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

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Running title: Temperature impacts on metabolic rates of stick insects.

**ABSTRACT**

Access to balanced nutrition enables optimum health and development, body repair, fat storage, increased fecundity and longevity. In this 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°C, 30°C, 35°C, 40°C and 42°C). Sex but not diet influenced respiration and metabolic rate. Male individuals had, on average, 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, but Eucalypt reared animals showing an opposite trend. Lifetime egg production by females was 150% higher on bramble compared to the other host plants. Incorporating fluctuating temperature ranges into experiments will further help understand the impact thermal stress will have on the growth, development, performance and survival of insects in a more variable climatic and nutritional landscape.

Keywords: metabolic rates; diet, temperature; climate change; sex; thermal stress; temperature stress point; stick insect; phasmid.
INTRODUCTION

Host plants influence the life history of insect herbivores in terms of 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 sub-optimal nutrition, and 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 specialised on a single host plant, and even preferring specific aged leaves (e.g. older leaves) when feeding (see Blüthgen and Metzner, 2007).

In this 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 since birth: tree lucerne (Chamaecyisis palmensis), bramble (Rubus fruticosus) and Eucalyptus species. These plant species have several chemical (e.g. macro and micro-nutrients) and morphological differences which can influence foliage digestibility, insect herbivore growth and physiology if the insects are allowed to specialise 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, tree lucerne and bramble are both introduced but commonly available.

Eucalyptus species (Myrtaceae) are native to Australia and are one component of E. tiaratum diet from eastern coastal forests (Brock and 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 and Fox, 1980; Ohmart and Edwards, 1991). Exposure to these chemicals when insects are feeding can decrease the food intake, and 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 due to its high leaf protein content (Borens and Poppi, 1990; Lambert et al., 1989; Lindeque and 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 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 (Buřičová et al., 2011; Gudej and Tomczyk, 2004) 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 and 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).

Here, we examined variations in the metabolic rate ($\dot{V}CO_2$) 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 *Extatosoma tiaratum* as our 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. There are 200 known phasmid species in Australia, out of the 3000 species identified globally (Brock and Hasenpusch, 2007), 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 and 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). *E. 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 which protects the egg from environmental hazards and predation by wasps (Hughes and Westoby, 1992). The first instar nymphs are ant mimics, which allow them to escape the ant nest after hatching (Bedford, 1978). *E. tiaratum* flick away their eggs so there is less certainty which plants will be accessible when the nymph hatches. This species of insect has been kept in captivity in Australia since the 1960s (Hadlington, 1966; Korboot, 1961) and Europe since the 1970s (Brock, 1992), so we know 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 and Fry, 1999). Being able 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.

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 (Clarke, 2006; Gillooly et al., 2001). Understanding the impact of temperature on an animal is vital to know, and methods such as thermolimit respirometry have been devised to assess changes in metabolic rates with a constant increase in the temperature ramping rate (Lighton and Turner, 2004). For insects, temperature exposure assessments have most commonly measured the critical endpoint when an animal loses muscular control (Critical Thermal Maximum, CT_{max}) ignoring the changes in metabolic rate up until this point.

Understanding how the metabolic rate of insects changes with exposure to increasing temperatures is vital: metabolic rate can be used as a measure of stress resilience (Krams et al., 2018), and to determine more realistic CT_{max} endpoints using thermolimit respirometry techniques (Lighton and Turner 2004). As the temperature rise, an insects metabolic rate (measured as $\dot{V}CO_2$ ml/h) also rises, until reaching a pre mortal plateau (Andrew et al., 2016; Lighton and Turner, 2004). Metabolic rate responses to temperature have been measured for a range of insects including ants (Andrew et al., 2016; Lighton and Turner, 2004), Helicoverpa caterpillars (Betz and Andrew, in review 5vi19), silkworms (Boardman and Terblanche, 2015), and beetles (Verberk and Bilton, 2015) among others (Neven, 2000).

We are interested in if host plant diets affect the metabolic rate of insects at different temperatures, and female fecundity. Specifically, in this study, we addressed the questions:

How do 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?

Does host plant influence stick insect fecundity as measured by egg production per female?

**MATERIAL AND METHODS**

**Stick insect’s 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-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
cage cages (245 x 245 x 630mm). 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°C to 25°C), but in a similar environment of enclosure size,
temperature, humidity and lighting over four months.

**Respirometry measurements**

We used flow-through respirometry to measure the metabolic rate ($\dot{V}_{\text{CO}_2}$) in adult *E.
tiaratum*. Atmospheric air was pumped via a HiBlow pump (HB40) through soda-lime and
Drierite (desiccant) columns to remove CO$_2$ and water (H$_2$O) from the air and then into two
mass flow control valves (Model 840, Sierra Side-Trak, Sierra Instruments Inc., Monterey,
USA) at a flow rate of 490 ml min$^{-1}$ which were regulated by a mass flow control unit (MFC-2,
Sable Systems). Air-flow was directed through the zero channel (cell A) of a calibrated (to
360 ppm CO$_2$ in Nitrogen) infrared CO$_2$-H$_2$O Analyzer (Li-7000, Li-Cor, Lincoln, NE, USA),
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 (Grant, GP200-R4),
programmed using LABWISE software to ramp temperatures at a rate of 0.25°C min$^{-1}$. Air
continued through the animal chamber into the analyser 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-second intervals. The LI-7000 software (Version 2.0.0,
LiCor) records output from the CO$_2$-H$_2$O analyser.

Baseline air measurements were taken at the beginning and end of each trial for five
minutes by using an identical setup as described above, but without the test animal in the
respirometry chamber to correct for analyser drift. After each baseline recording, animals
were weighed using a Mettler Toledo XP404S balance to 0.1 mg and placed into a 500 ml
polypropylene chamber for flow-through respirometry. Animals were allowed to settle for ten
minutes (enough time for them to stop vigorously moving inside the chamber), and the CO$_2$
readings on the analyser were stabilised before recordings began. The animal chamber was
then submerged in a water bath, which was programmed to generate an equilibration period of
five minutes at 25°C, followed by a ramp at 0.25°C min$^{-1}$ to 42°C, followed by an
The equilibration period at 42°C for five minutes. In total, each assay ran for 98 minutes. After this time, animals were removed from the water bath and re-weighed. Diet treatments were tested in a random sequence across six days. Three test animals were exposed to temperatures up 50°C to determine thermolimit respirometry CT_max (Andrew et al., 2016; Lighton and Turner, 2004). We found that CT_max was 46.36±0.19 (S.E.) °C, and as 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 program *ExpeData* Version 1.9.2 (Sable Systems Data acquisition and analysis software) to extract our data. The rate of CO₂ release in ppm was corrected for baseline analyser drift before been converted to ml CO₂ hour⁻¹ before any data analyses occurred. We also calculated the rate of CO₂ release, \( \dot{V}CO_2 \), at specific temperature ‘bins’ (25°C, 30°C, 35°C, 40°C 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 done 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 a 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 <0.5mm. Macro- and micro-nutrient analysis were performed on the pooled samples using a subsample of approximately 0.15-2.0g for each analysis. Carbon and nitrogen were measured using a TruSpec Series Carbon and Nitrogen Analyser (LECO Corporation, Michigan, USA). 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). 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. Due to a few deaths the numbers in each 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 analysis.

**Statistical Analysis**

A two-way ANOVA was carried out (using Datadesk 7, Data Description Inc) to test the effects of diet and sex on $\dot{VCO}_2$ (the rate of CO$_2$ released). As there was an effect of weight, $\dot{VCO}_2$ data was divided by the weight (mg) of each individual (units are ml/h/mg). Due to an effect of weight, an ANCOVA analysis with weight as a co-variable was inappropriate (Miller and Chapman, 2001). A two-way ANOVA was carried out (using Datadesk 7) to test the effects of diet and sex on the total $\dot{VCO}_2$. A two-way ANOVA (diet and sex) was also performed to test $\dot{VCO}_2$ of a 120 second period either side of each of five specific temperature ‘bins’ (25°C, 30°C, 35°C, 40°C, 42°C) and the temperature stress point (33.8±3.7 to 38.0±0.4°C). As 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: as the samples of frass and food were pooled for each diet. A $\chi^2$ test was used to assess the differences among macronutrients (%) and micronutrients (ug/g) among the three host-plant diets.

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

**RESULTS**

There was a significant effect of sex, diet and a significant interaction between sex and diet on *Extatosoma tiaratum* weight (Table 1a; Figure 1a). 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 $\dot{VCO}_2$.**
Male individuals had on average (± s.e.) a higher $\dot{V}CO_2$ (4.13±0.37 – 5.21±0.52 ml/h/mg) than females (3.17±1.71 – 5.11±1.32 ml/h/mg) for all three food types (Figure 1b). However, sex and diet did not have a significant effect on $\dot{V}CO_2$ for the whole sampling period (see Table 1b).

**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°C and 30°C) there was no significant difference (Table 2a,b, Figure 2a,b). 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 (Figure 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°C 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 ($P<0.0001$, Figures 2d and 2e). 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 did not differ significantly between diet types (Figure 2f; 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, $p = <0.00001$). Approximate digestibility of magnesium was negative for tree lucerne but positive for *Eucalyptus* sp. and negligible for bramble; was highly positive for sodium, and but highly negative for bramble and *Eucalyptus* sp.; and zinc 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 ($F_{2,6} = 31.15$, $P<0.001$; Figure 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 *Extatosoma 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. $\dot{V}CO_2$ 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, and 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 this study exhibited a continuous gas exchange cycle which supports previous work on the metabolic rates of *E. tiaratum* (Marais et al., 2005).

Metabolic rate ($\dot{V}CO_2$) is influenced by several variables including activity level, age, size, sex, feeding status and breeding status (Waters and 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 $\dot{V}CO_2$ as 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, identifying that it is a more nutritious food source out of the three tested, and 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. While 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 (Silcocks *pers. obs*.). 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; Niitepõld et al., 2014; Portman et al., 2015; Tan et al., 2013) as well as growth rates (Clissold et al., 2009). We found that nutrients varied between the different diets. While 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 due to the lack of nutrients available (Naya et al., 2007); and nutrient restriction may increase survival (Naya et al., 2007; Niitpöld 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 and Edwards, 1991) and leaf toughness (Malishev and Sanson, 2015). Newly flushed foliage lack physical defences that older leaves have and are generally higher in nitrogen (Ohmart and Edwards, 1991). Leaf toughness and herbivore leaf preference can affect rates of growth, development and performance of insects (Clissold et al., 2009; Clissold and Simpson, 2015; Sanson et al., 2001). In this 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 (Nosil et al., 2002; Papaj and Prokopy, 1988) and leaf quality choices (Sandlin and Willig, 1993). Specialists feeding on specific host plants prefer young leaves to old ones whereas generalist feeders prefer old leaves (Blüthgen and Metzner, 2007); E. tiaratum will eat most plant foliage offered to them (Brock and 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), and change their behaviour, as in the walking stick insect Timema cristinae (Nosil et al., 2002). Increased metabolic rate leads to an increased demand for energy resources (Dillon et al., 2010): here when male stick insects become exposed to temperatures at 40°C and higher, metabolic rate increased significantly across all food types. We know that elevated CO₂ 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 on 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 $\dot{V}\text{CO}_2$ for male and female individuals of E. tiaratum. Incorporating fluctuating temperature ranges into experiments (e.g. Ghaedi and Andrew, 2016; Holley and Andrew, 2019a, b) will help 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 and Terblanche, 2013; Harris et al., 2018; Hoffmann et al., 2019).

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**REFERENCES**


Table 1. The results of a two-way ANOVA testing the effects of sex and diet on the (a) adult weight, and (b) total metabolic rate ($\dot{V}CO_2$). Significant factors in **bold**.

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<th>Factor</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F-ratio</th>
<th>P-value</th>
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<tr>
<td>(a) Adult weight</td>
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<tr>
<td>Diet</td>
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<td>$3.47 \times 10^7$</td>
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<td>$5.17 \times 10^8$</td>
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<td>$6.58 \times 10^7$</td>
<td>$3.29 \times 10^7$</td>
<td>12.55</td>
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<td>Error</td>
<td>12</td>
<td>$3.14 \times 10^7$</td>
<td>$2.62 \times 10^7$</td>
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</tr>
<tr>
<td>(b) Total volume of CO$_2$ released</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>1.70</td>
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Table 2. The results of a two-way ANOVA testing the effects of diet and sex on the metabolic response at different temperatures (a-e) and the temperature stress point (f).

Significant factors in **bold**.

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<tr>
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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).

<table>
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<th>Sample type</th>
<th>Macronutrients</th>
<th>Micronutrients</th>
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<tr>
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<td>C (%)</td>
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<td>Tree Lucerne</td>
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</table>

*C=Carbon; N=Nitrogen; Ca=Calcium; K=Potassium; Mg=Magnesium; P=Phosphorus; S=Sulfur; Cu=Copper; Fe=Iron; Mn=Manganese; Na=Sodium; Zn=Zinc.*
Figure 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.

Figure 2 – The rate of CO$_2$ production ($\dot{V}$CO$_2$) by adult *Extatosoma tiaratum* at different temperatures: 25, 30, 35, 40, and 42°C and the temperature stress point. Different letters signify significant differences (ANOVA, $P > 0.050$).