, USA) collected
22 every 10 seconds using a custom-written Visual Basic version 6.0 data acquisition
23 program. Trials were run for 60 minutes, with a minimum 30 minute baseline run before
24 and after the respirometry trials. The wasps were restrained by a plug of tissue paper so
25 that they were immobile. None of the individuals measured showed any discernible
26 attempt to move in the metabolic chamber. Although Drierite has an established affinity
27 for CO₂, and has been suggested to increase washout times, (White et al., 2006)
28 demonstrated that Drierite is an appropriate desiccant to use for steady-state metabolic
29 measurements such as those undertaken in this study. Body masses were measured before
30 and after trials using a Mettler-Toledo AD245 electronic scale (accurate to 10⁻⁴ g), and
31 the difference recorded as a gravimetric measure of evaporative water loss (EWL).
32 Metabolic traces were analysed by a custom-written Visual Basic program to determine
33 the minimum 20-minute average for VCO₂ at each T_a , in order to record the most
34 accurate standard metabolic rate of the wasps.
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4 *Statistical analysis*
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8 Using ANOVA, we tested for differences in weight between sexes, and between and
9 within temperature trials. For both sexes, we used ANCOVA to compare the slope of the
10 log-transformed body mass to VCO₂ relationship at 25 °C to the log-transformed
11 relationship across all insects reported by Chown et al. (2007). Where the slopes were
12 found not to differ significantly, our metabolic rates were scaled by body mass^{0.75} as
13 suggested by the interspecific relationship presented in (Chown et al., 2007). Similarly,
14 EWL was scaled by body mass^{0.67} as suggested by the interspecific relationship presented
15 in (Chown et al., 1998; Edney, 1977).
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24 Modelling the nonlinear response of VCO₂ to T_a may enable an accurate
25 estimation of the point at which metabolism begins to decline at high temperatures
26 (Chown and Nicholson 2004; Withers, 1992). The curve was estimated using a
27 biexponential (Ritz and Streibig, 2008) function similar in form to the (Logan et al.,
28 1976; Logan et al., 1985) function:
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$$VCO_2 = y_0 \times (\exp^{k \times T_a} - \exp^{T_a - T_d})$$

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38 Where y_0 is the intercept of the curve at 0°C, k is the metabolic scaling exponent and T_d ,
39 the temperature at which the line begins to deviate from an unmodified exponential
40 relationship (analogous to the T_{opt} of Logan et al. (1976)). This curve approximates the
41 exponentially-modified Gaussian distributions explored by (Angilletta, 2006). The
42 resulting function describes a non-linear increase to a maximal threshold, followed by
43 decline, as exhibited by the metabolism of ectotherms in response to temperature (Chown
44 and Nicholson, 2004; Withers, 1992). We define this maximal threshold as M_TR
45 (Tomlinson and Phillips, 2012), the point where metabolic rate begins to decline,
46 calculated as the point in the function where the instantaneous rate of change is zero. A
47 statistical approach to determining this threshold is not well-established. (Tomlinson and
48 Phillips, 2012) used post-hoc tests of ANOVA comparisons, and linear regression
49 techniques, to estimate a range in which metabolism began to significantly decrease, but
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4 could not provide a precise estimate of M_{TR} . Similarly, segmented regression (Muggeo,
5 2003) offers a more refined estimate, but it is predicated upon linear change that is not
6 representative of the thermal physiology of ectotherms.
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10 The effects of sex and T_a on EWL were modelled using an exponential model

$$11 \text{ EWL} = y_0 \times \exp^{\frac{T_a}{k}}$$

12 where y_0 is the intercept of the curve at 0°C and k is the scaling exponent. Thermal
13 responses curves for both $V\text{CO}_2$ and EWL were fitted by non-linear least squares
14 regression.
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24 For both the relationships of $V\text{CO}_2$ and EWL with temperature, differences in coefficients
25 between the sexes were tested for using the ANOVA.nls curve comparison (Ritz and
26 Streibig, 2008). This method follows a curve-comparison approach, by fitting an average
27 (convergent) curve, and then testing for significant deviations from the convergent fit by
28 attributing unique coefficients for discriminant factors (sex in this case). The most
29 parsimonious curve is described by a combination of shared and unique coefficients.
30 Where no discrimination between sexes was found we report the convergent equation as
31 the most parsimonious fit.
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41 Water Use Efficiency was calculated by the division of metabolic rate (to
42 approximate metabolic water production) by evaporative water loss (following (Klok and
43 Chown, 1998; Zachariassen et al., 1987). The effects of sex and T_a on WUE were
44 analysed using ANOVA. Statistical analyses were performed in *R* version 3.0.3 using
45 generic packages. Data are presented as mean \pm S.E.
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Results

260 *Changes in body mass during respirometry*

Male wasps (0.181 ± 0.006 g) were significantly heavier than female wasps (0.103 ± 0.005 g; $F_{1,67} = 130.8$, $p = 2.20 \times 10^{-16}$), however, there was no difference in body mass within sexes between T_a treatments ($F_{4,67} = 0.805$, $p = 0.562$). The slope of the log body mass to log VCO_2 relationship for *Z. nigripes* males ($F_{1,394} = 0.006$, $p = 0.938$) and females ($F_{1,394} = 0.019$, $p = 0.892$) was not different to that of the data set presented in [Chown et al. \(2007\)](#), which included all studied insect groups.

270 *Effects of temperature on metabolism and water loss*

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The response of VCO_2 to temperature increased to a plateau between the temperatures of 28 and 30 °C (Figure 2A). Using the bi-exponential thermal response equations, M_{TR} was estimated at $T_a = 30.3$ °C for males and $T_a = 30.4$ °C for females. Below these thresholds there were significant exponential increases in metabolism between 12 and 28 °C, (Table 1; Figure 2). There was a significant effect of sex on the thermal response curves of VCO_2 (Table 1; ANOVA.nls $F_{71,72} = 97.1$, $p = 2.0 \times 10^{-16}$), but the most parsimonious thermal response curves estimated a common intercept. The biological relevance of this intercept is unclear as these wasps are unlikely to encounter or be active at temperatures as low as 0 °C. The relationship between T_a and VCO_2 for males was approximated by the relationship $VCO_2 = 0.12528 \times (e^{0.12 \times T_a} - e^{T_a - 28.6})$, while the relationship for females was $VCO_2 = 0.12528 \times (e^{0.08 \times T_a} - e^{T_a - 30.3})$.

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EWL increased exponentially between 12 and 32 °C, (Figure 2B; Table 1) with no significant differences in the relationship between T_a and EWL between sexes ($F_{70,72} = 0.845$, $p = 0.434$; Figure 2B; Table 1). The relationship between T_a and EWL for males was approximated by the relationship $EWL = 0.002 \times e^{0.11 \times T_a}$, while the relationship for

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4 females was $EWL = 0.0003 \times e^{0.17 \times T_a}$. The convergent thermal response curve of the two
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6 sexes was $EWL = 0.008 \times e^{0.13 \times T_a}$.
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10 290 There was no change in WUE between 12 and 28 °C for both sexes ($F_{2,40} = 1.34$,
11 $p = 0.272$; Figure 2C; Table 1), but females were less efficient than males (*i.e.* they lost
12 more water per unit metabolic expenditure; $F_{1,40} = 5.90$, $p = 0.0197$). For males, WUE
13 did not exhibit a clear change above M_{TR} , but it decreased for females between 28 and 30
14 °C (Figure 2C).
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21 Discussion

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24 Both sexes of *Z. nigripes* showed an exponential increase in metabolic rate until
25 approximately 29 °C and an exponential increase in evaporative water loss across all T_a .
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27 300 Unlike most published studies, where metabolism continues to increase up to the
28 maximum T_a studied, data for *Z. nigripes* demonstrate the predicted deviation from
29 increasing metabolic rate above a critical temperature (Chown and Nicholson, 2004;
30 Withers, 1992), suggestive of the high end of the insects' thermal tolerance. The
31 metabolic rate of both sexes showed a plateau between 28 and 30 °C. By solving the bi-
32 exponential performance equation for the point where the instantaneous rate of change
33 (slope) was zero, it was estimated that maximum metabolic rate would occur at
34 approximately 30 °C for both sexes. This estimated peak in VCO_2 (M_{TR} *sensu*
35 (Tomlinson and Phillips, 2012) is very low compared to most other insects studied (*e.g.*
36 Lighton and Bartholomew, 1998; Klok et al., 2004; Lighton and Turner, 2004) . The most
37 striking comparisons in our data are that, despite having a similar M_{TR} , females had
38 lower metabolic rate and lower WUE than males at all T_a by a substantial margin,
39 suggesting divergent physiological limitations.
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54 *Differences in metabolic rate between the sexes*

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57 Our data support the hypothesis that male *Z. nigripes* have higher metabolic rates
58 than females, which is consistent with males having higher metabolic rates as a result of
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4 higher basal requirements of an active, flying lifestyle rather than the more sedentary
5 lifestyle of the female. However, this finding raises questions as to the mechanism
6 underlying these differences. Differences in metabolic rate between sexes could
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8 320 potentially arise from sex-linked genetic traits or endocrine differences associated with
9 reproduction (similar to (Ferraro et al., 1992; Poehlman et al., 1997)). However, the most
10 parsimonious explanation is that the large flight muscles maintained by the males, but not
11 by females, cause mass-specific changes to metabolic rates that might be exacerbated by
12 the energetic requirements of flight muscles (Suarez, 2000; Suarez et al., 2005).
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21 If sexual dimorphism in SMR results from the maintenance of highly-active tissue
22 required for flight in males, then it would be predicted that the entire thermal response
23 profile might shift to a higher energetic level in males, with a higher intercept of a non-
24 linear curve. Since the exponent k represents the biochemical effect of T_a upon metabolic
25 rate, a consistent exponent might be expected between sexes, through natural selection
26 330 acting similarly on physiological thermal responses of both sexes. However, differences
27 in the exponent suggest that while their basal metabolic rates do not differ, the
28 biochemical responses to the thermal environment do. Different oxidation pathways,
29 potentially arising through the use of different substrates, lead to variation in metabolic
30 output, with oxidation pathways that release more energy also consuming fuel substrates
31 more rapidly (Suarez, 2012; Suarez et al., 1996; Suarez et al., 1990; Suarez et al., 1997).
32 As such, differences in oxidation pathways between the sexes could contribute to the
33 intra-specific differences that we observe here. However, without deeper study of these
34 biochemical pathways, we cannot discriminate between the possibilities that differences
35 in VCO_2 could arise from different fuel substrates or different selection pressures on
36 metabolic rate between the sexes.
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52 While there have been investigations of sexual dimorphism and flight energetics
53 in insects, published reports of the effects of sexual dimorphism on standard metabolic
54 rates are few. Nonetheless, these studies provide strong support for our interpretation of
55 the metabolic rate data for *Z. nigripes*. In studies focusing on flight energetics,
56 differences in body mass (Rajagopal and Bursell, 1966; Terblanche et al., 2004) or flight
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4 muscle mass (Bursell and Kuwengwa, 1972; Darveau et al., 2014) have been the only
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6 350 detected cause of differences in metabolic rate between the sexes. However, there is
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8 strong evidence for higher standard metabolic rates in the more active sex in other groups
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10 of terrestrial invertebrate. (Rogowitz and Chappell, 2000) recorded higher RMRs in the
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12 males of two species of beetles (*Phoracantha* spp.) that actively seek and compete for
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14 mates by running up and down tree-trunks, compared to females that more sedately
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16 explore foraging and oviposition sites. (Tanaka and Ito, 1982; Watson and Lighton, 1994)
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18 recorded higher metabolic rates for male than female spiders (Lycosidae and Linyphiidae
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20 respectively), and male tarantulas (Theraphosidae) have been reported to have higher
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22 metabolic rates at rest (Shillington, 2005) and during locomotion (Shillington and
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24 360 Peterson, 2002b) than females. As illustrated by these examples, higher metabolic rate is
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26 observed where males actively search for females (Rogowitz and Chappell, 2000;
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28 Shillington, 2005; Shillington and Peterson, 2002a; Tanaka and Ito, 1982; Watson and
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30 Lighton, 1994), and sometimes compete for them (Rogowitz and Chappell, 2000). It has
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32 been generally concluded that this pattern results from selection for highly active males
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34 (Rogowitz and Chappell, 2000; Shillington, 2005; Shillington and Peterson, 2002b;
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36 Watson and Lighton, 1994), requiring elevated RMR in concert with the maintenance of
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38 energetically active tissues.

39 *Differences in EWL between the sexes*

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42 370 Although patterns of water loss resulting from temperature variation are
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44 informative for understanding physiological adaptations to thermal environments (Chown
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46 and Nicholson, 2004; Withers, 1992), there is a relative paucity of such studies in the
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48 entomological literature. We are not aware of any studies of EWL in the context of sexual
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50 dimorphism (in the hymenoptera or any other insect group), so expectations must be
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52 made based upon interspecific comparisons across a diverse range of organisms. The
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54 lower metabolic rate of female compared to male *Z. nigripes* implies lower metabolic
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56 water production (Woods and Smith, 2010) and potentially lower EWL. Theoretically,
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58 fossorial taxa should lack adaptations to low relative humidity and high temperature
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60 variability (Gray, 1968; Mason et al., 2013), leading to the prediction of higher
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4 380 susceptibility of female *Z. nigripes* to water loss than the aerial males. Our data clearly
5 refute these expectations, as EWL is similar between the sexes. The similarity in EWL
6 may arise because both sexes experience the same environmental conditions during their
7 prolonged mating flights. As these are likely to be the most physiologically challenging
8 conditions a female experiences, selection on EWL may be strongest during this activity.
9 The higher WUE in males is likely to result from a similar EWL and the high rate of
10 metabolic water production inferred to result from higher SMR in males.
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18 *Thermal sensitivity*

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22 390 As originally defined, M_{TR} was inferred where there was a significant reduction
23 in metabolism between one T_a and the subsequent, higher T_a (Tomlinson and Phillips,
24 2012). The calculation of M_{TR} described here, as the point where the nonlinear thermal
25 response curve flattens, or turns from increase to decrease, is conceptually consistent with
26 the original definition of (Tomlinson and Phillips, 2012). However, the method used here
27 allows a precise estimation of M_{TR} , rather than establishing a range of possible
28 temperatures where metabolism begins to decline. While not implying the lethality of the
29 critical thermal maximum (CT_{max}), M_{TR} does imply sensitivity where even small
30 increases in T_a will rapidly approach the CT_{max} . The compromise of metabolic capacity
31 should also compromise water regulation via the spiracles, increasing insensible water
32 loss, leading to a significant increase in EWL and a rapid decrease in WUE. The thermal
33 metabolic peak at approximately 30 °C for *Z. nigripes* (supported by frequent death at T_a
34 = 32 °C) was very low compared to values recorded for other insects, which range from
35 between >30 °C to >45 °C (Addo-Bediako et al., 2000; Lighton and Bartholomew, 1988;
36 Tomlinson and Phillips, 2012). Further, while the M_{TR} of *Z. nigripes* is higher than the
37 average temperatures within its geographic range during late spring and early summer
38 (Hijmans et al., 2005), they are low given the occasionally high temperatures that can
39 occur. Records for November indicate that at the meteorological station closest to our
40 collection site, there is on average 3.8 days per year above 30°C with extreme
41 temperatures reaching 36.9 °C (Australian Bureau of Meteorology online database;
42 www.bom.gov.au).
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6 Given their low M_{TR} , *Z. nigripes* probably avoids lethal high temperatures by its
7 spring flight phenology (Phillips et al., 2014), remaining underground in a cocoon for the
8 majority of the year after consuming their scarab beetle hosts (Ridsdill Smith, 1970). As
9 such, they are expected to conform more to the expectations of thermal specialists
10 described by (Angilletta, 2009) than to exhibit activity in a broad range of environmental
11 conditions. Investigation of CT_{max} (or M_{TR}) in a range of thynnine wasp species from
12 arid, temperate and tropical regions with a range of emergence times is needed to resolve
13 whether thynnine wasps have evolved greater resilience to high temperatures in response
14 to variation in climate or if they rely on behavioural mechanisms.
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22 23 24 **Conclusions**

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27 We have shown that the thermal response of metabolic rate of female *Z. nigripes*
28 is different to that of males. These differences are likely to have arisen through divergent
29 selection pressures on the two sexes arising from their contrasting life history. However,
30 the mechanisms underlying these differences in metabolic rate remain to be established.
31 We suggest that studies of sexual differences in insect taxa with marked sexual
32 dimorphism may provide interesting new pathways for the investigation the adaptations
33 associated with different ecological niches. In species with pronounced physiological
34 differences between the sexes, differences in how males and females interact thermo-
35 energetically with their physical environment may lead to differences in fitness. Given
36 the recent interest in understanding the role of physiological adaptations in determining
37 biogeographic processes and species' distribution (Chown et al., 2004; Gaston, 2009;
38 Gaston et al., 2009), these findings raise the possibility of the sexes playing differing
39 430 roles in limiting the distribution of some species.
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53 **Acknowledgments**

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57 440 We thank Professor Phillip Withers and Hai Ngo for the use of metabolic equipment. The custom-written
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Table 1: Coefficients of non-linear regressions describing metabolic rate (VCO₂) and water loss rate (EWL) in response to temperature. y_0 is the intercept of the curve at 0°C, k is the exponential scaling exponent and M_{7R} is the temperature where metabolic rate peaks and begins to decline. The comparison column reports the ANOVA.nls curve comparisons between a nonlinear model partitioning the coefficients by sex, and the convergent fit of the partitioned data. Statistical significance means that the partitioning contributed to greater fit and that the sexes exhibit a different relationship with Ta. Where a single value is reported for both sexes, this is the unpartitioned value resulting from the convergent curve, which was not significantly better resolved by partitioning.

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		<i>Zaspilothynnus nigripes</i>		Comparison
		♂	♀	
VCO₂	$y_0 \pm \text{S.E}$ (mL.g ^{-0.75} ,h ⁻¹)	0.125±0.060		F _{71,72} = 97.1 p = 2.0×10 ⁻¹⁶
	$k \pm \text{S.E}$	0.125±0.017	0.082±0.018	
	$T_d \pm \text{S.E}$ (°C)	28.6±0.62	30.3±1.08	
	M_{7R} (°C)	30.30 °C	30.37 °C	
EWL	$y_0 \pm \text{S.E}$ (mg.g ^{-0.67} ,h ⁻¹)	8.36×10 ⁻⁵ ±7.90×10 ⁻⁵		F _{70,72} = 0.845 p = 0.434
	$k \pm \text{S.E}$	0.134±0.572		

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Figure 1: A) *Zaspilothynnus nigripes* ♂ (left) and ♀ (right) showing extreme sexual dimorphism where males are volitant, while females are flightless, and are carried by the male to a food source during copulation (B). Photographs by A) S. Tomlinson and B) K. Smith.

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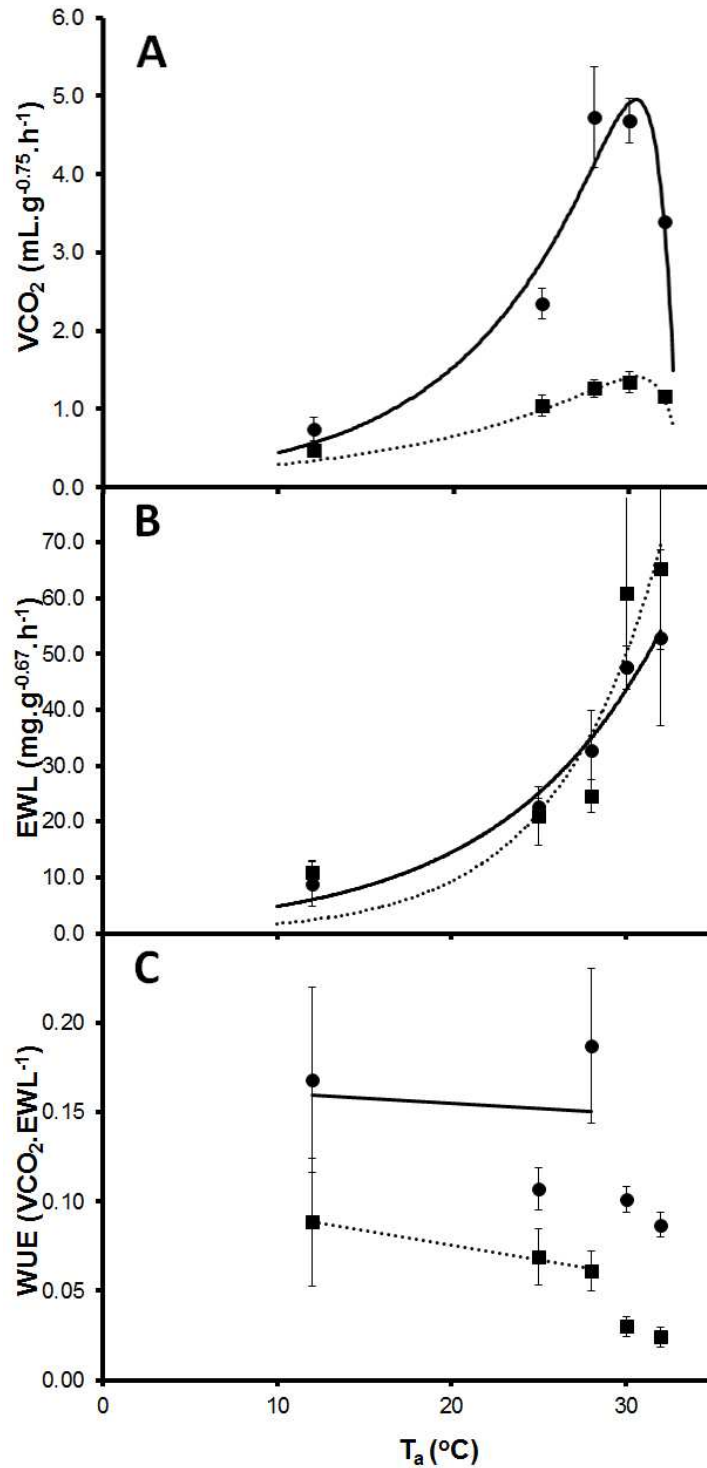


Figure 2: Responses of mass-corrected metabolic rates (A), evaporative water loss (B) and water use efficiency (C) of *Zaspilothynnus nigripes* ♂ (●) and ♀ (■) across T_a range 12 – 32 °C. The lines represent non-linear fits for metabolic and water loss rates, and the linear estimates of WUE for males (solid) and females (dashed). Values are presented as means \pm S.E.M.

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