Physiological Entomology

Physiological Entomology (2020) **45**, 7–15 DOI: 10.1111/phen.12310

Impacts of temperature on metabolic rates of adult *Extatosoma tiaratum* reared on different host plant species

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Abstract. Access to balanced nutrition enables optimum health and development, body repair, fat storage, increased fecundity and longevity. In the present study, we assessed the responses of a generalist leaf feeder (the phasmid *Extatosoma tiaratum*) reared continuously on one of three host plants, tree lucerne (*Chamaecyisus palmensis*), bramble (*Rubus fruticosus*) and *Eucalyptus* species, in a low fluctuating temperature environment until adulthood. Once all individuals reached adulthood, we exposed each individual to a ramping temperature event (starting at 25 °C and ramping the temperature at 0.25 °C min^{-1}) and assessed their metabolic rates ($\dot{V}_{CO_2}$) responses at specific temperature 'bins' (25, 30, 35, 40 and 42 °C). Sex but not diet influenced respiration and metabolic rate. Male individuals, on average, had a higher $\dot{V}_{CO_2}$ than females. Sex and diet were significant influences on $\dot{V}_{CO_2}$ at different temperatures. Metabolic rates at lower temperatures were not affected by sex or diet type. At 35 °C, metabolic rates were influenced by sex and diet, with males reared on bramble and tree lucerne having a higher metabolic rate than females reared on the same foodplant, whereas Eucalypt reared animals showing an opposite trend. Lifetime egg production by females was 150% higher on bramble compared with the other host plants. Incorporating fluctuating temperature ranges into experiments will further help to understand the impact that thermal stress will have on the growth, development, performance and survival of insects in a more variable climatic and nutritional landscape.

Key words. Climate change, diet, metabolic rates, phasmid, sex, stick insect, temperature, temperature stress point, thermal stress.

Introduction

Host plants influence the life history of insect herbivores in terms of the time taken to reach maturity, longevity and fecundity (Clissold *et al.*, 2009). For herbivorous insects to feed on plants, they have adapted to deal with suboptimal nutrition, as well as to resist and overcome plant defences; however, this comes at the cost of body repair, fat storage, survival, development and reproductive output (Arnó *et al.*, 2009; Fürstenberg-Hägg *et al.*, 2013). Generalist insect feeders (i.e. those insects that can feed on a range of plants across multiple families) may rely on one host plant once they have started feeding, becoming specialized on a single host plant, and even preferring specific aged leaves (e.g. older leaves) when feeding (Blüthgen & Metzner, 2007).

In the present study, we assessed the responses of a generalist leaf feeder, the phasmid *Extatosoma tiaratum* (Macleay, 1826; Phasmatodea; Phasmatidae), reared on one of three host plants subsequent to birth: tree lucerne (*Chamaecyisus palmensis*), bramble (*Rubus fruticosus*) and *Eucalyptus* species. These plant species have several chemical (e.g. macro- and micronutrients) and morphological differences that can influence foliage digestibility, insect herbivore growth and physiology if the insects are allowed to specialize feeding on a specific host plant throughout their lives. These species are also commonly used to rear *E. tiaratum*, even though they have very different native ranges: *Eucalyptus* is native to Australia, whereas

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tree lucerne and bramble are both introduced but commonly available.

Eucalyptus species (Myrtaceae) are native to Australia and are one component of the *E. tiaratum* diet from eastern coastal forests (Brock & Hasenpusch, 2009). They have tough adult leaves and a chemical profile based on terpene compounds (Moore *et al.*, 2004), as well as phenols and tannins (Macauley & Fox, 1980; Ohmart & Edwards, 1991). Exposure to these chemicals when insects are feeding can decrease the food intake, as well as the ability to digest proteins and cell wall carbohydrates. Tree lucerne (Fabaceae) is native to the Canary Islands and was introduced into Australia as fodder for livestock as a result of its high leaf protein content (Lambert *et al.*, 1989; Borens & Poppi, 1990; Lindeque & Rethman, 1998). As a food source for insect herbivores, it does have mechanical feeding deterrents on the leaves (hairs) and a chemical deterrent is the alkaloid sparteine (Ventura *et al.*, 2000). The introduction of brambles (Rosaceae) from the British Isles into Australia occurred in the 1840s; primarily for their for fruit (Blood, 2001). Brambles quickly escaped into the wild, becoming a significant weed, particularly in south-eastern Australia, and invaded forests where *E. tiaratum* was naturally found (CRC for Australian Weed Management, 2003). It is now considered to be an invasive weed. Brambles are highly nutritious but have extensive mechanical defences, including spikey leaves, as well as thorns and prickles (Bazely *et al.*, 1991; Pellissier, 2013). Their leaves contain secondary metabolites such as flavonoids, tannins and ellagic acid (Gudej & Tomczyk, 2004; Buřičová *et al.*, 2011), which can deter some insects from feeding (War *et al.*, 2012) or may reduce their metabolic efficiency.

Insects reared on different host plants throughout their life may exert different natural history preferences. For example, the apple maggot fly (Diptera: Tephritidae) females had a higher oviposition rate on either apple or hawthorn depending on prior exposure to the particular fruit (Papaj & Prokopy, 1988). Highly polyphagous species also show a host plant preference once they start developing. For example, the caterpillars of *Colias philoice* (Lepidoptera: Pieridae) feed on a range of Fabaceae host plants but show a feeding preference for a host that it has prior experience with (Karowe, 1989).

In the present study, we examined variations in the metabolic rate ($\dot{V}_{\mathrm{CO_2}}$) of adults when individuals feed on one of three host plants from birth until adulthood in a low fluctuating temperature environment. We used adult male and female individuals of *E. tiaratum* as a model to investigate whether diet affects the metabolic rate of these animals reared in captivity. Phasmids, or stick and leaf insects, occur worldwide, mostly in tropical regions. Out of the 3000 phasmid species identified globally (Brock & Hasenpusch, 2007), there are 200 known species in Australia that are primarily herbivorous and have a hemimetabolous life cycle. The study species are a sexually dimorphic species, with the spiny wingless females considerably larger and fatter than the winged males (Zborowski & Storey, 2003). The females also have abdominal margins with flattened plates and legs, which resemble leaves with spines, whereas the males are mottled in colour to mimic lichen (Brock, 2001). *Extatosoma tiaratum* can reproduce both sexually and asexually via parthenogenesis. Their eggs resemble seeds with an elaiosome, which are attractive to ants for this lipid-rich appendage, and this egg feature appears to be an adaptation for burial by ants that protects the egg from environmental hazards and predation by wasps (Hughes & Westoby, 1992). The first-instar nymphs are ant mimics, which allow them to escape the ant nest after hatching (Bedford, 1978). *Extatosoma tiaratum* flick away their eggs and so there is less certainty regarding which plants will be accessible when the nymph hatches. This species of insect has been kept in captivity in Australia from the 1960s onwards (Korboot, 1961; Hadlington, 1966) and Europe from the 1970s onwards (Brock, 1992) and it is known that they gain all their nutritional requirements from feeding on the leaves of a variety of host plants, including native species of *Acacia*, *Callicoma*, *Eucalyptus*, *Melaleuca* and *Leptospermum*. Introduced species such as holme oak, rose, bramble and guava are also palatable to these insects (Brock & Fry, 1999). The ability to adapt to feeding on a variety of plant species is determined by host plant exposure at an early age. It may be challenging to transfer individuals to a different plant species in the later stages of their development.

The metabolic rate is a measure of performance for all organisms. It measures the rate at which an organism transforms energy and resources changes with temperature exposure and body mass (Gillooly *et al.*, 2001; Clarke, 2006). Understanding the impact of temperature on an animal is vital knowledge. Accordingly, methods such as thermolimit respirometry have been devised to assess changes in metabolic rates with a constant increase in the temperature ramping rate (Lighton & Turner, 2004). For insects, temperature exposure assessments have most commonly measured the critical endpoint when an animal loses muscular control (critical thermal maximum, $\mathrm{CT_{max}}$) ignoring the changes in the metabolic rate up until this point. Understanding how the metabolic rate of insects changes with exposure to increasing temperatures is vital: the metabolic rate can be used as a measure of stress resilience (Krams *et al.*, 2018), as well as to determine more realistic $\mathrm{CT_{max}}$ endpoints using thermolimit respirometry techniques (Lighton & Turner, 2004). As the temperature rise, an insects metabolic rate (measured as $\dot{V}_{\mathrm{CO_2}}$ $\mathrm{ml\,h^{-1}}$) also rises, until it reaches a premortal plateau (Lighton & Turner, 2004; Andrew *et al.*, 2016). Metabolic rate responses to temperature have been measured for a range of insects, including ants (Lighton & Turner, 2004; Andrew *et al.*, 2016), *Helicoverpa* caterpillars (Betz and Andrew, unpublished data), silkworms (Boardman & Terblanche, 2015) and beetles (Verberk & Bilton, 2015), amongst others (Neven, 2000).

We were interested in whether host plant diets affect the metabolic rate of insects at different temperatures and female fecundity. Specifically, the present study investigated: (i) how metabolic rates vary between an adult male and female *Extatosoma tiaratum* exposed to increasing temperatures after being reared on different host plant species throughout their lives and (ii) whether the host plant influences stick insect fecundity as measured by egg production per female.

Materials and methods

Stick insects and their feeding treatments

Adult *Extatosoma tiaratum* feeding on a mixed diet of tree lucerne, *Eucalyptus* spp., *Acacia* spp., *Agonis* and holme oak laid the eggs used in this experiment. Nymphs hatched from eggs laid in May to June 2015, with emergence occurring in October 2015. Water was provided twice daily via hand-misting of each cage. Eighteen individuals (nine males and nine females) were reared from their first-instar to adulthood for one generation on one of three different host plants (three replicate individuals per sex per host plant): bramble (*Rubus fruticosus*), *Eucalyptus* sp. and tree lucerne (*Chamaecytisus palmensis*) in separate plastic insect cages (245 × 245 × 630 mm). Food was made available *ad libitum* to each individual used in this study before the sampling period and included a variety of leaf ages on freshly cut foliage. All animals in the trial were reared simultaneously at varying seasonal room temperatures (ranging from 12 to 25 °C) but in a similar environment of enclosure size, temperature, humidity and lighting over 4 months.

Respirometry measurements

We used flow-through respirometry to measure the metabolic rate ($\dot{V}_{CO_2}$) in adult *E. tiaratum*. Atmospheric air was pumped via a HiBlow pump (HB40; Techno Takatsuki (Japan) through soda-lime and Drierite (desiccant) (W. A. Hammond Drierite co. Ltd, Xenia, Ohio) columns to remove CO_2 and water (H_2O) from the air and then into two mass flow control valves (Model 840, Sierra Side-Trak; Sierra Instruments Inc., Monterey, California) at a flow rate of 490 mL min^{-1}, which were regulated by a mass flow control unit (MFC-2; Sable Systems Inc.). Air-flow was directed through the zero channel (cell A) of a calibrated (to 360 ppm CO_2 in nitrogen) infrared CO_2-H_2O Analyzer (Li-7000; Li-Cor, Lincoln, Nebrska), and then over the test animal in its respirometry chamber. The respirometry chambers were put into a double plastic bag and plunged into a programmable water bath (GP200-R4; Grant Instruments, U.K.), programmed using LABWISE (Grant Instruments) to ramp temperatures at a rate of 0.25 °C min^{-1}. Air continued through the animal chamber into the analyzer through a second channel (Cell B), which recorded the difference in CO_2 concentration of the air before and after it flowed through the animal chamber, at 1-s intervals. The application software, version 2.0.0, provided with the Li-7000 Analyzer (LiCor) records output from the CO_2-H_20 analyzer.

Baseline air measurements were taken at the beginning and end of each trial for 5 min using an identical set-up, as described above, although without the test animal in the respirometry chamber to correct for analyzer drift. After each baseline recording, animals were weighed using a XP404S balance (Mettler-Toledo, Switzerland) to 0.1 mg and placed into a 500-mL polypropylene chamber for flow-through respirometry. Animals were allowed to settle for 10 min (allowig sufficient enough time for them to stop vigorously moving inside the chamber) and the CO_2 readings on the analyzer were stabilized before the recordings began. The animal chamber was then submerged in a water bath, which was programmed to generate an equilibration period of 5 min at 25 °C, followed by a ramp at 0.25 °C min^{-1} to 42 °C, then followed by an equilibration period at 42 °C for 5 min. In total, each assay ran for 98 min. After this time, animals were removed from the water bath and re-weighed. Diet treatments were tested in a random sequence across 6 days. Three test animals were exposed to temperatures up 50 °C to determine thermolimit respirometry CT_{max} (Lighton & Turner, 2004; Andrew *et al.*, 2016). We found that the mean ± SE CT_{max} was 46.36 ± 0.19 °C and, because we did not want to kill the animals in this experiment, we pushed them to as close as possible to their CT_{max} without death.

Data extraction

We used the data acquisition and analysis software EXPEDATA, version 1.9.2 (Sable Systems Inc.) to extract our data. The rate of CO_2 release in ppm was corrected for baseline analyzer drift before been converted to ml CO_2 h^{-1} before any data analyses occurred. We also calculated the rate of CO_2 release, $\dot{V}_{CO_2}$, at specific temperature 'bins' (25, 30, 35, 40 and 42 °C with 0.5 °C variation either side of the specific temperature). We identified the temperature when each individual became stressed (the 'temperature stress point') when a breakpoint was reached in the $\dot{V}_{CO_2}$ curve: this was carried out visually assessing each datafile in *Expedata* for a distinct change in the curve trend as the temperatures were ramping.

Approximate digestibility of nutrients

The frass and representative leaf samples from each separate insect cage were collected for 1 week over the same period when the respirometry measurements were made (see below) and stored in the freezer. Both the leaf and frass samples for each diet were pooled together and oven-dried at 80 °C until a constant sample weight was obtained and then ground to a particular size of < 0.5 mm. Macro- and micronutrient analysis were performed on the pooled samples using a subsample of approximately 0.15–2.0 g for each analysis. Carbon and nitrogen were measured using a TruSpec Series Carbon and Nitrogen Analyzer (LECO Corporation, Benton Harbor, Michigan). The other nutrients (calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, sulphur and zinc) were measured using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Model 725 Radical Viewed ICPOES with a mass flow controller; Agilent, Australia). The approximate digestibility of each food type was calculated by subtracting the nutrients contained in the frass from the nutrients contained in the leaves.

Egg production by females

We also calculated the lifetime production of eggs by females. Initially, five females and four males were kept in each of three insect cages and fed consistently on one of the three diets throughout their lives. As a result of a few deaths, the numbers in each were reduced (but no less than two males and three females). Once they reached adulthood, we counted and removed eggs from the cages. The number of eggs produced/female/cage was used for the analysis.

Statistical analysis

A two-way analysis of variance (ANOVA) was carried out (DATADESK, version 7; Data Description Inc., Ithaca, New York) to test the effects of diet and sex on $\dot{V}_{CO_2}$ (the rate of CO_2 released). Because there was a relationship with weight, $\dot{V}_{CO_2}$ data was divided by the weight (mg) of each individual (units are mL h^{-1} mg^{-1}). As a result of a relationship of weight with $\dot{V}_{CO_2}$, an analysis of covariance with weight as a covariable was inappropriate (Miller & Chapman, 2001). A two-way ANOVA was carried out (using DATADESK, version 7) to test the effects of diet and sex on the total $\dot{V}_{CO_2}$. A two-way ANOVA (diet and sex) was also performed to test $\dot{V}_{CO_2}$ of a 120 s period either side of each of five specific temperature ’bins’ (25, 30, 35, 40 and 42 °C), as well as the temperature stress point (33.8 ± 3.7 to 38.0 ± 0.4 °C). Because the temperatures were ramped at 0.25 °C min^{-1}, this is a 1 °C temperature ‘bin’ with 0.5 °C variation either side of the specific temperature.

For approximate digestibility, the samples of frass and food were pooled for each diet. A chi-squared test was used to assess the differences among macronutrients (%) and micronutrients (μg g^{-1}) among the three host plant diets.

For egg production by females, we analyzed using a one-way ANOVA with the number of eggs produced/ female reared on each diet out using SIGMAPLOT, version 14 (Systat Software Inc., Chicago, Illinois).

Results

There was a significant effect of sex and diet, as well as a significant interaction between sex and diet, on *E. tiaratum* weight (Fig. 1a and Table 1). Females feeding on bBramble were 217% heavier than females on *Eucalyptus* sp. ($P < 0.0001$) and 653% heavier than males feeding on Bramble ($P < 0.0001$); females feeding on tree lucerne were also 183% heavier than females feeding on *Eucalyptus* sp. ($P = 0.0013$) and 661% heavier than males feeding on Tree Lucerne ($P < 0.0001$); and females feeding on *Eucalyptus* sp. were 328% heavier than males feeding on *Eucalyptus* sp. ($P = 0.0049$).

All individuals of the stick insect species *Extatosoma tiaratum* tested for their metabolic response displayed a cyclical gas exchange pattern. Overall, as the temperature was ramped higher, there was a greater gas exchange for each individual.

Sex influences overall $\dot{V}_{CO_2}$

Male individuals had a higher mean ± SE $\dot{V}_{CO_2}$ (4.13 ± 0.37–5.21 ± 0.52 mL h^{-1} mg^{-1}) than females (3.17 ± 1.71–5.11 ± 1.32 mL h^{-1} mg^{-1}) for all three food types (Fig. 1b). However, sex and diet did not have a significant effect on $\dot{V}_{CO_2}$ for the whole sampling period (Table 1).

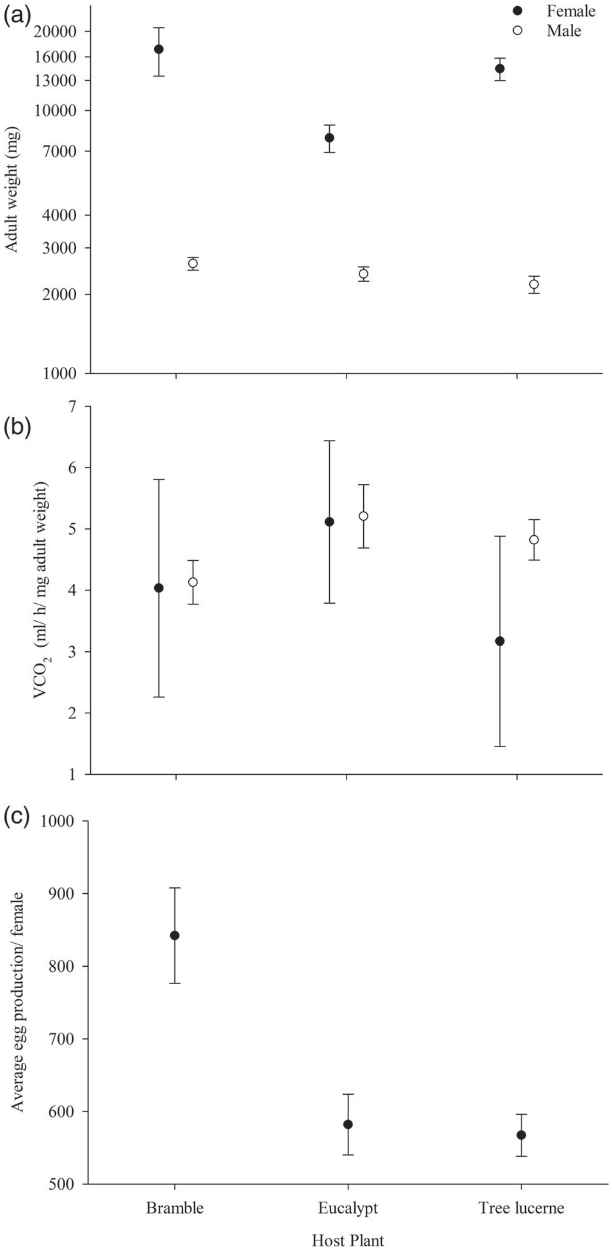

Fig. 1. *Extatosoma tiaratum* adult weight (a), adult rate of CO_2 production ($\dot{V}_{CO_2}$) (b) and average egg production/female (c), fed on one of three different diets: bramble, *Eucalyptus* sp. or tree lucerne.

Sex and diet influences on $\dot{V}_{CO_2}$ at different temperatures

We tested $\dot{V}_{CO_2}$ of both sexes of *E. tiaratum* at different ‘binned’ temperatures and found that, at lower temperatures (25 and 30 °C), there was no significant difference (Fig. 2a,b and

Table 1. The results of a two-way analysis of variance testing the effects of sex and diet on adult weight and total metabolic rate ($\dot{V}_{CO_2}$)

Factor	d.f.	SS	MS	F-ratio	P-value
Adult weight					
Diet	**2**	**6.95×10^7**	**3.47×10^7**	**13.26**	**< 0.0001**
Sex	**1**	**5.17×10^8**	**5.17×10^8**	**197.36**	**< 0.0001**
Diet × Sex	**2**	**6.58×10^7**	**3.29×10^7**	**12.55**	**< 0.0001**
Error	12	3.14×10^7	2.62×10^7		
Total volume of CO_2 released					
Diet	2	5.08	2.54	1.82	0.20
Sex	1	1.70	1.70	1.23	0.29
Diet × Sex	2	2.43	1.21	0.87	0.44
Error	12	16.72	1.39		

Significant factors are indicated in bold.

Table 2). At 35 °C, males feeding on bramble and tree lucerne had a higher $\dot{V}_{CO_2}$ than the females in the same diet type. There was a significant interaction (Fig. 2c) between diet and sex for females feeding on *Eucalyptus* sp. compared with females feeding on tree lucerne ($P < 0.0001$) and bramble ($P = 0.009$). There were also significant differences found between male and female individuals feeding on bramble ($P < 0.038$) and those feeding on tree lucerne ($P < 0.001$). At the highest temperatures (40 and 42 °C), sex was found to be significantly different with $\dot{V}_{CO_2}$ in male individuals significantly higher than those in female individuals ($P < 0.0001$) (Fig. 2d,e). Temperature stress points ranged from (33.8 ± 3.7 to 38.0 ± 0.4 °C) and were not significantly higher for males than females and also did not differ significantly between diet types (Fig. 2f and Table 2).

Approximate digestibility of nutrients

Of the seven macronutrients assessed (Table 3), all showed relatively consistent changes among the diets, and there was no significant difference among diets and percentage of each macronutrient ($\chi^2 = 0.98$, d.f. $= 12$, $P = 0.99$). Of the five micronutrients assessed (Table 3), there was a significant difference between diet and micronutrient concentration ($\chi^2 = 398.5$, d.f. $= 8$, $P \leq 0.00001$). The approximate digestibility of manganese was negative for tree lucerne but positive for *Eucalyptus* sp. and negligible for bramble. The approximate digestibility of sodium was highly positive for tree lucerne but highly negative for bramble and *Eucalyptus* sp. The approximate digestibility of zinc was positive for bramble, negative for *Eucalyptus* sp. and negligible for tree lucerne.

Egg production by females

Stick insect females feeding on bramble produced nearly 150% more eggs per female than females feeding on *Eucalyptus* and tree lucerne ($F_{2,6} = 31.15$, $P < 0.001$) (Fig. 1c). Female stick insects reared on *Eucalyptus* and tree lucerne produced a similar number of eggs (574 ± 33.2 eggs).

Discussion

We investigated the metabolic rate response ($\dot{V}_{CO_2}$) of adult *E. tiaratum* when exposed to thermolimit respirometry, after being reared on three different host plant species in captivity throughout their lives. We found that males had a higher total $\dot{V}_{CO_2}$ than females for all three host species. $\dot{V}_{CO_2}$ values at lower temperatures were not affected by sex or diet type. At higher temperatures, $\dot{V}_{CO_2}$ was affected by the sex of the animal and the diet that they were reared on. At 35 °C, $\dot{V}_{CO_2}$ was affected by sex and diet. Males that fed on bramble, as well as tree lucerne, had a higher $\dot{V}_{CO_2}$ than females reared on the same host plant diet, whereas females had a higher $\dot{V}_{CO_2}$ than males reared on the *Eucalyptus* sp. diet. We also found that all stick insect individuals in the present study exhibited a continuous gas exchange cycle, which supports previous work on the metabolic rates of *E. tiaratum* (Marais *et al.*, 2005).

The metabolic rate ($\dot{V}_{CO_2}$) is influenced by several variables, including activity level, age, size, sex, feeding status and breeding status (Waters & Harrison, 2012). Identifying a difference in $\dot{V}_{CO_2}$ between insects reared on different food sources may indicate that nutrition has an impact on morphology, fitness and energy budget availability (Terblanche *et al.*, 2004, 2005). In crickets, males have a higher $\dot{V}_{CO_2}$ because they have more demanding performance activities, such as calling and aggressive behaviour (Kolluru *et al.*, 2004). Male *E. tiaratum* have costs associated with flight and seeking out the wingless females to mate with. Metabolic rates at higher temperatures may be affected by other factors other than food nutrient content, such as reproductive costs in *E. tiaratum* females. However, egg production was higher for the females reared on bramble, indicating that it is a more nutritious food source out of the three tested, and also that egg production does not influence metabolic rate.

All animals in this trial were of a similar age (i.e. adults) and were reared in the same environment. The impact of nutrition availability was visibly more marked in females between groups than for males. Although males attained final moult and maturity over the three diet groups before females, the females fed on bramble reached maturity faster than females fed on *Eucalyptus* sp. (S. C. Silcocks, personal observation). Weights of female individuals varied between each diet group, probably as a result of having different nutrient availability. Diet quality can have an impact on physiological functions and reproductive outputs (Naya *et al.*, 2007; Tan *et al.*, 2013; Niitepõld *et al.*, 2014; Portman *et al.*, 2015), as well as growth rates (Clissold *et al.*, 2009). We found that nutrients varied between the different diets. Although carbon amounts were similar across the diets, nitrogen was highest in the tree lucerne leaves. Food quality can impact on the physiological and life history of some insects (Naya *et al.*, 2007). High protein diets improve body condition and fecundity, whereas high carbohydrate diets can reduce reproductive output as a result of the lack of nutrients available (Naya *et al.*, 2007), and nutrient restriction may increase survival (Naya *et al.*, 2007; Niitepõld *et al.*, 2014).

Although we did not measure leaf characteristics and physiology, we know that herbivores make preference decisions of *Eucalyptus* leaf-feeding based on leaf age (Ohmart & Edwards, 1991) and leaf toughness (Malishev & Sanson, 2015). Newly

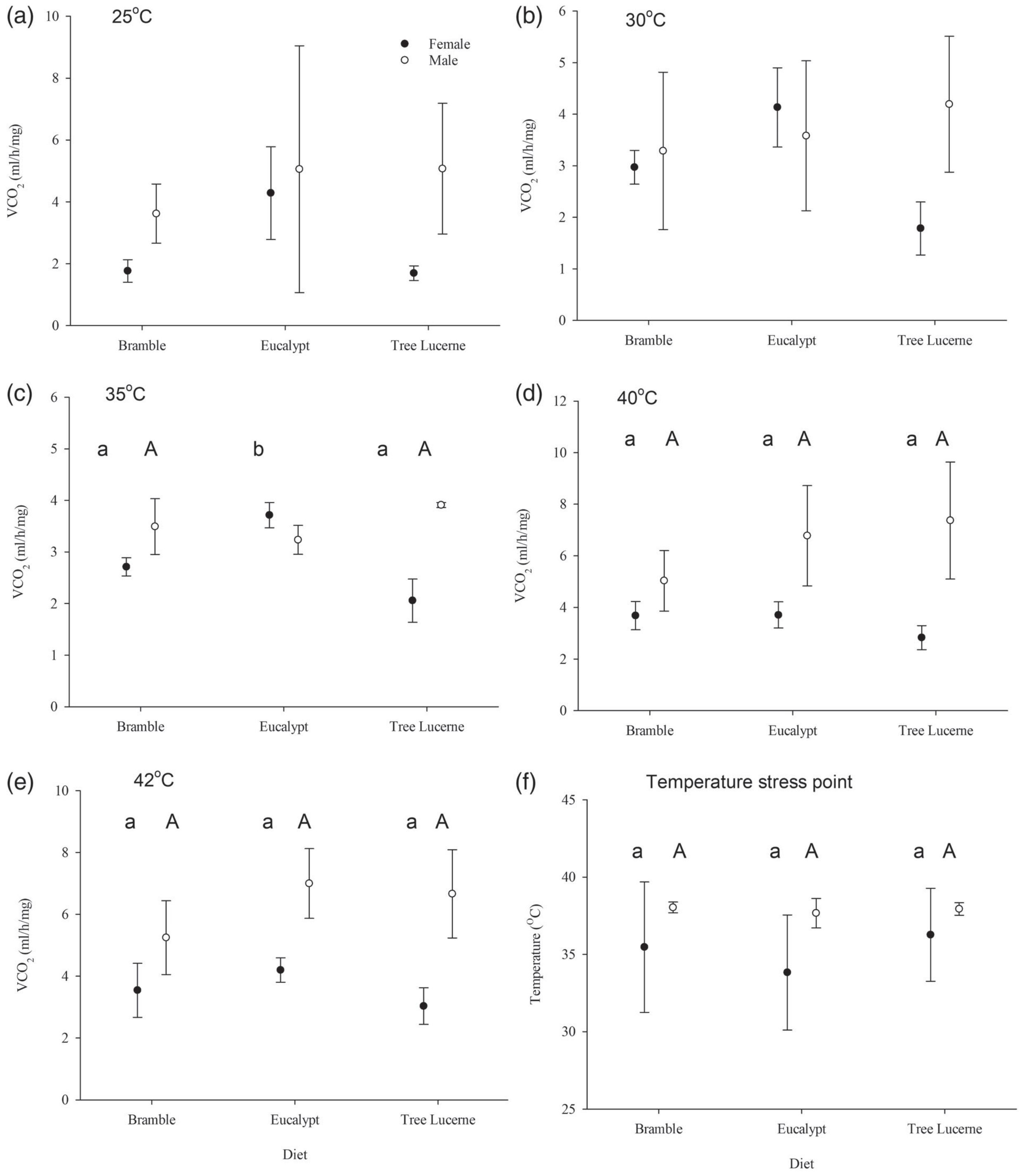

Fig. 2. The rate of CO_2 production ($\dot{V}_{CO_2}$) by adult *Extatosoma tiaratum* at different temperatures: (a) 25, (b) 30, (c) 35, (d) 40 and (e) 42°C, as well as the (f) temperature stress point. Different letters indicate significant differences (analysis of variance, $P > 0.050$).

flushed foliage lack physical defences that older leaves have and are generally higher in nitrogen (Ohmart & Edwards, 1991). Leaf toughness and herbivore leaf preference can affect rates of growth, development and performance of insects (Sanson *et al.*, 2001; Clissold *et al.*, 2009; Clissold & Simpson, 2015). In the present study, we did not assess individual leaf preferences among the host plant types. For other host plant species, the age of the leaf is critical. Young leaves have higher chemical defences against herbivores (Junker *et al.*, 2008) and host plant preference can also influence mating choices (Papaj & Prokopy,

Table 2. The results of a two-way analysis of variance testing the effects of diet and sex on the metabolic response at different temperatures, as well as the temperature stress point

	Factor	d.f.	SS	MS	*F*-ratio	*P*-value
25 °C	Diet	2	12.08	6.04	1.53	0.26
	Sex	1	18.03	18.03	4.56	0.054
	Diet × Sex	2	5.13	2.56	0.65	0.54
	Error	12	47.46	4.00		
30 °C	Diet	2	2.60	1.30	1.09	0.37
	Sex	1	2.37	2.37	1.98	0.18
	Diet × Sex	2	6.95	3.47	2.91	0.09
	Error	12	14.31	1.19		
35 °C	Diet	2	0.79	0.39	3.70	0.06
	Sex	**1**	**2.33**	**2.33**	**21.89**	**< 0.0001**
	Diet × Sex	**2**	**4.09**	**2.05**	**19.25**	**< 0.0001**
	Error	12	1.28	0.11		
40 °C	Diet	2	2.71	1.36	0.73	0.50
	Sex	**1**	**40.04**	**40.04**	**21.68**	**< 0.0001**
	Diet × Sex	2	7.61	3.81	2.06	0.17
	Error	12	22.16	1.85		
42 °C	Diet	2	4.42	2.21	2.21	0.15
	Sex	**1**	**33.07**	**33.07**	**33.07**	**< 0.0001**
	Diet × Sex	2	2.81	1.40	1.40	0.28
	Error	12	12.00			
Temperature stress point	Diet	2	5.89	2.95	0.42	0.66
	Sex	1	32.54	32.54	4.67	0.052
	Diet × Sex	2	3.55	1.78	0.26	0.78
	Error	12	83.62	6.97		

Significant factors are indicated in bold.

Table 3. Approximate digestibility (subtraction of nutrients contained in the frass from nutrients contained in leaves) of macronutrients and micronutrients from three different diet plants (Bramble, *Eucalyptus* sp. and Tree Lucerne)

	Macronutrients							Micronutrients				
Sample type	C (%)[a]	N (%)	Ca (%)	K (%)	Mg (%)	P (%)	S (%)	Cu ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Na ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)
Bramble	−2.64	1.01	0.47	−0.32	−0.24	0.15	0	2.5	−144	9	−317.8	9.6
Eucalyptus	−3.02	0.28	0.17	−1.3	−0.12	0.1	−0.05	2.2	−51.6	228.1	−511.8	−16.7
Tree Lucerne	−2.52	1.32	0.06	−1.49	−0.08	0.1	−0.07	5.3	−61.3	−93.1	225.4	−1

[a] C, carbon; N, nitrogen; Ca, calcium; K, potassium; Mg, magnesium; P, phosphorus; S, sulphur; Cu, copper; Fe, iron; Mn, manganese; Na, sodium; Zn, zinc.

1988; Nosil *et al.*, 2002) and leaf quality choices (Sandlin & Willig, 1993). Specialists feeding on specific host plants prefer young leaves to old ones, whereas generalist feeders prefer old leaves (Blüthgen & Metzner, 2007). *Extatosoma tiaratum* will eat most plant foliage offered to them (Brock & Hasenpusch, 2009).

Host plant usage will play a key role in enabling stick insects to adapt to a warmer and more variable climate. For some species, populations that use different host plants may diverge in morphology (body shape and size), as well as change their behaviour, as in the walking stick insect *Timema cristinae* (Nosil *et al.*, 2002). An increased metabolic rate leads to an increased demand for energy resources (Dillon *et al.*, 2010). In the present study, when male stick insects become exposed to temperatures at 40 °C and higher, the metabolic rate increased significantly across all food types. Elevated CO_2 levels reduce the nutrient value of leaves and this leads to higher consumption of foliage by herbivores (DeLucia *et al.*, 2012). For the Phasmatodea, there has been a depauperate amount of research carried out regarding how they will respond to climatic change: either directly via climate or indirectly via host plant chemistry change (Andrew *et al.*, 2013). We have demonstrated that higher temperatures can result in an increase in $\dot{V}_{CO_2}$ for male and female individuals of *E. tiaratum*. Incorporating fluctuating temperature ranges into experiments (Ghaedi & Andrew, 2016; Holley & Andrew, 2019a, 2019b) will help us to understand the impact that exposure to thermal extremes will have on the growth, development, performance and survival of insects in a changing climate (Andrew, 2013; Andrew & Terblanche, 2013; Harris *et al.*, 2018; Hoffmann *et al.*, 2019).

Acknowledgements

We thank the Melbourne Zoo for providing the eggs of *E. tiaratum*, as well as the enclosures used to rear them. Nicolas Meyer prepared leaf and frass samples for nutrient analysis. Partial funding for this research came from the Australian Research

Council grant DP160101561 to N. R. A. There authors declare that they have no conflicts of interest.

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Accepted 1 October 2019