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HUMIDITY AND TEMPERATURE EFFECTS ON RESPIRATORY PATTERN IN THE WORKER CASTE OF THE TERMITE *HODOTERMES MOSSAMBICUS* (HAGEN)

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A Dissertation submitted to the Faculty of Science, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master of Science

Johannesburg, 2013



School of Animal, Plant and Environmental Science
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Declaration

I declare that this Dissertation is my own, unaided work. It is being submitted for the degree of Master of Science at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



Isabelle Maxine Inder

15 day of August 2013 in Johannesburg

Abstract

The evolutionary genesis and the current adaptive significance of the use of the discontinuous gas exchange cycle (DGC) for respiration by insects is the subject of intense debate. Most current research centers on three adaptive hypotheses and one non-adaptive hypothesis; these are the hygric hypothesis, the chthonic hypothesis, the oxidative-damage hypothesis and the emergent-property hypothesis respectively. Workers of the harvester termite, *Hodotermes mossambicus* were selected as a model to test three of these hypotheses. The respiratory patterns of workers, investigated using flow-through respirometry, were obtained at 0 % relative humidity (RH), 100 % RH, at 100 % O_2 and under varying temperature to evaluate the assumptions of the various hypotheses. A change in ambient humidity had no impact on metabolic rate (VCO_2), coefficient of variation (CV) or the pattern of gas exchange but only influenced the amount of water loss experienced by workers. Major workers exposed to hyperoxia (100 % O_2) responded by increasing spiracular control and constriction through the use of cyclic gas exchange thereby protecting their interior against the toxic effects of O_2. As VCO_2 increased in response to increasing temperature, the gas exchange pattern displayed by workers transitioned from a modified DGC through cyclic to continuous gas exchange. A true DGC, defined as showing all three phases and a CV value close to 2, was not expressed under any of the experimental conditions. The results of this study support the oxidative-damage and emergent-property hypotheses but not the hygric hypothesis. The workers of *H. mossambicus* spend only brief periods above ground before returning to the refuge of their underground nests and as such there is probably little selective advantage to the DGC for limiting respiratory water loss. The conclusion drawn from the study of termite workers is that changes in respiratory patterns are most likely an emergent property of the insects' nervous and respiratory systems and spiracular control also serves to limit oxidative damage.

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CHAPTER 1: Introduction to Insect Respiratory Patterns

1.1 General Introduction

Insects maintain the highest mass-specific rates of oxygen consumption in the animal kingdom. They achieve this through the use of an efficient respiratory system, the tracheal system (Maina, 2002). The tracheal system consists of an extensive network of air-filled tubes, the tracheae, which branch throughout the body and essentially allow for the rapid movement of oxygen and carbon dioxide between the insect's tissues and the surrounding environment (Moerbitz & Hetz, 2010; Matthews & White, 2011; Harrison *et al.*, 2012). This system opens to the external environment via the spiracles, small pores that occur in pairs, usually one pair per segment in the thorax and abdomen, located on the lateral margins of the body (Bradley, 2006; Matthews & White, 2011; Harrison *et al.*, 2012). Spiracles contain valve-like structures that allow them to close, thus giving insects the ability to restrict gas exchange with the environment.

Insects are known to exhibit a variety of gas exchange patterns (Contreras & Bradley, 2010). These patterns vary between species, between individuals of the same species and even within an individual (Karise *et al.*, 2010). They also have been shown to vary with time, environment and behaviour (Bradley, 2006). Three distinct patterns have been identified: discontinuous, cyclic and continuous. The most enigmatic of these is the discontinuous gas exchange cycle (DGC). This pattern is distinctive in that it displays periodic breathing; a cycle of repeated spiracular openings and closings that results in the intermittent release of carbon dioxide (Bradley, 2006; Karise *et al.*, 2010; Chown, 2011).

Discontinuous gas exchange is characterized by the behaviour of the spiracles, which produce a unique, three-phase pattern of gas exchange (Schneiderman, 1960; Shelton & Appel, 2001; Chown *et al.*, 2006; Chown, 2011). A typical DGC consists of a closed phase (C), a period when the spiracles are sealed and no gas exchange occurs between the environment and the respiratory surfaces (Shelton & Appel; 2001; Chown & Nicolson, 2004; Forster & Hetz, 2010). During this phase O_2 in the tracheal system is consumed and quickly falls to the oxygen threshold while CO_2 accumulates slowly. When the oxygen threshold is reached the spiracles begin to open intermittently, an action described as spiracular fluttering (F). CO_2 still accumulates in this phase while O_2 oscillates around its threshold ensuring adequate oxygen delivery at safe levels. Eventually the CO_2 threshold is reached and the spiracles open widely to release a burst of CO_2. This phase is typically known as the open or burst phase (O/B) (Bradley, 2007; Forster & Hetz, 2010; Chown, 2011).

The discontinuous gas exchange pattern is thought to have evolved independently a minimum of five times in the Insecta (Marais *et al.*, 2005). Of the 18 orders that have had species studied, so far DGC has only been found in the cockroaches, locusts, grasshoppers, beetles, moths, bees and ants (Marais *et al.*, 2005; Chown, 2011). Why these particular insects should display this unique pattern has sparked several questions. What is the evolutionary genesis and current adaptive significance of DGC? That is, what is the underlying mechanism driving the production of DGCs and is this mechanism the same among all insects? This pattern of gas exchange has been the subject of intensive research, scrutiny and heated debate within the field of insect respiratory physiology. Years of research has resulted in several hypotheses but as of yet no consensus.

At present four major hypotheses, three adaptive and one non-adaptive, have been proposed to account for the origin of DGC. The first is the hygric hypothesis, which states that DGC serves to reduce respiratory water loss (Chown *et al.*, 2006). All terrestrial animals face a gas-water tradeoff, as it is a fundamental cost of living that gas exchange with the environment results in a loss of water. The

higher the rates of gas exchange the higher the rate of water loss (Woods & Smith, 2010). In insects, tracheal water is lost through open spiracles, therefore by closing the spiracles for long stretches, as in DGC; water loss through this avenue should theoretically be reduced (Levy & Schneiderman, 1966; Shelton & Appel, 2001). However the contribution of respiratory water loss to overall water loss and therefore the likelihood of it being subject to selection is contentious (Addo-Bediako *et al.*, 2001; Chown, 2002).

Some studies have found that several groups of beetles from arid environments have reduced their cuticular water permeability to the point where respiratory water loss constitutes the major avenue of water loss (Edney, 1977; Zachariassen, 1991; Zachariassen, 1996). However, the vast majority of studies have found that on average respiratory water loss contributes less, and in some cases much less than 20 % to overall water loss even when the spiracles are kept open (Shelton & Appel, 2001; Chown, 2002; Gibbs & Johnson, 2004). Thus there is contention as to whether modulation on such a small contribution would confer a fitness advantage. If it does and all insects are at risk of dehydration, it begs the question: why are there so few insects that employ DGC? This hypothesis has been subjected to further scrutiny with the finding that for insects, water loss per unit O_2 consumed were up to 8-fold higher than predicted by the universal model for water costs of gas exchange (Woods & Smith, 2010). This inconsistency with the model is thought to be a consequence of DGC. The need to expel sufficient CO_2 requires the spiracles to be open longer or more frequently than is necessary for the uptake of adequate O_2. Thus DGC seems to necessitate a larger water cost than is necessary for gas exchange (Woods & Smith, 2010; Chown, 2011). Insects are also found to abandon DGC under conditions when water conservation is most necessary such as when insects are exposed to high temperatures and during dehydration (Hadley & Quinlan, 1993; Chappell & Rogowitz, 2000; Bosch *et al.*, 2000; Williams *et al.*, 2010). However despite these findings this hypothesis continues to gain support and is thought to be, if not the evolutionary genesis of DGC, at least the mechanism or reason for its continued maintenance (White *et al.*, 2007).

The chthonic hypothesis theorizes that the decoupling of O_2 and CO_2 exchange produces an enhanced ability to exchange gases in hypercapnic (high CO_2) and hypoxic (low O_2) environments (Lighton, 1998; Shelton & Appel, 2001). Insects build up CO_2 when the spiracles are closed thereby creating an increased gradient for outward diffusion when they eventually open. At the same time tracheal O_2 levels decrease during the C phase, therefore increasing the gradient for O_2 uptake during the O phase (Gibbs & Johnson, 2004). This hypothesis has gained credibility as many of the insects that have been found to express DGC are fossorial, that is they inhabit or are at some stage of their life cycle under the influence of hypercapnic and hypoxic conditions (Lighton, 1998; Gibbs & Johnson, 2004). However, a study by Chown and Holter, (2000) on the beetle *Aphodius fossor*, which inhabits moist to wet dung pats where O_2 is low and CO_2 is high, found that the beetles abandoned DGC when exposed to declining O_2 concentrations. In response to decreasing oxygen, the C phase of DGC declined in length until it eventually disappeared. The closed phase is shortened because the amount of O_2 in the body is reduced following the open phase in hypoxic environmental conditions (Harrison *et al.,* 2006; Bradley, 2007). Similarly, White *et al.,* (2007) found no significant relationship between DGC duration and environmental PO_2, i.e. environmental oxygen availability was found to have no significant effect on DGC.

This finding also has implications for the third adaptive hypothesis, the oxidative-damage hypothesis (Hetz & Bradley, 2005). Oxygen is necessary for metabolism but acts as a toxic chemical to cells and tissues even at low concentrations. The oxidative-damage hypothesis states that DGC serves to reduce the supply of oxygen to the tissues and therefore prevent oxygen toxicity (Hetz & Bradley, 2005; Lighton & Ottesen, 2005). Insects have been shown to react to elevated ambient O_2 levels by constricting their spiracles (Lighton & Ottesen, 2005). This phenomenon is referred to as the hyperoxic switch (Lighton *et al.,* 2004). This oxidative-damage hypothesis was formulated on the basis that the F phase of DGC regulates O_2 at a low, constant level (Lighton, 1998; Chown *et al.*, 2006).

However, the open phase of DGC represents a vital flaw in this hypothesis. It is during this phase that the spiracles are open, oxygen floods in and internal oxygen reaches almost atmospheric levels, the very thing DGC is meant to prevent under this hypothesis (Lighton, 2007).

The emergent-property hypothesis states that DGC is an emergent property or consequence of interactions between the O_2 and CO_2 set points that regulate spiracular function, i.e. opening and closing (Chown & Holter, 2000; Chown *et al.*, 2006; White *et al.*, 2007). It has been proposed that the gas exchange patterns in insects are not discrete respiratory forms but instead represent a continuum that reflects the balance between O_2 demand and supply (Bradley, 2007). When insect metabolic rate is increased, the increased O_2 demand must be accommodated by increasing spiracular conductance, i.e. opening the spiracles (Bradley, 2007; Moerbitz & Hetz, 2010). Thus DGCs emerge when the gas exchange system has little demand placed on it and the spiracles can remain closed for long periods of time (Marais *et al.*, 2005; Contreras & Bradley, 2010). The balance between CO_2 production and elimination are equally important in influencing spiracular conductance and therefore the pattern of gas exchange. The control of internal CO_2 levels is crucial for acid-base homeostasis, which is important for ideal protein function (Forster & Hetz, 2010). Insects have been shown to transition from discontinuous, cyclic and continuous respiration as a response to increasing temperature, which results in increased metabolic rate and aerobic demand (Bradley, 2006; Contreras & Bradley, 2010; Contreras & Bradley, 2011).

Although, the emergent-property hypothesis gives a simple and elegant explanation for the existence of DGC, it does not address why this system of interacting set points evolved. New findings provide an adaptive reason for the existence of this mechanistic component of DGC. The above hypothesis states that both CO_2 and O_2 are involved in controlling spiracular behaviour. The CO_2 threshold for spiracular opening is sensed at the spiracular muscle while the O_2 threshold is sensed at the segmental ganglia of the central nervous system (Hustert, 1975; Chown, 2011; Matthews & White, 2011). Thus the brain does not

govern spiracular opening and insect thoracic and abdominal ganglia are responsible for the gas exchange pattern displayed. These ganglia have been shown to possess spontaneous rhythm generators and chemosensitivity to O_2 and CO_2 (Hustert, 1975). One thing that all insects displaying DGCs have in common is reduced or absent brain activity (Matthews & White, 2011). Brain activity is energetically expensive, large brains in particular are energetically draining (Chown, 2011). Thus energetic considerations should promote the downregulation of metabolic rate and a reduction in brain activity. Respiratory control will then be relinquished to the segmental ganglia of the thorax and abdomen resulting in a discontinuous pattern of gas exchange. This incorporation of the non-adaptive hypothesis with an adaptive energy savings one is referred to as the neural hypothesis (Chown, 2011). It states that DGCs are merely an emergent property of the insect's nervous system, which occur when energetic constraints favour the downregulation of neural activity (Matthews & White, 2011).

The wide range of environments occupied by insects that display DGCs and the dissimilar ways in which insects from different taxa react to environmental conditions, as well as their diverse life strategies makes identifying a common adaptive benefit of DGC difficult (Terblanche *et al.*, 2008; Matthews & White, 2011). It might be that several factors exist that select simultaneously for DGC or that a pre-existing DGC could be pressed into service for multiple purposes. White *et al.*, (2007) found a significant positive relationship between DGC duration and habitat temperature as well as an important interaction between habitat temperature and precipitation. According to their study reducing water loss while ensuring adequate O_2 delivery under desiccating conditions is likely to be the feature selecting for or maintaining DGCs. Thus it is these features; water loss/availability, temperature and oxidative damage that I will be focusing on in my study on the respiratory patterns used by workers of *Hodotermes mossambicus* (Hagen) in hope of adding further clarity to the arguments of the evolution and maintenance of the DGC.

1.2 Rationale

Studies on termite respiratory physiology and gas exchange patterns have focused on damp-wood termites and dry-wood termites (Shelton & Appel, 2000; Shelton & Appel, 2001; Lighton & Ottesen, 2005). Of these termites species studied none have been shown to exhibit a true DGC but cyclic respiration is common. Chown, (2011), characterizes cyclic gas exchange by its regular peaks and troughs of gas exchange, however no closed phase is shown. Bradley, (2007), defines cyclic gas exchange by O phases in which CO_2 is released in large bursts, followed by an F phase.

For this study I chose the grass-harvester termite species, *H. mossambicus* to provide clarification in the study of DGC. The workers of this species forage mostly during the day while exposed to desiccating temperature and humidity conditions (Coaton & Sheasby, 1975; Myburgh, 1989; Uys, 2002). Unlike the workers in most termite species, these workers possess a dark pigmentation which serves to protect them against harmful and potentially lethal UV radiation (Nel & Hewitt, 1969); therefore any additional protection from desiccation due to exposure to the sun could potentially provide a survival advantage. Workers of *H. mossambicus* are continually exposed to desiccating ambient conditions (Nel & Hewitt, 1969). If they exhibit DGC in response to these conditions this would provide further support to the hygric hypothesis.

Hodotermes mossambicus is a subterranean species found at depths of a few centimeters up to 6 meters. When not foraging, galleries leading to the soil surface are plugged with mud (Coaton & Sheasby, 1975; Myburgh, 1989; Uys, 2002). Thus, the workers are likely operating under the selective pressures of hypoxia and hypercapnia as a result of their colony depth and lack of direct oxygen exchange. They are therefore likely candidates of exhibiting DGC in order to positively influence respiratory gas concentration gradients in accordance with the chthonic hypothesis. Although it appears that workers are exposed to both hypoxic and hyperoxic conditions, workers exposed to O_2 concentrations ranging from 2 % to 25 %, under experiemental conditions did not exhibit DGC (Inder,

2010). This corresponds with the findings of White *et al*., (2007) where environmental O_2 was found not to affect respiratory pattern.

A study by Lighton and Ottesen (2005), found that individuals of *Zootermopsis nevadensis,* a damp-wood termite, did not express DGC in response to hyperoxia but did, however, restrict their spiracular area under elevated oxygen concentrations. They proposed that full DGC expression was not in effect as the O phase would flush the intestinal microorganisms residing within the gut of this species of termite with harmful concentrations of O_2. These intestinal microbes are anaerobic and exposure to high/normal O_2 concentrations would result in their death (Lighton & Ottesen, 2005). Workers of *H. mossambicus,* however, do not possess these anaerobic intestinal microbes and instead are fed by 5^{th} larval instars (Watson, 1973). Hence these termites were thought to provide a more suitable subject to test the possible significance of O_2 toxicity avoidance as a mechanism for DGC.

In an initial study (Inder, 2010); the respiratory pattern of workers of *H. mossambicus* exhibited a cyclic gas exchange pattern when recorded at 25 °C. Contreras and Bradley, (2010), found that insects exposed to low temperatures exhibited a DGC. As metabolic rate increased, periods of spiracular closure decreased and insects showed a more cyclic pattern. As metabolic rate increased further under the highest experimental temperatures, periods of spiracular closure decreased even more and continuous respiration was displayed. Therefore, under the emergent-property hypothesis the experimental temperature of 25 °C would represent an intermediate metabolic rate (Bradley, 2007). Thus, if this hypothesis is to be believed workers of *H. mossambicus* exposed to lower temperatures should exhibit a DGC.

1.3 Aims and Objectives

Firstly, I aim to test the hygric, emergent-property and oxidative-damage hypotheses of the discontinuous gas exchange cycle in the workers of *H. mossambicus*. Secondly, I wish to ascertain what environmental condition/s

(humidity, temperature and oxygen) result in the expression of DGC in this species. By testing these hypotheses I hope to add further insight into the evolutionary genesis and current adaptive significance of DGC in insects.

1.4 Research Questions

- What is the water loss rate and critical hydration level for workers of *H. mossambicus*? (Chapter 3)
- What is the effect of environmental water availability (relative humidity) on the respiratory pattern of workers? (Chapter 4)
- What is the effect of environmental temperature and hence metabolic rate (MR) on the respiratory pattern of workers? Specifically, does low temperature (low MR) induce a DGC? (Chapter 5)
- What is the effect of hyperoxia (100 % oxygen) on the respiratory pattern of workers? (Chapter 6)

CHAPTER 2: Methods and Materials

2.1 Study Species

Hodotermes mossambicus is a southern African, grass harvesting species, often considered a pest, which is widely distributed and frequently occur in areas of low rainfall. This species is subterranean and can occur up to 6 m underground. Colonies are found primarily on savannas. Foraging in this species generally takes place at night during summer and by day in the dry winter months, and is done by the worker caste (Coaton & Sheasby, 1975; Uys, 2002; Myburgh, 1989). The worker caste is the only caste to leave the colony and they can be separated into the major and minor workers, which are differentiated by head width and perform slightly different tasks (Watson, 1973). Major workers are responsible for cutting the plant material while the minor workers are responsible for the transport of material (Duncan & Hewitt, 1989).

2.2 Study Area

Actively foraging workers of *Hodotermes mossambicus* were collected in the field in the Highveld area from Emmerentia Dam, South-West entrance (-26.160264 S, 27.998067 E) in Johannesburg. Workers were collected between the months of May and November 2011 and April and May 2012, between 9 am and 3 pm. Temperature and humidity were measured during each collection (PH1000 Zeal Thermohygrometer). Termites were housed in Petri dishes with moist cotton wool and placed in a temperature chamber maintained at 25 °C. Termite workers are unable to feed themselves and as such were left unfed. However, water was provided in the form of moist cotton wool. All measurements were performed within a period of 2 weeks after collection. During this period starvation was shown to have no significant impact on measurements of VCO_2.

2.3 Experimental Design and Protocol

2.3.1 Water Loss

Three aspects of water balance were determined for both major and minor workers: total body water (TBW), critical hydration level (CHL) and water loss in $ml.h^{-1}$. The gravimetric method was used for determining rates of water loss.

Individual termites were placed in pre-weighed, perforated Eppendorf tubes (1.5 ml) and weighed (Libror AEG-455M) prior to the experiment to determine their initial or wet mass. The termites were then placed in a desiccator where relative humidity (RH) was 27 %. The desiccator was kept at a constant temperature of 30 °C within a temperature-controlled chamber (Labcon low temperature incubator Model L.T.I.E). Termites were weighed hourly, their masses were recorded and their coordination was assessed. Termites that showed a lack of coordination i.e. they could not right themselves when turned over, were removed from the desiccator, weighed and then dried to a constant mass at 60 °C in an oven (Hadley, 1994). After four days, the termites were weighed and this weight was recorded as dry mass. The water loss rates of ten major and ten minor workers were assessed using this method.

Total body water is the difference between wet and dry mass of the termite. Critical hydration level was defined as the percentage of total body water left at the point of loss of coordination. The following formulas were used and the values are expressed as percentages:

Total body water = $((M_W – M_D)/ M_W) \times 100$
Water content at death = $((M_{LC} – M_D)/ M_{LC}) \times 100$
Critical hydration level = $((M_W - M_{LC})/ (M_W – M_D)) \times 100$

Where M_W is the initial or wet mass of the termite, M_D is the dry mass of the termite and M_{LC} is the mass of the termite at loss of coordination.

Average water loss rate for both major and minor workers was determined using least squares regression. In addition, during flow through respirometry experiments all termites were weighed prior and following each experiment. These values were used to calculate water loss rate under moving air as follows:

Water loss rate ($g.h^{-1}$) = ($M_{Before} - M_{After}$)/time of recording

2.3.2 Respirometry

General

A flow through respiratory system was used to determine the respiratory pattern and metabolic rate of the major and minor workers of *Hodotermes mossambicus* in both dry air and humidified air. Respirometry measurements were taken between the hours of 9 am and 8 pm in order to take account of the possible influence of circadian rhythm. The dry air experiments were set up as shown in Fig. 2.1. Room air was scrubbed of water and carbon dioxide (CO_2) through the use of a Drierite/Ascarite column before being pumped through the respirometry chamber, which contained the termite. The CO_2 released by the termite was then measured by a CO_2 analyzer (Licor LI7000). Emissions of CO_2 were recorded every second over a period of 2 hours using a data acquisition system (ExpeData, Sable Systems). The flow rate of the air was kept constant at 50$ml.min^{-1}$ by a mass flow controller (MFC, Sable Systems MFC2 mass flow control unit). Experiments at 100% relative humidity (RH) were similarly set up (Fig. 2.2); however room air was scrubbed of only CO_2 before being bubbled through a flask of distilled water to humidify the air. The air was then scrubbed of water using a magnesium perchlorate scrubber before entering the CO_2 analyzer.

Ten termites from each of the worker castes were used for each trial. Individual termites were placed in the respirometry chamber and given 10 minutes to adjust to the set-up and air humidity before measurements started. A baseline measurement of the air stream was taken for 5 minutes before and after each

termite was placed in the respiratory chamber to control for analyzer drift. Experiments were run at a constant temperature of 25 °C unless otherwise stated.

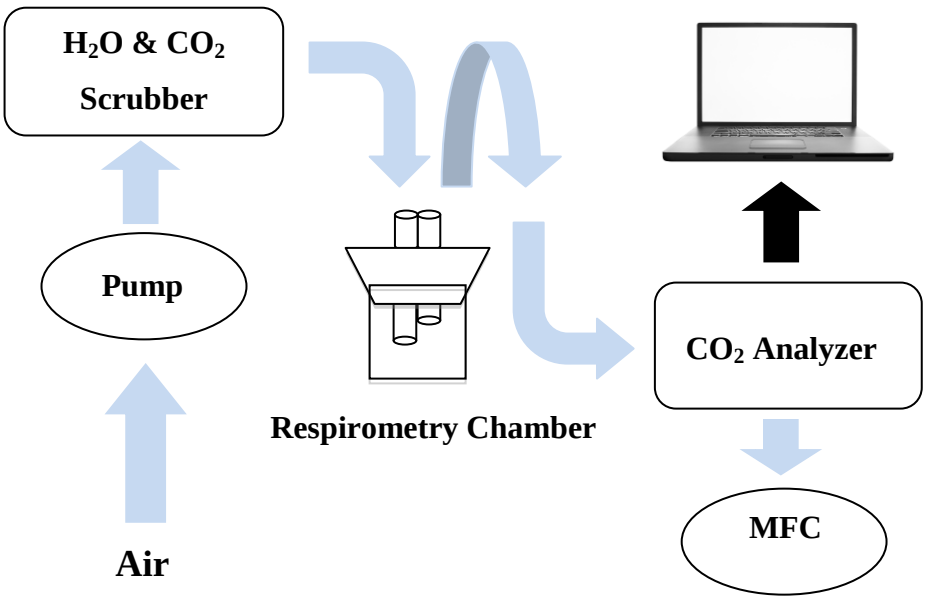


Figure 2.1: Diagram showing the experimental set-up of the flow-through respirometry system used to determine the gas exchange pattern at 0 % RH. MFC = mass flow controller.

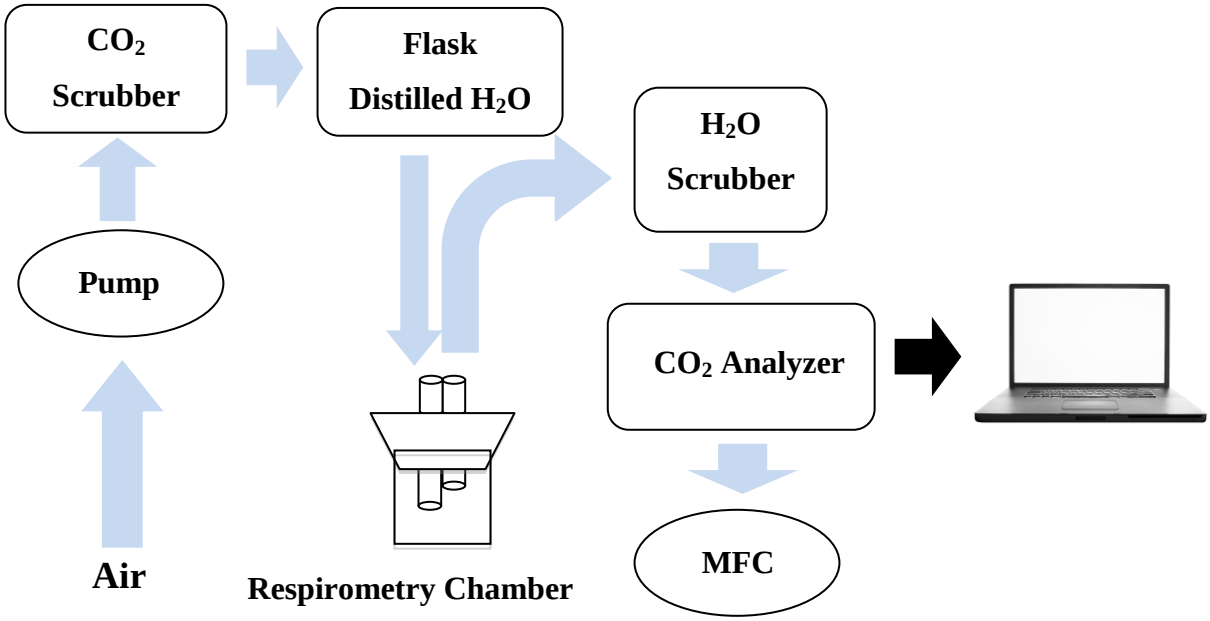


Figure 2.2: Diagram showing the set-up of the flow-through respirometry system used to determine the gas exchange pattern at 100 % RH. MFC = mass flow controller.

Temperature

Flow-through respirometry was used to assess CO_2 output under different temperatures. The following temperatures were used: 5 °C, 10 °C, 15 °C, 25 °C and 35 °C. Individual termites were only used once at an assigned temperature. Only major workers were assessed in this experiment. Ten major workers were measured at each temperature. Experiments were run using humid air (100 % RH) as it was found that termites survived for longer under humid conditions. The experimental set-up is similar to that shown in Figure 2.2 with the experimental termite and humidifier placed in a controlled temperature chamber set at the temperature of the experiment (Fig. 2.3). As before termites were given 10 minutes to adjust to the temperature before data collection began. CO_2 emission data were collected for a period of two hours. Baseline CO_2 was obtained before and after the recording.

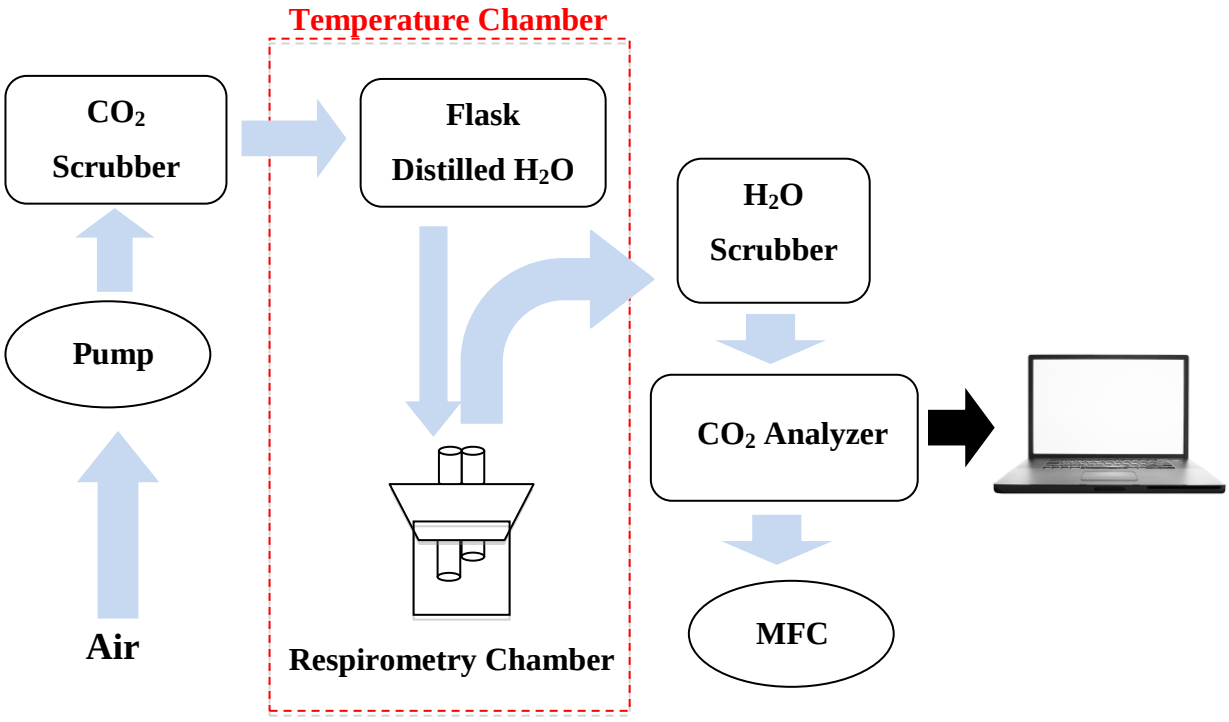


Figure 2.3: Diagram showing the set-up of the flow-through respiratory system used to determine the gas exchange pattern at varying temperature. MFC = mass flow controller.

Hyperoxia

A flow-through respirometry system was used in order to determine periods of spiracular closure. The experiment was set up as described above (Fig. 2.3); however the airstream was replaced with 100 % O_2. Major workers were exposed to hyperoxic conditions for a period of 2 hours at 25 °C. This experiment was done in both dry air and humid air. Ten major workers were used for each treatment. A five-minute baseline CO_2 reading was taken before and after each run. Termites were given 10 minutes to adjust to the new oxygen conditions before the start of the experiment.

Activity

Termite activity levels were monitored in all respirometry experiments. In the humidity experiments activity was monitored both visually, through the use of a video camera (HD Hero Go Pro), and through use of an activity detector (Sable Systems AD-2-R). The detector consists of two fiber-optic wires with a beam of

light traveling between the two ends. The wires were set up on either side of the respirometry chamber. When a termite crosses the beam, this causes fluctuations in the light level. These fluctuations are recorded as volts over time. Activity is recorded as the variance of activity from zero. During the other respirometry experiments only the activity detector was used to monitor activity, as it was too dark in the temperature chamber to film the termites. These traces of activity were used to clarify that the peaks of CO_2 were a result of the use of DGC and not a result of high activity. It is worth mentioning here that the termites used in the experiments were always active, to varying degrees, and were never seen at rest or inactive. This phenomenon has been observed in other species of termites (Lighton & Ottesen, 2005).

2.4 Data Analysis

2.4.1 Respirometry

Before traces were analyzed, drift correction was performed using a function in ExpeData. After this, CO_2 output was converted from parts per million (ppm) to VCO_2 or metabolic rate (ml $CO_2.g^{-1}.h^{-1}$) using the following formula:

$VCO_2 = ((C1/1\ 000\ 000) \times 50\ ml.min^{-1} \times 60)/mass\ of\ termites\ in\ grams$

Where C1 is $ppmCO_2$, 50 $ml.min^{-1}$ is the flow rate and 60 is used to convert minutes to hours. A linear relationship of mass loss was assumed, thus the mass of the termite was calculated as the average of the initial and final mass of the termite.

The different patterns of gas exchange observed under varying humidity and temperature as well as under hyperoxia were identified by looking at the CO_2 release over time. Once the pattern was identified a portion of the trace was extracted for data analysis. This portion had to be between 25 to 40 minutes long and be extracted after the first half hour of data collection and before the last half hour. These half hour portions of data were excluded as during the first half hour,

the insect was still adjusting to the experimental conditions and in the last half hour, the condition of the termites had a tendency to deteriorate. Extracted portions had to be representative of the general trend of the trace and activity had to be constant. Metabolic rate was calculated for this section by averaging the VCO_2 of the section of data. Metabolic rate was then averaged for each treatment. Coefficient of variance (CV) was also calculated for each section and averaged per treatment. CV is a measure of the amount of variation or cycling in a trace; a low CV value (close to 0) represents very little variation and a more continuous gas exchange pattern while a high CV value (greater than 1 and closer to 2) represents a highly variable trace and a more discontinuous gas exchange pattern. CV was calculated using the following formula:

CV = standard deviation / mean

2.4.2 Objectively defining spiracular closure

The method described below, used to indicate periods of probable spiracular closure, has been previously described by Contreras and Bradley in 2010 and again in 2011. In this method, termites were exposed to hyperoxic conditions (high oxygen) at 25 °C while VCO_2 was measured. Previous studies have shown that prolonged exposure to hyperoxia results in a prolonged close phase (Lighton *et al*., 2004). To achieve hyperoxic conditions, experiments were carried out as described above (Section 2.3.2). The lowest VCO_2 values (minimum of 150s of continuous data) for each individual were selected using the NADIR function in ExpeData. NADIR identifies the lowest values within selected periods of data. These data were then averaged. All data points below this threshold value were considered to be associated with a period of spiracular closure.

During the course of the experiments, it was found that under certain experimental conditions this method did not realistically detect spiracular closure. However, in all figures the cut-off threshold was included to give a rough estimation of periods of spiracular closure.

2.4.3 Statistical Analysis

Statistical analyses were performed in R (R version 2.11.1). All values are reported as mean ± standard deviation. Differences between the water balance characteristics of major and minor workers were calculated using student t tests. Analysis of covariance (ANCOVA) was used to test the influence of body mass on total body water, critical hydration level and water loss rate. Differences between means of the different humidity and temperature treatments were compared using analysis of variance (ANOVA). When ANOVA was significant a Tukey Post Hoc test was performed. ANCOVA was used to test the influence of body mass, water loss, time of day and days since collection on metabolic rate and CV. Student t tests were used to analyze differences between the means in the hyperoxic experiments. When necessary, non-parametric tests were used but in general parametric tests were used as these are more robust. Box plots also known as box and whisker plots were used to graphically depict groups of numerical data. Box plots indicate the following numerical characteristics of the given data: the smallest observation, the lower quartile, the median value, the upper quartile, and the largest observation. A boxplot also indicates outliers within the data.

CHAPTER 3: Water Balance

3.1 Introduction

3.1.1 General Introduction

Hodotermes mossambicus is a subterranean species of termite. Their underground nests are deep and complex, consisting of multiple connected hives (Coaton & Sheasby, 1975). As a result of this depth and complexity, vertical migration allows for the existence of a temperature and humidity refuge. As soil depth increases, the temperature of the soil decreases until it reaches a constant while soil moisture and access to the water table create a humid environment. Thus behavioral regulation of water loss rate is possible. Foragers, however, have fewer options available to them as they are exposed to the temperature and humidity of the external environment (Lighton & Feener, 1989; Duncan & Lighton, 1994). Over the course of one or more foraging trips, foragers will have to contend with high temperatures and dry air conditions, which may result in dehydration, loss of coordination and even death. It is therefore likely that foragers or workers of *H. mossambicus* are under strong selection for the ability to minimize water loss because water stress may limit their foraging ability and foraging time.

Foragers perform a vital and continuous role in any successful termite colony and water loss by foragers may have important consequences for colony fitness. Water balance considerations have a profound influence on foraging efficiency; foragers affected by water stress may become incapable of cutting and transporting grass. Foraging areas of a single colony of *H. mossambicus* can be anywhere between 0.5 and 3.1 ha (van der Linde *et al.*, 1989). This large area is essential because foraging can strip the land directly surrounding foraging ports of all plant matter, therefore workers have to go further to provide for the colony. Water stress can result in reduced foraging time for workers and therefore reduced foraging

distance. If body water content falls below a critical value, foragers may lose coordination and be lost to the colony. All these factors ultimately impact colony fitness and survival (Lighton & Feener, 1989; Feener & Lighton, 1991; Duncan & Lighton, 1994; McKechnie & Wolf, 2004). Foragers, therefore, must employ various mechanisms to remain in positive water balance.

3.1.2 Desiccation Resistance

Insects from habitats of differing water availability show considerable variation in their tolerance to desiccation, resistance to desiccation and their initial body water content (Gray & Chown, 2008). Insects respond to desiccation in a variety of ways. They can simply tolerate the loss of water by increasing the amount of water that they are able to lose before death, they can avoid desiccating conditions through movement to more suitable microhabitats or they can resist desiccation through increasing their initial body water content and decreasing their water loss rates (Chown & Nicolson, 2004; Bazinet *et al*., 2010). Most insects employ a variety of these adaptive mechanisms (Hadley, 1994). By defining an insect's water balance characteristics we can determine the mechanisms the insect employs to remain in positive water balance under varying environmental conditions.

3.1.3 Water Balance

Despite the various mechanisms employed to reduce water loss, be they behavioural, morphological or physiological, some water loss is inevitable and this water needs to be replaced in order to sustain proper functioning of the insect (Yoder *et al*., 2006). There are four basic avenues through which water can be obtained: drinking, uptake via feeding, absorbing atmospheric water vapour and finally through the production of metabolic water (Edney, 1957; Hadley, 1994). Most insects drink freestanding water if it is available, however, the majority of water is taken in with food. Without access to freestanding water, the water content of food becomes especially important (Edney, 1957; Hadley, 1994). However, while harvester termites might gather both dry and green grass, they eat dry grass, which has low water content. Some insects have the ability to actively absorb water vapour from the atmosphere even when the air is well below the

point of saturation (Nicolson, 1980). The larvae of the mealworm, *Tenebrio molitor* have the ability to absorb water through their rectum; however this form of water uptake is a process that requires the expenditure of a great deal of energy to overcome water thermodynamic activity (Hadley, 1994). Metabolic water is derived from the metabolism or complete oxidation of food. This avenue of water uptake is thought to only provide a small or negligible portion of the water required to maintain positive water balance (Hadley, 1994). However, in large flying bees, such as *Xylocopa* and *Bombus*, metabolic water production is substantial (Nicolson, 2009). Drier conditions can stimulate greater metabolism and therefore the production of greater quantities of metabolic water (Edney, 1957). During periods of dehydration, the cockroach *Periplaneta* has been known to draw on reserve lipids (Nicolson, 1980). Hence this avenue of water uptake can prove valuable in preventing further dehydration.

Resisting desiccation through the uptake of water is a viable strategy. However, desiccation resistance through the selective modification of the channels of water loss still remains controversial. Excretion, cuticular transpiration and respiratory transpiration constitute the major avenues of water loss in insects (Hadley, 1994). The feces of termites are more or less dehydrated when expelled, all excess water is resorbed within the digestive tract and the excrement takes the form of dry pellets (Krishna & Weesner, 1969). Thus excretory water loss is minimal.
The cuticle is a noncellular, multilayered membrane that makes up the body covering of insects (Hadley, 1994). The cuticle is made up primarily of chitin, proteins and lipids. The outer layer or epicuticle is composed of lipoproteins and it is this layer that serves as a barrier to water diffusion (Lease & Wolf, 2010). However, despite this degree of waterproofing, cuticular transpiration remains a major avenue of water loss and in fact contributes the largest component of total water loss (Lighton & Feener, 1989).

Respiratory water loss is the other major avenue of water loss, although its contribution to overall water loss, and hence its importance to water balance, is an area of much research and controversy. Respiratory water loss contributes less,

and in many cases much less, than 20 % of total water loss. Modulation on such a small amount is thought unlikely to confer a fitness benefit (Chown, 2002). Others argue that even a small savings in water, especially when cuticular water loss has already been reduced, might be significant (Lighton *et al*., 1993; Bosch *et al*., 2000; Chown, 2002).

All terrestrial animals face the conflicting needs of obtaining oxygen and simultaneously preventing water loss. This conflict is thought to be resolved in insects through the intermittent closing of the spiracles (Chown & Nicolson, 2004; Bradley, 2007; Woods & Smith, 2010). This pattern of gas exchange is commonly known as the discontinuous gas exchange cycle (DGC). When the spiracles are closed, the avenue of respiratory transpiration is cut off and hence respiratory water loss is reduced. However, whether this is in fact the case has been disputed. DGC is not universally expressed in insects and insects experiencing water stress readily abandon this pattern of gas exchange (Bosch *et al*., 2000). In fact, DGC seems to incur a greater level of water loss than is necessary during gas exchange. For insects, water losses per unit O_2 were up to 8-fold higher than predicted by Woods and Smith's (2010) universal model for water costs. This inconsistency was thought to be a consequence of the discontinuous respiratory pattern. It seems that spiracles are required to open longer to rid the insect of sufficient CO_2 than is necessary to take up adequate O_2 (Section 1.1). Yet despite these findings the role of gas exchange pattern and metabolic rate in water loss is still largely a matter of contention. Chown (2011) has proposed that the high water loss associated with DGC might produce strong selection for modification of the components of DGC, such as having a prolonged closed phase. Metabolic rate may well be down regulated as a mechanism to reduce respiratory water loss (Chown *et al*., 2011)

3.2 Aim and Research Objectives

The aim of the experiment was to determine the water balance characteristics of the worker caste, both major and minor, of *H. mossambicus*. In particular, body water content, maximum tolerable water loss (critical hydration level) and the rate of water loss were determined. This information was used to compare water loss

under varying humidity and temperature conditions and ultimately to test the hygric hypothesis of the discontinuous gas exchange cycle.

3.3 Results

3.3.1 Water content and Critical hydration level

The mean wet body masses of major and minor workers were found to be 0.067 ± 0.009 g and 0.046 ± 0.008 g respectively. Expressed as a percentage of wet body mass, the water contents of the two castes of workers were, 76.42 ± 1.19 % and 77.63 ± 3.14 %, respectively. Without correcting for possible mass effects, there was no significant difference evident between the water contents of the two castes ($t_{(18)} = 1.73$, $p = 0.27$). An ANCOVA revealed no significant mass effects, the relationship between water content and initial mass between major and minor workers were found to differ but not significantly (Fig. 3.1)($F_{(1,18)} = 1.58$, $p = 0.23$). Initial mass was found to have a non-significant effect on water content ($F_{(1, 18)} = 0.62$, $p = 0.81$).

Loss of co-ordination occurred at less than 50 % of body weight remaining for both worker castes. Critical hydration level (CHL) was 42.36 ± 7.01 % of total body water for major workers. Not surprisingly, minor workers were slightly less resilient, i.e. they could withstand the loss of less water, with a CHL of 47.73 ± 6.21 %. This difference however, was not significant ($t_{(18)} = 2.145$, $p = 0.127$). Using ANCOVA to correct for mass effects, initial mass was found to have no significant effect on CHL ($F_{(1, 18)} = 0.015$, $p = 0.905$).

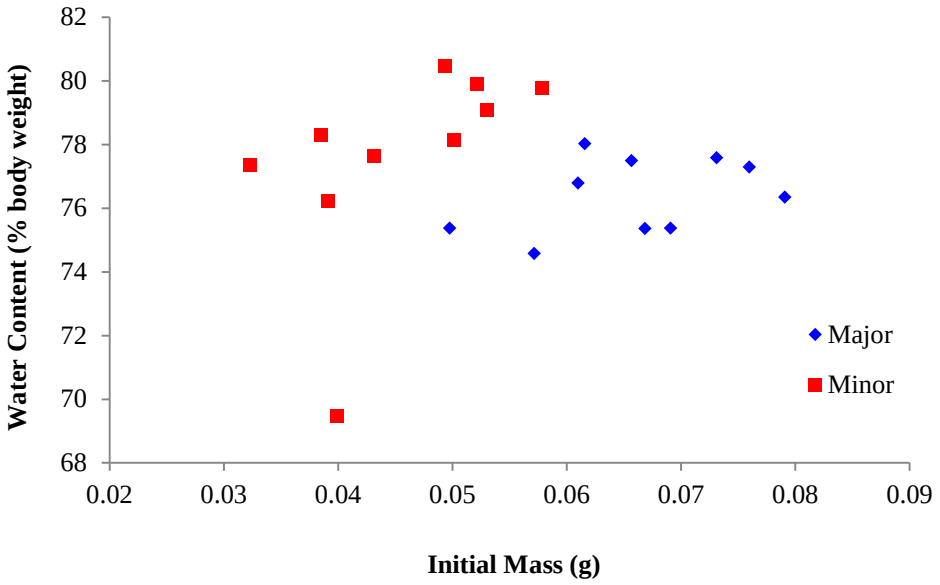


Figure 3.1: The relationship of water content and initial mass for major and minor workers of *H. mossambicus*. Major workers: water content= 50.13 x initial mass + 73.11 (R^2 = 0.142, p>0.05); minor workers: water content = 201.98 x initial mass + 68.43 (R^2 = 0.271, p>0.05).

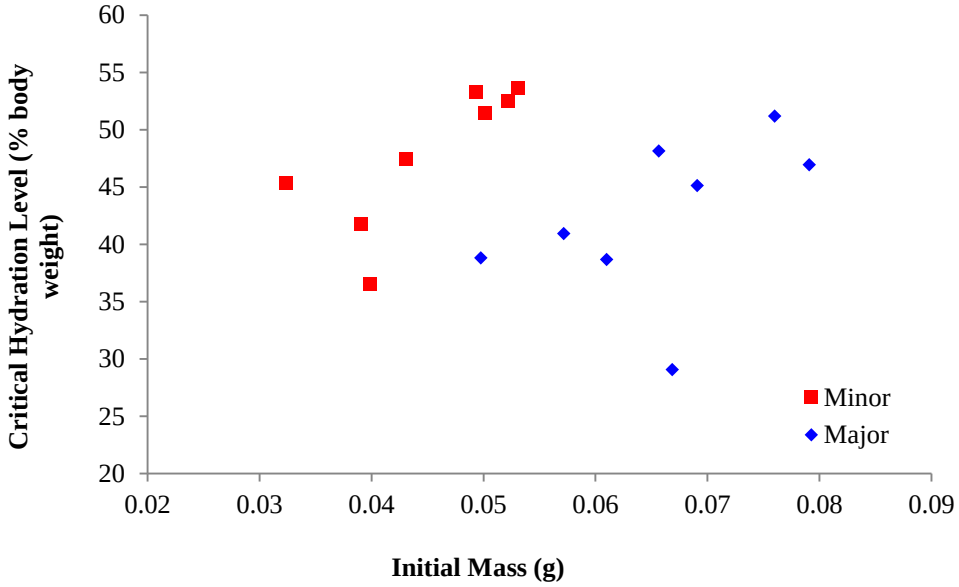


Figure 3.2: The relationship between critical hydration level and initial mass for major and minor workers of *H. mossambicus*. Major workers: CHL= 357.91 x initial mass + 18.89 (R^2 = 0.241, p>0.05); minor workers CHL = 641.12 x initial mass + 18.95 (R^2 = 0.271, p>0.05).

3.3.2 Water Loss Rate

Major workers lost water steadily over time until the 8th hour. Minor workers showed a similar trend and slope; however, their water loss stopped increasing at the 6th hour of the experiment (Fig. 3.3). After the 6th and 8th hour, only two minor and major workers had not reached the point of loss of co-ordination. This value was considered too low to be statistically relevant so measurement of water loss was discontinued. Major workers lost water at a rate of 0.0029 ± 0.0005 $ml.h^{-1}$ (R^2= 0.994), while minor workers lost water at a rate of 0.0024 ± 0.0008 $ml.h^{-1}$ (R^2= 0.983). The rates of water loss in the two worker castes did not differ significantly ($t_{(18)}$ = 2.179, p=0.225). An ANCOVA was performed to test the effect of mass on water loss rate (Fig. 3.4). It was found that initial mass had a non-significant effect on WLR ($F_{(1, 18)}$= 4.4716, p= 0.058). This result could be a consequence of the small sample size, n=10.

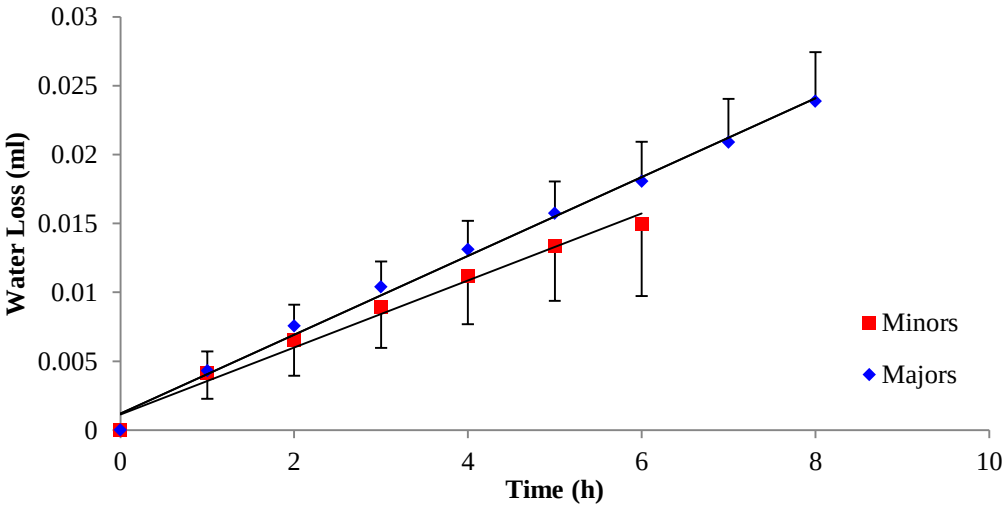


Figure 3.3: Rate of water loss over a period of ten hours for major and minor workers of *H. mossambicus*. Error bars show standard deviation.

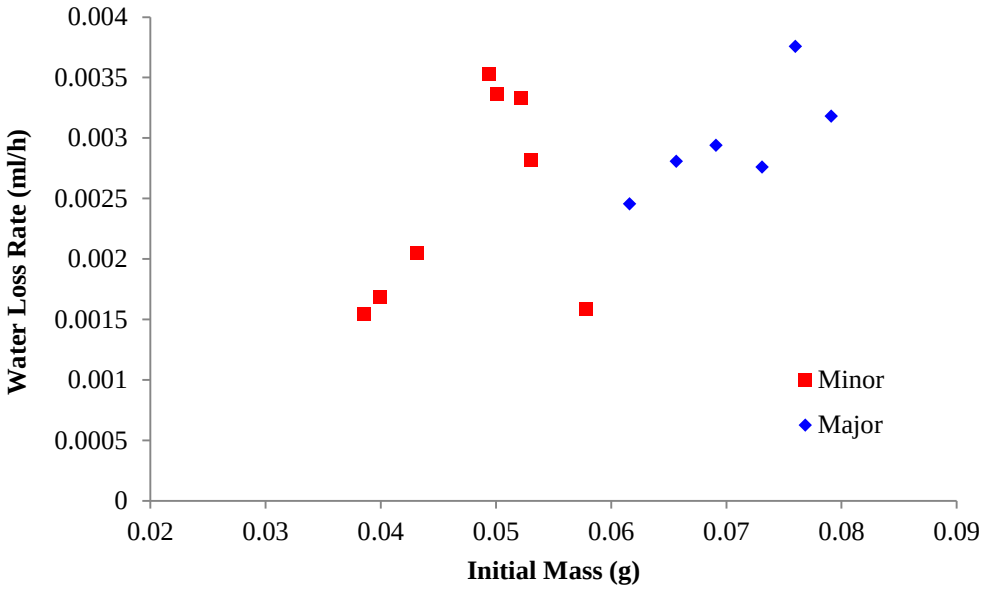


Figure 3.4: The relationship between water loss rate (WLR) and initial mass for major and minor workers of *H. mossambicus*. For major workers WLR= 0.051 x initial mass – 0.0006 (R^2 = 0.560, p>0.05) and for minor workers WLR = 0.053 x initial mass – $4E^{-05}$ (R^2 = 0.173, p>0.05).

3.4 Discussion

Table 3.1: Water balance characteristics of several termite species. (BWC is body water content and T is temperature). Values are means ± SD.

Species	Mass (mg)	Caste	BWC (%)	Water Loss Rate ($mg.g^{-1}.h^{-1}$)	T (°C)	Source
Incisitermes minor (Hagen)		Workers		5.22 ± 1.87	20	*a*
		Workers		17.96 ± 2.55	40	*a*
Coptotermes formosanus (Shiraki)		Female alates	55.68 ± 0.42		30	*b*
Reticuliitermes flavipes (Kollar)		Workers	75.88 ± 0.36		30	*b*
Macrotermes carbonarius (Hagen)	11.25 ± 0.22	Minor workers	79.87 ± 0.35	72.11 ± 2.16	30	*c*
	24.91 ± 0.71	Major workers	82.72 ± 0.72	58.51 ± 2.49	30	*c*
Macrotermes gilvus (Hagen)	5.14 ± 0.14	Minor workers	79.60 ± 0.58	97.80 ± 1.81	30	*c*
	9.30 ± 0.32	Major workers	81.30 ± 0.40	87.21 ± 2.27	30	*c*
Hodotermes mossambicus (Hagen)	67.00 ± 9.00	Major workers	76.42 ±1.19	44.01 ± 7.59	30	*d*
	46.00 ± 8.00	Minor workers	77.63 ± 3.14	52.63 ± 17.54	30	*d*

a is Shelton & Appel, 2000, *b* is Sponsler & Appel, 1990, *c* is Hu *et al*., 2012 and *d* is this study.

The body water content of workers of *H. mossambicus* is comparable to the workers of other termite species (Table 3.1) and does not deviate from the typical average percentage body water content of 70 % for insects (Hadley, 1994). Minor workers have slightly higher body water content, although this difference was found not to be significant. This result is unusual, however when considering the difference in body mass between the two castes. Body mass and size generally influence body water content (Hadley, 1994). This was found not to be the case for this species. Initial mass had a non-significant influence on body water content.

The mean body water content of *H. mossambicus* is, however slightly lower than the *Macrotermes* species. This could be a result of the different environmental conditions that the different species are exposed to. Differences in body water content exist between species and within species, between instars and individuals and in the same individual over time, with factors such as life history stage, age, sex, body mass, physiological state as well as environmental conditions having a profound influence (Wharton, 1985; Hadley, 1994). For example, insects maintained at a higher relative humidity will present with higher body water levels. This can be a result of either hygroscopic absorption of water through the cuticle or the lower transpiration rate created by the reduced water gradient (Hadley, 1994). Workers of *H. mossambicus* forage above ground in the open and are exposed to a mean temperature and relative humidity of 26.8 ± 4.17 °C and 32.6 ± 9.39 % RH, while foraging. These values represent the averages of the recorded temperature and humidity during collections, i.e. when workers were seen foraging. Workers of *Macrotermes carbonarius* also forage in the open, however they are widely distributed across Southeast Asia and forage under a higher range of relative humidity, from 70 % to 86 % RH, while workers of *Macrotermes gilvus* forage below ground where humidity and temperature can be tightly controlled (Hu *et al*., 2012).

The critical hydration levels of most insects fall between 20 % and 50 %, with most species being able to cope with a loss of approximately 35 % of their water

content (Lighton & Feener, 1989; Yoder *et al*., 2006). Workers of *H. mossambicus* were able to tolerate a loss of almost 60 % of their body water before loss of coordination and/or death; hence they fall outside the upper limit of the published range. Minor workers of *H. mossambicus* share similar desiccation tolerance to that of major workers, i.e. they are both capable of tolerating a similar degree of water loss. Critical hydration level did not scale with body mass and was not affected by the initial body mass. This result is unexpected as critical hydration level was found to scale linearly with body mass in tsetse flies (Lighton & Feener, 1989), although there is no evidence that this relationship holds true for termite species.

The majority of termites lost co-ordination after 8 hours for the major workers and 6 hours for the minor workers. Water loss rates of the two castes are very similar. This result is unusual. One would expect minor workers to have a higher rate as a result of their higher surface area to volume ratio (Duncan & Lighton, 1994). Water loss rate was found to scale with body mass; however, initial mass was found not to have a significant effect on water loss rate. Minor workers could potentially be relying on a behavioral mechanism to resist water loss. A study by Duncan & Hewitt (1989), found that foraging trips by minor workers were generally short, with the longest duration being recorded as 260 s, while major workers made foraging trips of a longer duration, the longest made was 1158 s. Minor workers are therefore only exposed to desiccating conditions over brief periods of time before returning to the refuge of the underground galleries. Thus a physiological mechanism to resist or tolerate desiccation stress may be unnecessary. The similarities in water balance characteristics of major and minor workers can perhaps be explained by the overlap in their duties. Both major and minor workers cut and transport material, the only difference being the percentage of time they spend doing either activity. Major workers spend more time cutting material while minor workers spend more time transporting it (Duncan & Hewitt, 1989). The performance of similar duties under the same conditions will not produce differential selection pressure and thus the different castes will possess similar water balance characteristics.

When converted to mg $H_2O.g^{-1}.h^{-1}$ and compared to the water loss rates of other termite species (Table 3.1), the water loss rate of workers of *H. mossambicus* is found to be much lower than the *Macrotermes* species and higher than workers of *Incisitermes minor*. *I.minor* is a western dry-wood termite found mostly in South-West USA, particularly in California. These differences could be attributed to differences in habitat or food consumption (Hadley, 1994). Zachariassen *et al.*, (1988) found that insects from dry habitats were found to lose water at lower rates than species from humid habitats. Elaterid beetles of the species *Tetralobus rotundifrons*, which inhabit dry savannas, have rates of water loss lower (0.089 % of weight/hour) than that of closely related species *T. schuckardi* (0.555 % of weight/hour), which live in humid forests. This corresponds to findings by Addo-Bediako *et al*., (2001); they found that water loss rates and precipitation are positively related at a global scale. This pattern does not always hold true, however exceptions are generally a result of microhabitat use within the environment (Zachariassen *et al*., 1988). High rates of water loss or desiccating conditions can also be compensated for by high dietary water intake. Termites such as *H. mossambicus* and *I. minor*, feed on dry grass and dry wood, respectively, with little moisture, thus their dietary water intake would not be able to compensate for a large water loss rate and hence they require other means to resist or tolerate desiccation (Zachariassen *et al*., 1988).

CHAPTER 4: The Hygric Hypothesis

4.1 Introduction

The hygric hypothesis states that discontinuous gas exchange is an adaptation to reduce respiratory water loss (Chown *et al*., 2006). This hypothesis has and continues to garner the most support. Phylogenetic studies performed by White *et al*., (2007) and Marais *et al*., (2005), found that species from dry habitats are more likely to display DGCs than those from wet habitats, i.e. DGCs are associated with xeric environments. Other studies showed that desert beetles exhibit DGCs and that DGCs result in reduced respiratory water loss (Duncan & Byrne, 2000; Duncan *et al*., 2002) and insect respiratory patterns respond to changes in environmental humidity (Schimpf *et al*., 2009). Yet, despite this support, the hygric hypothesis has been disputed on several grounds. Evidence is mounting that DGC does not result in water savings but in water costs. The universal model for water costs of gas exchange (Woods & Smith, 2010) found, that for insects, water losses per unit O_2 consumed were 8-fold higher than predicted. This deviation from the model was attributed to DGC. Another study showed that while oxygen availability and metabolic rate influence respiratory pattern, environmental humidity does not (Terblanche *et al*., 2008). Clearly, whether using DGC does in fact result in reduced respiratory water loss remains to be resolved. DGCs appear to have evolved independently at least five times (Marais *et al*., 2005). Thus, there are at least five potential explanations for the genesis and maintenance of DGC. It might be that DGC represents a common solution to several problems, which would certainly explain the inconsistency between insects displaying DGC.

The hygric hypothesis essentially predicts that insects undergoing water stress should exhibit DGC in order to conserve water. Water stress can be defined in terms of insect hydrational status and the ambient relative humidity to which the

insect is exposed. Insect spiracles are sensitive to the hydration state of both the insect and the surrounding atmosphere (Terblanche *et al*., 2008). Dehydrated insects have been shown to exhibit increased spiracular control, while certain insects exhibit spiracular control in response to the changing relative humidity of the ambient air (Terblanche *et al*., 2008, Schimpf *et al*., 2009). It is this observed spiracular sensitivity that brought about the hygric hypothesis (Schneiderman, 1960).

If the hygric hypothesis represents the adaptive function of discontinuous gas exchange and the hydration status of the insect is controlled for, DGCs should be present under low humidity and abandoned at high humidity where it is no longer required for the conservation of respiratory water. More specifically, insects exposed to low relative humidity should have shorter open phases than those exposed to high relative humidity. Thus DGCs should be highly regulated and controlled under dry conditions (Terblanche *et al*., 2008; Schimpf *et al*., 2009). Studies on the effect of humidity on gas exchange patterns have, however, produced many conflicting results. Although DGC has been observed in desiccating conditions, DGC may also be employed when ambient relative humidity is high (Harrison *et al*., 2012; Contreras & Bradley, 2011; Fielden & Lighton, 1996). Despite this conflict, studies suggest that DGCs do in fact reduce respiratory water loss. Acute exposure to low humidity reduced CO_2 burst volume during the open phase for moth pupae, *Samia cynthia* (Terblanche *et al*., 2008), while chronic exposure of cockroaches, *Nauphoeta cinerea*, to low humidities reduced the duration of the open phase and resulted in lower rates of water loss (Schimpf *et al*., 2009).

Insects are capable of modifying their morphology and physiology in response to changes in their environment in order to allow for improved function in new ambient conditions (Schimpf *et al*., 2009). This process is known as phenotypic plasticity or acclimation response. Through observations of the acclimation response of an insect's gas exchange patterns in response to a range of environmental conditions we can describe the function of the discontinuous gas

exchange cycle. Thus, through the observed responses in respiratory pattern to changes in relative humidity we can evaluate the influence of relative humidity and therefore comment on the validity of the hygric hypothesis.

4.2 Aims and Research Objectives

The aim of this experiment was to determine the effects of low and high relative humidity on the metabolic rate, gas exchange pattern and water loss of major and minor workers of *Hodotermes mossambicus*.

4.3 Results

4.3.1 Rate of Carbon Dioxide Emission

The mean rate of CO_2 emission (VCO_2) or metabolic rate for major and minor workers under a relative humidity (RH) of 100 % was found to be 0.138 ± 0.054 ml $CO_2g^{-1}h^{-1}$ and 0.121 ± 0.066 ml $CO_2g^{-1}h^{-1}$, respectively. At 0 % RH the VCO_2 was found to be 0.112 ± 0.087 ml $CO_2g^{-1}h^{-1}$ and 0.244 ± 0.172 ml $CO_2g^{-1}h^{-1}$ respectively (Fig. 4.1). On inspection of the box plot (Fig. 4.1) it is noticeable that the distribution of VCO_2 for the different groups is similar, with the exception of minor workers at 0 % RH which had the largest range. Using an ANOVA, the means of the different groups were found to be similar ($F_{(3, 27)}$=0.772, p=0.536).

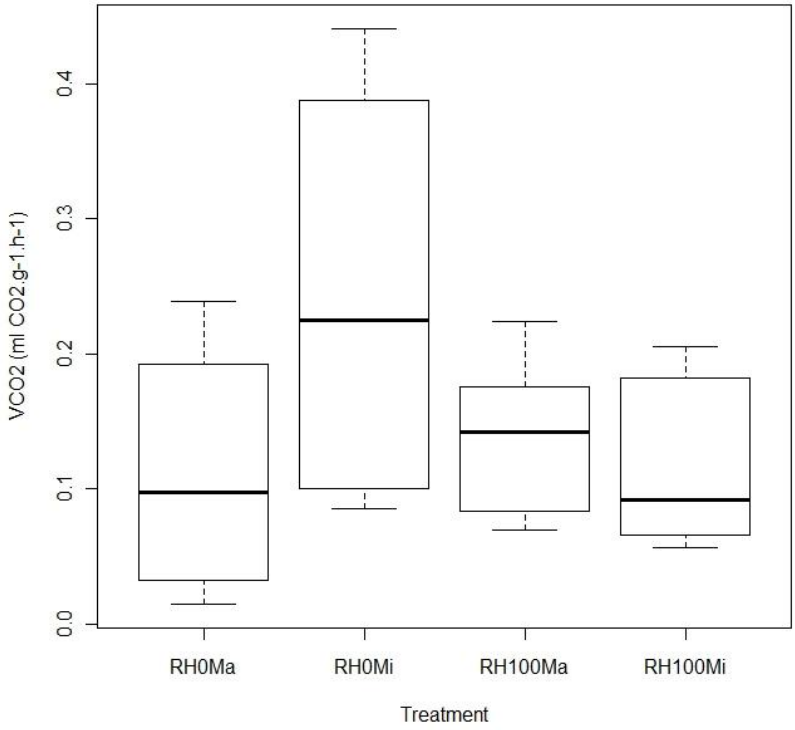


Figure 4.1: Box plot comparing the medians and variance of VCO_2 for major and minor workers under 100 % and 0 % relative humidity (RH). RH0Ma is major workers at 0% RH (n=11), RH0Mi is minor workers at 0 % RH (n=4), RH100Ma is major workers at 100 % RH (n=9) and RH100Mi is minor workers at 100 % RH (n=7).

4.3.2 Coefficient of Variation

The coefficient of variation (CV) gives an indication of the level of cycling in a CO_2 trace (See Section 4.4, paragraph 2). The means of the CV for the different groups are as follows; for major workers 0.353 ± 0.125 at 100 % RH and 0.801 ± 0.627 at 0 %, for minor workers 0.203 ± 0.058 at 100 % RH and 0.381 ± 0.309 at 0 % RH. Termites under the relative humidity of 0 % have a tendency towards higher values of CV than termites under 100 % RH, but not significantly so (Fig. 4.2). Termites at 0 % RH also experience greater ranges of CV than those at 100 % RH, i.e. the traces of termites at 0 % RH are highly variable. This is also illustrated in the CO_2 emission traces (Fig. 4.3 and Fig. 4.4). For workers at 0 % RH two typical patterns appear. The first is continuous with a great deal of pumping displayed and the second, although still continuous, shows periods of

spiracular closure where VCO_2 emission does not occur for short periods (Fig. 4.3). The level of cycling is much higher in the 0 % RH traces (Fig. 4.3) than in the 100 % RH traces (Fig. 4.4). At 100 % RH, the majority of traces display cyclic gas exchange (Fig. 4.4), however minor workers had a tendency towards more continuous patterns. Traces of major workers at 0 % RH spend more time below the cut-off threshold for spiracular closure than major workers at 100 % RH (Fig. 4.3 and 4.4). There is also a difference in activity level between the two treatments (Fig. 4.3 and 4.4). The 0 % RH traces, in general, showed a much higher level of activity than 100 % RH traces. Exceptions to this were the traces of the major workers at 0 % RH (Fig. 4.3). At 0 % RH, minor workers displayed higher activity levels than major workers.

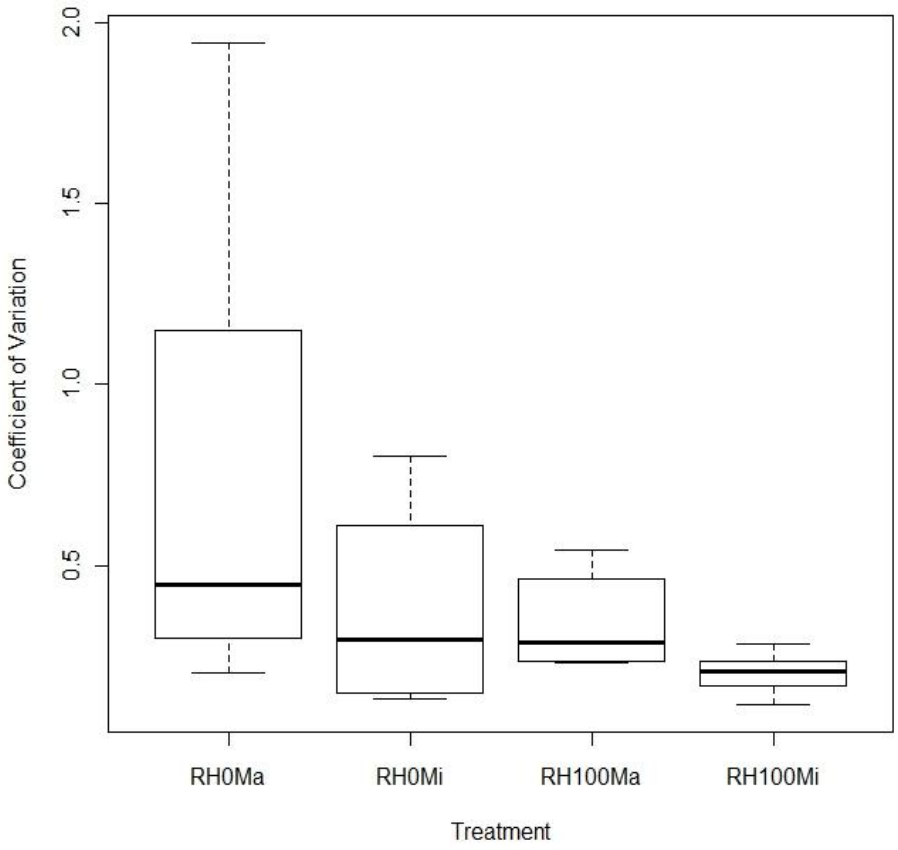


Figure 4.2: Box plot comparing the medians and variance of coefficient of variation (CV) for major and minor workers under 0 % and 100 % relative humidity. RH0Ma is major workers at 0 % RH (n=11), RH0Mi is minor workers at 0 % RH (n=4), RH100Ma is major workers at 100 % RH (n=9) and RH100Mi is minor workers at 100 % RH (n=7).

The Kruskal-Wallis test to determine whether the means of the different groups were equal showed that they were not (chi-squared=9.536, p=0.023). A Tukey post-hoc test showed that only RH100Mi differed significantly from RH0Ma (p=0.023). All other groups shared similar means.

An ANCOVA was performed to test the influence of various factors on CV. The average mass for the different groups is as follows: for major workers 0.061± 0.012 g at 100 % RH and 0.046 ± 0.005 g at 0 % RH, for minor workers 0.032 ± 0.01 g at 100 % RH and 0.019 ± 0.003 g at 0 % RH. The effect of mass was not

significant ($t_{(30)} = 1.798$, $p = 0.085$). The temperature during the experiment was found to influence the CV value ($t_{(30)} = 3.417$, $p = 0.002$). As temperatures increased, CV values increased as well (Fig. 4.5). A correlation analysis showed that CV and temperature are correlated ($S = 3064.427$, $p = 0.034$). The temperatures at which the groups were tested were found to differ significantly ($F_{(3, 27)} = 25.07$, $p < 0.0001$). A post-hoc test was run and the temperature at which workers at 0 % RH were tested differed significantly from all groups tested at 100 % RH (Table 4.1). This confirms the observations of Figure 4.5, where workers at 0 % RH were tested at temperatures 2-4 °C higher than the other groups. Days since collection, i.e. the number of days between collection in the field and the running of the experiment, was found to have no influence on CV ($t_{(30)} = -1.073$, $p = 0.294$).

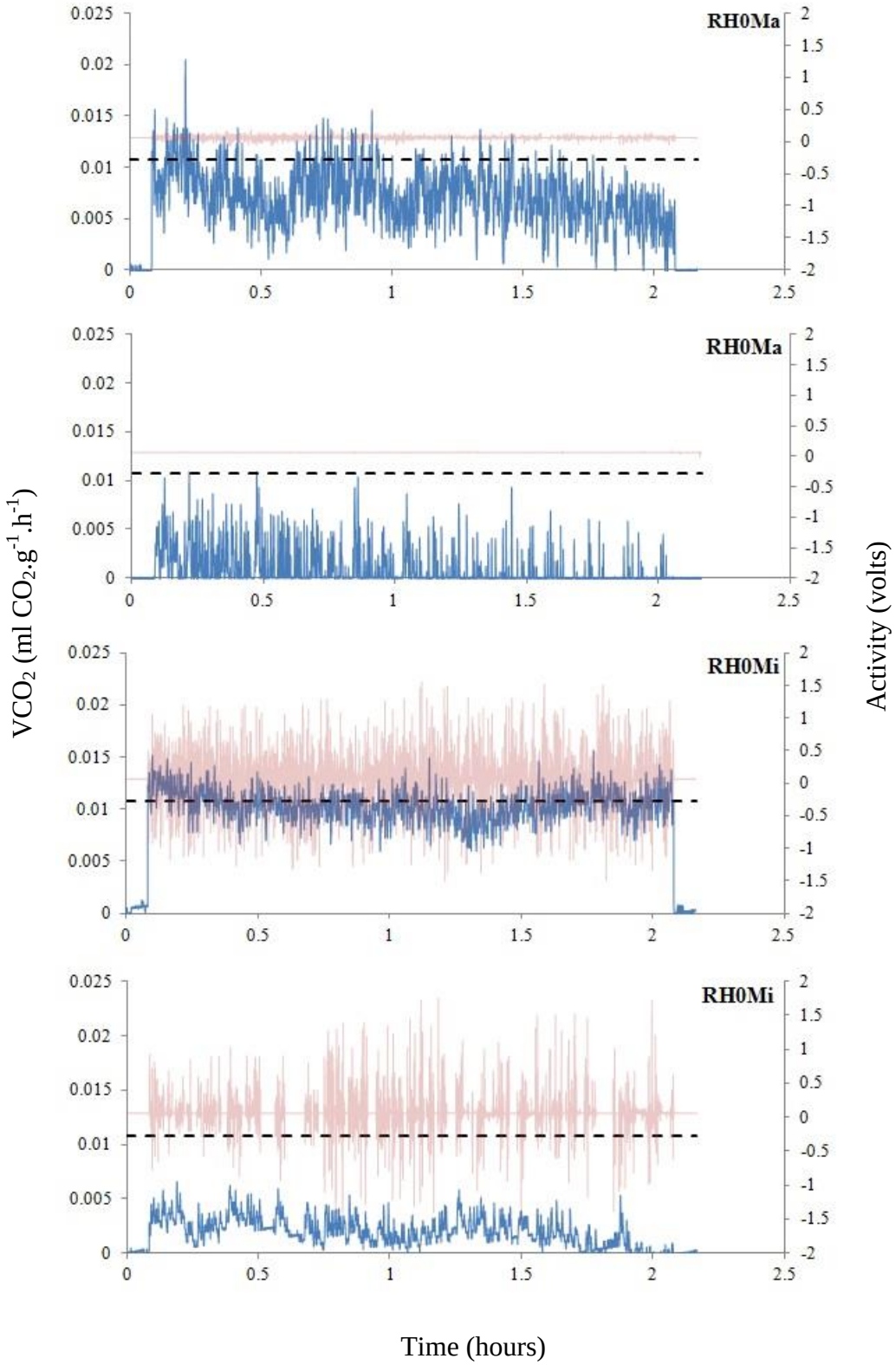


Figure 4.3: Typical CO_2 traces of major and minor workers at 0 % RH. The blue series shows VCO_2, the red series shows activity and the black dotted line represents the cut-off threshold for spiracular closure.

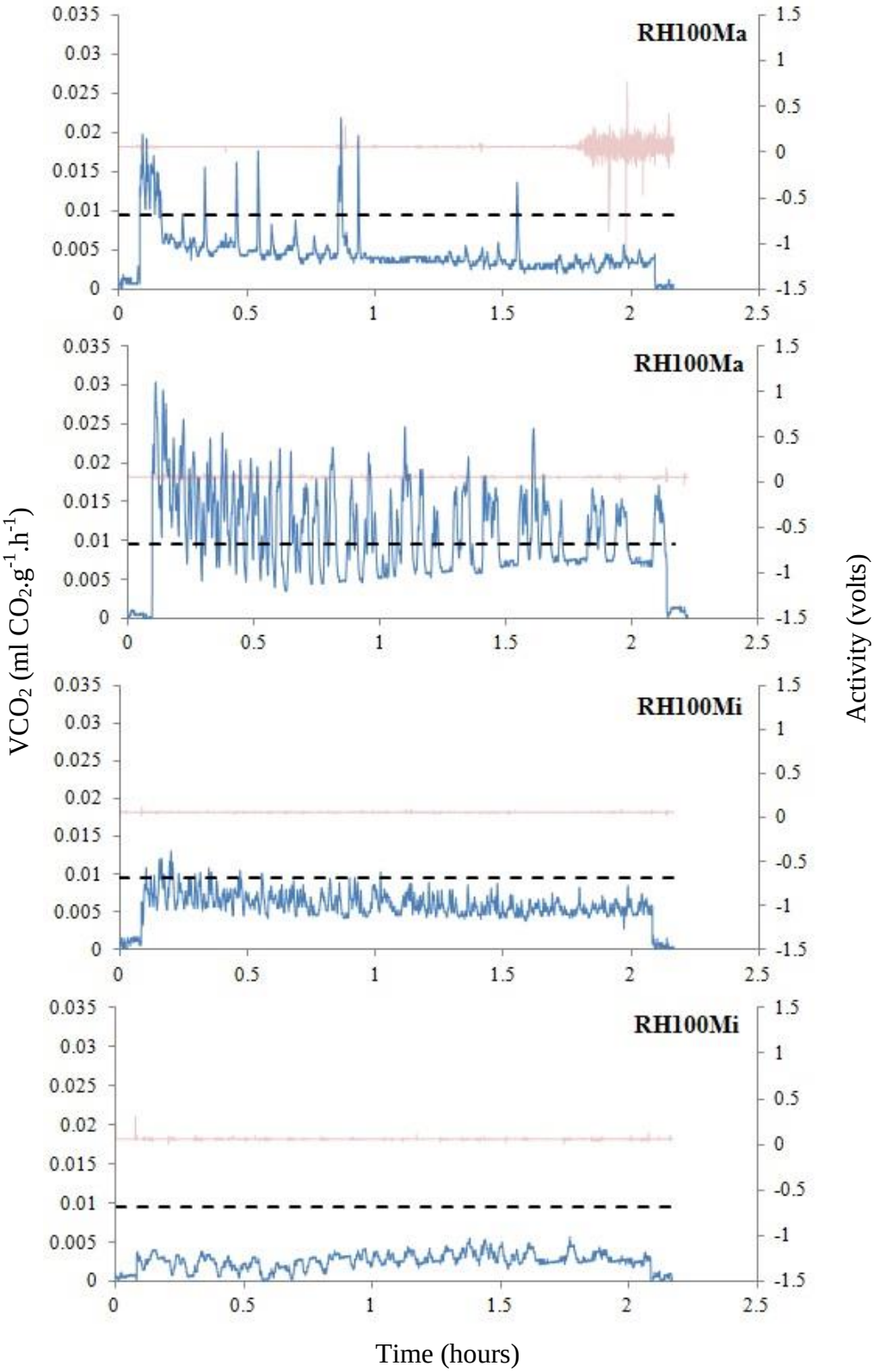


Figure 4.4: Typical CO_2 traces of major and minor workers at 100 % RH. The blue series shows VCO_2, the red series shows activity and the black dotted line represents the cut-off threshold for spiracular closure.

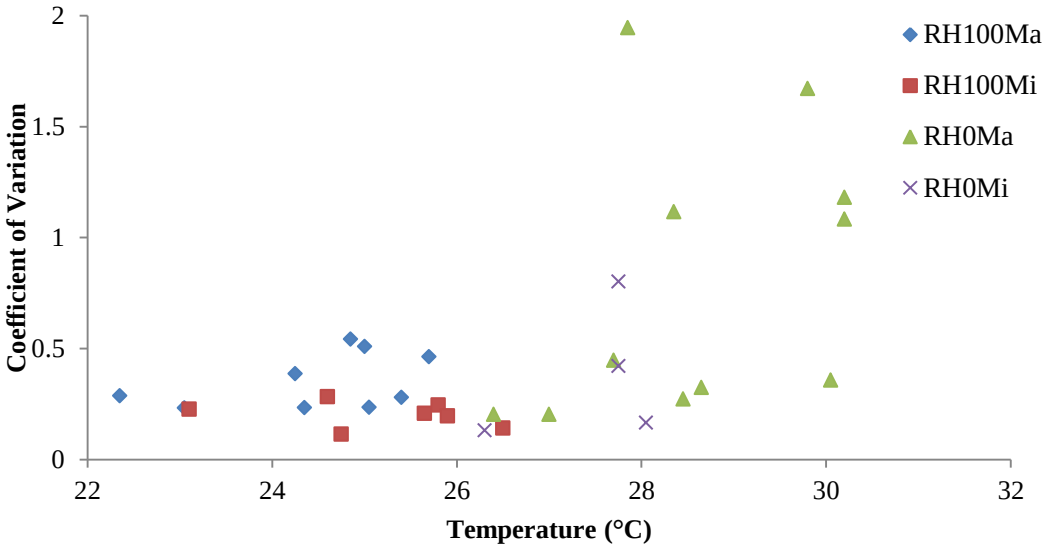


Figure 4.5: Graph of the influence of temperature on coefficient of variation (CV) across the different experiment groups. See text for details.

Table 4.1: The p-values of temperature as calculated from the Tukey post-hoc test. Significant values are indicated in bold.

	RH0Ma	RH0Mi	RH100Ma	RH100Mi
RH0Ma		0.356	**$1.0e^{-7}$**	**$1.04e^{-5}$**
RH0Mi			**0.001**	**0.021**
RH100Ma				0.595
RH100Mi				

4.3.3 Mass Loss

The mean mass loss experienced, after two hours, for major workers at 100 % and 0 % RH are $-1.122e^{-4} \pm 0.001$ g and $2.345e^{-4} \pm 0.001$ g respectively. For minor workers at 100 % and 0 % RH, the mean loss, after 2 hours, is 0.005 ± 0.001 g and 0.002 ± 0.000 g respectively. Major workers measured at 100 % RH experiment seem to experience, on average, a weight gain. The box plot (Fig.4.6) shows that termites in the 0 % RH experiment lose more mass over the same time period than termites in the 100 % RH experiment. However, termites at 0 % RH lose less mass over the same time period than termites in the water loss

experiment, 0.008 ± 0.002 g for major workers and 0.007 ± 0.003 g for minor workers over an equivalent 2 hour period (Chapter 3, Fig. 3.3). An ANOVA indicated that the four groups were significantly different ($F_{(3, 27)}$=65.855, p=6.161e^{-12}). Almost all groups were found to be significantly different from each other except for those shown in Table 4.2, as indicated by a post hoc Tukey test. ANCOVAs were run to test the influence of mass, temperature and days since collection on mass loss. None of these factors were found to influence mass loss.

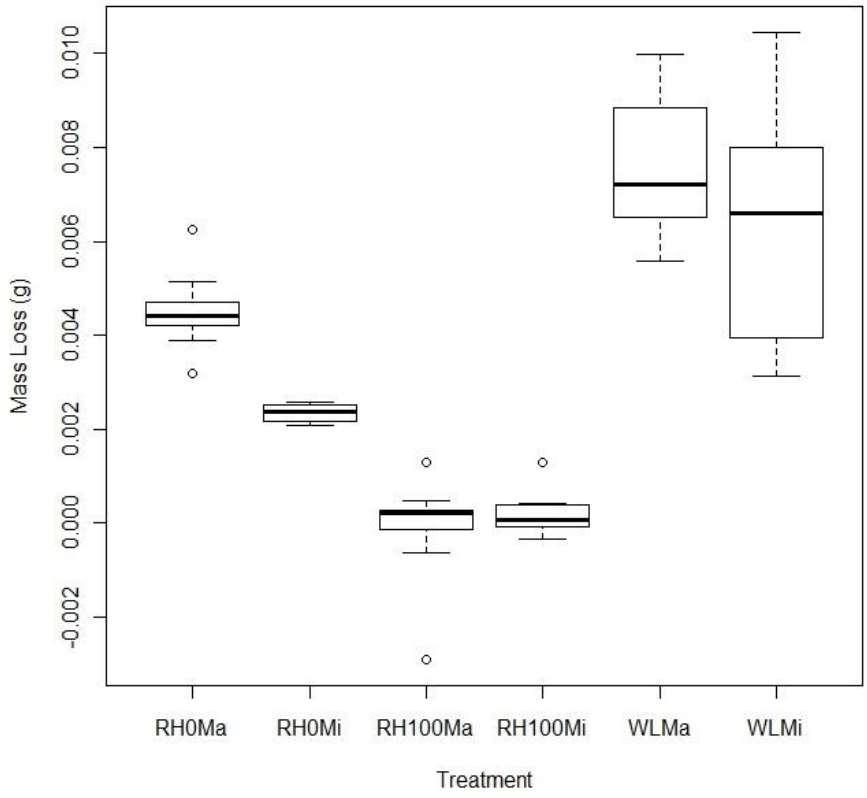


Figure 4.6: Box plot comparing the medians and variance of mass loss experienced over a period of two hours by termites at 0 % and 100 % relative humidity, where flow rate was 50 ml.h^{-1}. RH0Ma is major workers at 0 % RH (n=11), RH0Mi is minor workers at 0 % RH (n=4), RH100Ma is major workers at 100 % RH (n=9), RH100Mi is minor workers at 100 % RH (n=7). For comparison the water loss of termites under still air, for an equivalent two-hour period, are included (Chapter 3). WLMa is major workers and WLMi is minor workers (N=10). The open circles represent outliers in the data.

Table 4.2: Table of p-values of mass loss rates as calculated from the Tukey post-hoc test. Significant values are indicated in bold.

	RH0Ma	RH0Mi	RH100Ma	RH100Mi	WLMa	WLMi
RH0Ma		0.15	**2.00E-07**	**5.70E-06**	**3.68E-04**	**3.80E-02**
RH0Mi			0.086	0.231	**6.20E-06**	**3.08E-04**
RH100Ma				0.997	**<<0.05**	**<<0.05**
RH100Mi					**<<0.05**	**<<0.05**
WLMa						0.632
WLMi						

4.4 Discussion

The mass specific mean rate of CO_2 emission was found to not differ between termite workers under the influence of differing environmental humidities. This result is similar to the findings of Contreras and Bradley, (2011), who found that the semi-aquatic insect, *Aquarius remigis*, when placed in humid and dry air showed no statistically significant differences in metabolic rate. However, they also found that respiratory pattern did not change significantly between humidity treatments. This was not the case in workers of *H. mossambicus*.

The coefficient of variance, which is a numerical evaluation used to estimate respiratory pattern (Shelton & Appel, 2000; Lighton & Ottesen, 2005), changed with ambient relative humidity, although this change was not significant for most of the groups tested. The significant difference between major workers at 0 % RH and minor workers at 100 % RH is likely a consequence of the tendency of minor workers to display continuous respiration regardless of environmental humidity. CV values can vary from near 0 for continuous gas exchange patterns to >2 in insects displaying DGC (Lighton, 1990). A typical termite CV ranges from 0.25 to 0.5 (Shelton & Appel, 2001). Termites at 0 % RH displayed a large range of CV values (Fig. 4.2) and were found to display respiratory patterns ranging from continuous to continuous with periods of spiracular closure. This variation in CV and respiratory pattern could be a consequence of the variation in temperature

(Fig. 4.5). Studies have shown that changes in temperature influence the pattern of gas exchange displayed (Bradley, 2007; Contreras & Bradley, 2010; Contreras & Bradley, 2011; Basson & Terblanche, 2011). Although usually an increase in temperature results in an increase in VCO_2, which was not the case in this study.

The increase of cycling in termites at 0 % RH and the subsequent lack of DGC could be explained by the increase in activity level. Insects are highly aware of the abiotic conditions they experience at any given time. When these conditions are altered, insects can respond through swift changes in position, orientation or activity in order to return to some environmental optimum (Chown *et al*., 2011). Termites of *H. mossambicus* are exceptionally prone to water loss, especially workers that are exposed to the ambient temperature and humidity of the external environment. Workers are no more efficient at conserving water than the larvae living in the ideal conditions of the underground hive and thus they have evolved behaviour that reduces their exposure (Nel & Hewitt, 1969; Duncan & Lighton, 1994). Workers forage for approximately five minutes before returning below ground, the longest foraging trip observed for major and minor workers was 19 min 18 s and 4 min 20 s respectively. Foragers seek refuge from desiccating conditions underground (Nel & Hewitt, 1969: Duncan & Hewitt, 1989). Thus, in this study termites might be attempting to escape the severely desiccating conditions, resulting in increased activity. This also explains why minor workers experience much higher levels of activity than major workers at 0 % RH. They are smaller in mass and therefore have a higher surface area to volume ratio and are more prone to desiccation and water stress.

Termites at 0 % RH displayed traces with a great deal of pumping (Fig. 4.3). These results agree with those of Fielden and Lighton, (1996). The tick, *Dermacentor andersoni*, when exposed to dry air, exhibited patterns with more frequent but smaller CO_2 bursts. Cockroaches exposed to low humidities were also found to exchange respiratory gases for shorter durations (Schimpf *et al*., 2009). However, DGC was observed under both dry (0 % RH) and wet conditions (100 % RH). Termites at 100 % RH displayed very small ranges of CV values and

consistently exhibited a cyclic pattern of gas exchange. Minor workers had a tendency towards a more continuous pattern. Activity levels were lower at 100 % RH than 0 % RH. This is likely as a result of the more favourable environmental conditions. Termites were no longer experiencing water stress and therefore had no need to display a physiological or behavioural response to desiccation (Chown *et al*., 2011).

Termite workers in dry air were found to lose more mass than termites in humid air. Even when termites were exhibiting brief periods of spiracular closure (defined by VCO_2 and not the cut-off threshold), spiracular opening was still more frequent in dry air than in humid air, thus continually exposing the respiratory passages to the external atmosphere and desiccation. However, the mass loss experienced in these experimental conditions, with flowing air, did not exceed those of the water loss studies in still air (Chapter 3) where mass loss after two hours for major and minor workers was 0.008 g and 0.007 g, respectively. This indicates that termites at 0 % RH are doing something to curtail water loss. The closing of spiracles does not appear to be responsible for reduced water loss. Termites exhibiting a closed phase at 0 % RH shared similar water loss values with those exhibiting continuous respiration at 0 % RH. The increased pumping, that is discrete cyclic events involving rapid spiracular movements, could be responsible for reduced water loss. The pumping of spiracles results in minimized spiracular area and low partial pressure differences and thus diffusive loss of CO_2 and water is reduced (Duncan *et al*., 2002). The minimized water loss could also just be a consequence of any kind of spiracular control. Lighton, (2007), argued that any spiracular control regimen would be selected to conserve water, which makes sense when you consider that respiratory water loss is the fundamental cost of gas exchange and therefore all insects are under selective pressure to reduce this cost.

Major and minor workers were seen to lose similar amounts of water in humid air but not in dry air. This is possibly as a result of the small sample size of RH0Mi, a result of the removal of outliers, which displayed strange patterns, and the

difficulty of finding minor workers as opposed to major workers foraging at the field site. Nel, (1968) found no evidence of active control over water loss in *H. mossambicus*. In his study, water loss was found to be greatly dependent upon temperature and relative humidity. Termites lost an average of 6.43 mg at 0 % RH compared to 1.45 mg at 95 % RH, over a period of 6 hours at 25 °C. Several other studies have showed similar results: an increase in water loss with a decrease in ambient relative humidity (Fielden & Lighton, 1996; Zachariassen, 1991). However, Schimpf *et al*., (2009) found lower rates of mass loss in cockroaches in dry air, while Roberts *et al*. (1994) found that water loss rates increased at high humidities in the lubber grasshopper, *Romalea guttata*. This increase was thought to be a consequence of an increase in cuticular permeability but it did not preclude the elevation of water loss through other channels.

Humidity has a strong effect on respiratory pattern and water loss. The results of this study indicate that workers of *H. mossambicus* do not actively control or regulate water loss through DGC but they might do so through the use of other respiratory patterns. Using this evidence, it seems unlikely that the reduction of respiratory water loss is the original mechanism underlying the existence of DGCs. However, this is not to say that DGCs serve no purpose in reducing respiratory water loss.

CHAPTER 5: The Emergent-Property Hypothesis

5.1 Introduction

Insect tracheal respiration is renowned for its remarkable efficiency. Insects are capable of extremely high rates of oxidative catabolism and maintain the highest mass specific rates of O_2 consumption in the animal kingdom. The tracheae are capable of supplying 10 times more oxygen per gram of tissue than blood capillaries (Maina, 2002; Lighton & Ottesen, 2005; Matthews & White, 2011). The oxygen needs of an animal are reflected by the respiratory efficiency of their gas exchange system; whereby a highly efficient system would suggest the capacity for a highly energetic lifestyle in which maximum O_2 delivery can occur at high metabolic demands (Maina, 2002; Moerbitz & Hetz, 2010). Physiological systems are selected to maximize performance. Bradley (2006) stated that it would therefore seem counter-intuitive to possess a physiological mechanism, i.e. the ability to restrict spiracular conductance and induce periodic gas exchange, which impedes the rate of oxygen delivery to the tissues thus reducing the metabolic capabilities of the insect.

Aerobic metabolism confers great advancements in the production of metabolic energy; however, this increased metabolic capacity comes at a cost. The utilization of oxygen results in the production of reactive oxygen species that cause deleterious effects within the tissues and are highly toxic (Maina, 2002; Bradley, 2006; Moerbitz & Hetz, 2010). This oxidative damage is reduced when aerobic supply and aerobic demand is matched and O_2 is being consumed at the same rate at which it is being supplied to the tissues. A problem occurs when O_2 demand is low, such as, when the insect is at rest, but the respiratory system is still supplying O_2 at maximum capacity (Chown, 2011). Thus necessitating the existence of a physiological mechanism that reduces O_2 supply.

This would suggest that the type of gas exchange employed, be it discontinuous, cyclic or continuous, and is dictated by the interaction between an insect's metabolic rate and the capacity of the respiratory system to deliver oxygen to this metabolically active tissue (Contreras & Bradley, 2011). If O_2 entry exceeds the rate of O_2 being consumed by aerobic metabolism, the spiracles will close and the closed phase of discontinuous gas exchange will be observed (Section 1.1). This closed phase is required to lower the internal partial pressure of O_2 around the tissues to a safe level. This low level of O_2 is maintained during the flutter phase of DGC, where the spiracles open and close rapidly. The length of this F phase is determined by the rate at which CO_2 accumulates as a result of metabolism within the tissues. When CO_2 reaches a critical level the spiracles open (Forster & Hetz, 2010). As metabolic rate increases this critical CO_2 level is reached more rapidly until the closed phase becomes so short that it is no longer experimentally demonstrable and a continuous gas exchange pattern is displayed (Bradley, 2007). By decreasing the time over which the spiracles are closed, the volume of O_2 entry and CO_2 release are adjusted to meet metabolic demands (Contreras & Bradley, 2010; Contreras & Bradley, 2011).

Under conditions of low metabolic demand, DGC arises as a consequence of interactions between the O_2 and CO_2 set points that govern spiracular opening (Marais *et al*., 2005; Chown, 2011). This concept forms the basis of the emergent-property hypothesis. An implication of this hypothesis is that cycle frequency increases with metabolic rate such that the gas exchange pattern displayed by an insect transitions from discontinuous to cyclic to continuous as metabolic rates increase (Bradley, 2006; Bradley, 2007; White *et al*., 2007; Moerbitz & Hetz, 2010). Conditions that affect metabolic rate, such as temperature and activity, will therefore influence respiratory patterns through their effects on the rate of O_2 consumption and carbon dioxide production.

5.2 Aims and Research Objectives

This experiment was designed to manipulate metabolic rate, using temperature, to test whether the gas exchange pattern transitions with increasing metabolic rate as

stated by the emergent-property hypothesis. This experiment investigated the effect of temperature on metabolic rate, gas exchange pattern and water loss.

5.3 Results

5.3.1 Rate of Carbon Dioxide Emission

The mean rate of CO_2 emission or metabolic rate for major workers at different temperatures is as follows: 0.075 ± 0.001 ml $CO_2g^{-1}h^{-1}$ for 5 °C, 0.088 ± 0.001 ml $CO_2g^{-1}h^{-1}$ for 10 °C, 0.1 ± 0.002 ml $CO_2g^{-1}h^{-1}$ for 15 °C, 0.385±0.004ml $CO_2g^{-1}h^{-1}$ for 25 °C and 0.336 ± 0.002 ml $CO_2g^{-1}h^{-1}$ for 35 °C (Fig. 5.1). On inspection of the box plot (Fig. 5.1) it is discernable that two distinct groups exist within which the different temperature treatments share similar VCO_2 distributions. The first of these groups is made up of 5 °C, 10 °C and 15 °C, termite workers exposed to these temperatures share a lower VCO_2 than termites exposed to temperatures in the second group, that is, 25 °C and 35 °C. Using a Kruskal Wallis test, a non-parametric equivalent of an ANOVA, the means of the different temperature treatments were found to be significantly different (chi-squared = 18.503, p=0.001). A post-hoc test was run and the results confirmed the groupings seen in the box plot (Fig. 5.1); 5 °C, 10 °C and 15 °C were not significantly different from each other but all were found to be significantly different from 25 °C and 35 °C, while 25 °C and 35 °C were not significantly different from each other (Table 5.1).

ANCOVA was performed to test the influence of various factors on VCO_2. The average mass for workers at each temperature was: 0.035 ± 0.004 g for 5 °C, 0.037 ± 0.006 g for 10 °C, 0.044 ± 0.006 g for 15 °C, 0.047 ± 0.01 g for 25 °C and 0.038 ± 0.006 g for 35 °C. Mass was found to have no significant effect on the VCO_2 expressed ($t_{(28)}$=0.622, p=0.542). Days since collection was also found to have no influence on VCO_2 ($t_{(28)}$=-0.332, p=0.744). This result was a consequence of the higher VCO_2 experienced at those temperatures. The effect of mass loss on VCO_2 was also tested and found to be non-significant ($t_{(28)}$=0.597, p=0.559).

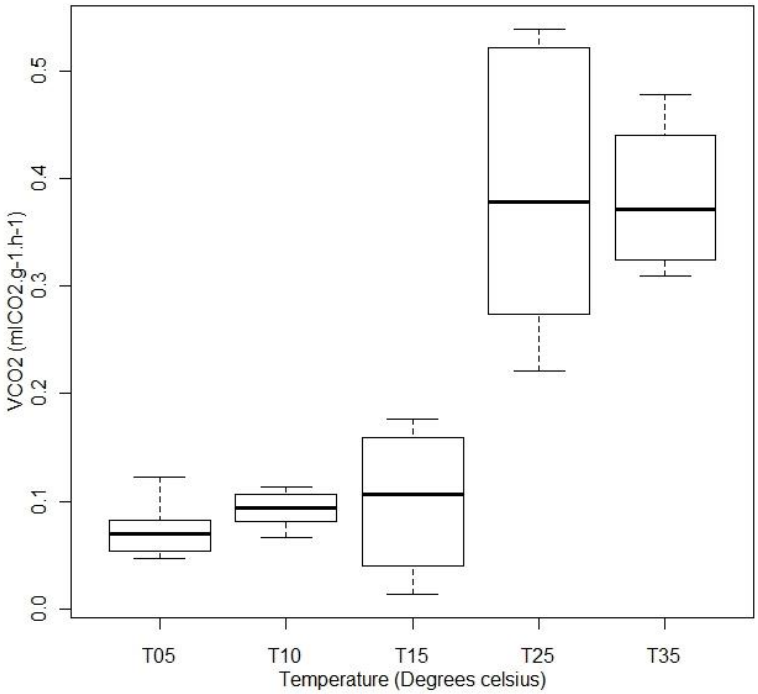


Figure 5.1: The medians and variance of metabolic rate as a function of temperature (T05 n=5, T10 n=6, T15 n=7, T25 n=6 and T35 n=5) in major workers using a box plot.

Table 5.1: Table showing the p-values calculated from the Tukey post-hoc test on major worker VCO_2 at different temperatures. Significant values are indicated in bold.

	5 °C	10 °C	15 °C	25 °C	35 °C
5 °C		0.997	0.984	**$1.35e^{-5}$**	**$6.49e^{-5}$**
10 °C			0.1	**$3.09e^{-5}$**	**$1.4e^{-4}$**
15 °C				**$1.27e^{-5}$**	**$7.79e^{-5}$**
25 °C					0.1
35 °C					

5.3.2 Coefficient of Variation

The means of the CV for the different temperature groups are as follows; 0.637 for 5 °C, 0.427 for 10 °C, 0.581 for 15 °C, 0.236 for 25 °C and 0.137 for 35 °C. Figure 5.2 shows that CV has a tendency to decrease with increasing temperature,

that is, that the level of cycling increases or becomes more continuous with increasing temperature. This trend is also illustrated in the CO_2 emission traces (Fig. 5.3). Termites at 5 °C and 10 °C had discontinuous patterns of gas exchange (as the CO_2 emissions fall well below the cut-off threshold and have periods were VCO_2 goes to zero). However, all three periods, which define a DGC, were not present. Termites at 15 °C shows the greatest range of CV values and the pattern of gas exchange expressed at this temperature were highly variable, going from discontinuous to continuous (Fig. 5.2). Termites at 25 °C and 35 °C displayed continuous gas exchange only. There was also a distinct difference in activity level between the five temperature groups. The activity level was similar for 5 °C and 10 °C and increased slightly at 15 °C. Activity level increased greatly at 25 °C and 35 °C (Fig. 5.3). A Kruskal Wallis test was performed to determine whether the CV means for the different groups were equivalent. The groups were found to differ significantly (chi-squared=17.628, p=0.001). After a Tukey post-hoc test was performed, it was found that the CV values for termites at 35 °C differed significantly from those at 5 °C (p=0.014) and 15 °C (p=0.03).

ANCOVA was performed to test the influence of various factors on CV. Mass and mass loss were found to have no significant effect on CV ($t_{(28)}$=-1.562, p=0.1356 and $t_{(28)}$=-0.455, p=0.654 respectively). Days since collection was also found to have no significant effect on CV ($t_{(28)}$=0.360, p=0.723), however 15 °C was significant. This result was a consequence of the large difference between number of days since collection for 15 °C and the other temperatures. The termites tested under 15 °C were tested up to 10-12 days after collection compared to the highest of 6 days for the other groups.

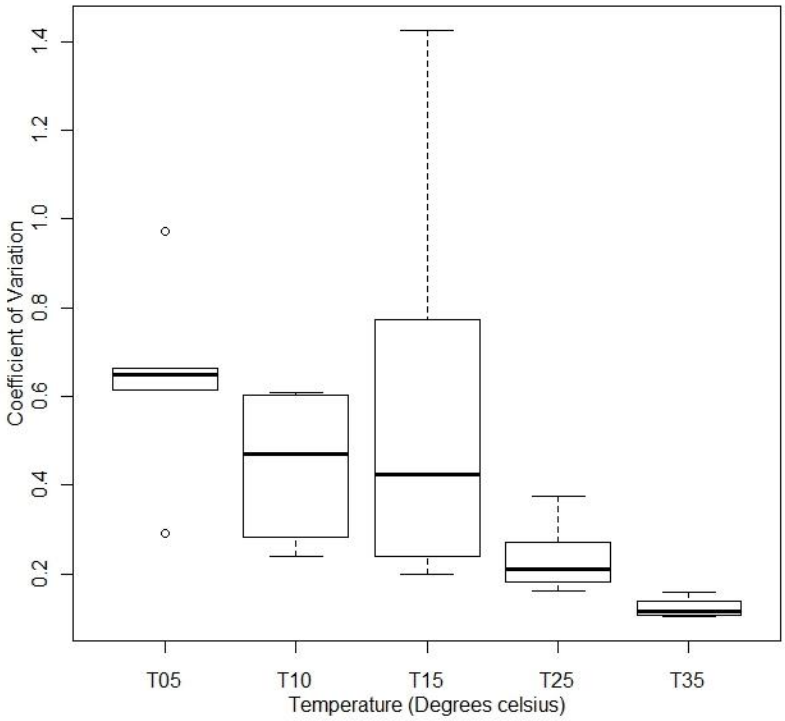


Figure 5.2: The medians and variance of coefficient of variation as a function of temperature for major workers using a box plot. The open circles represent outliers in the data.

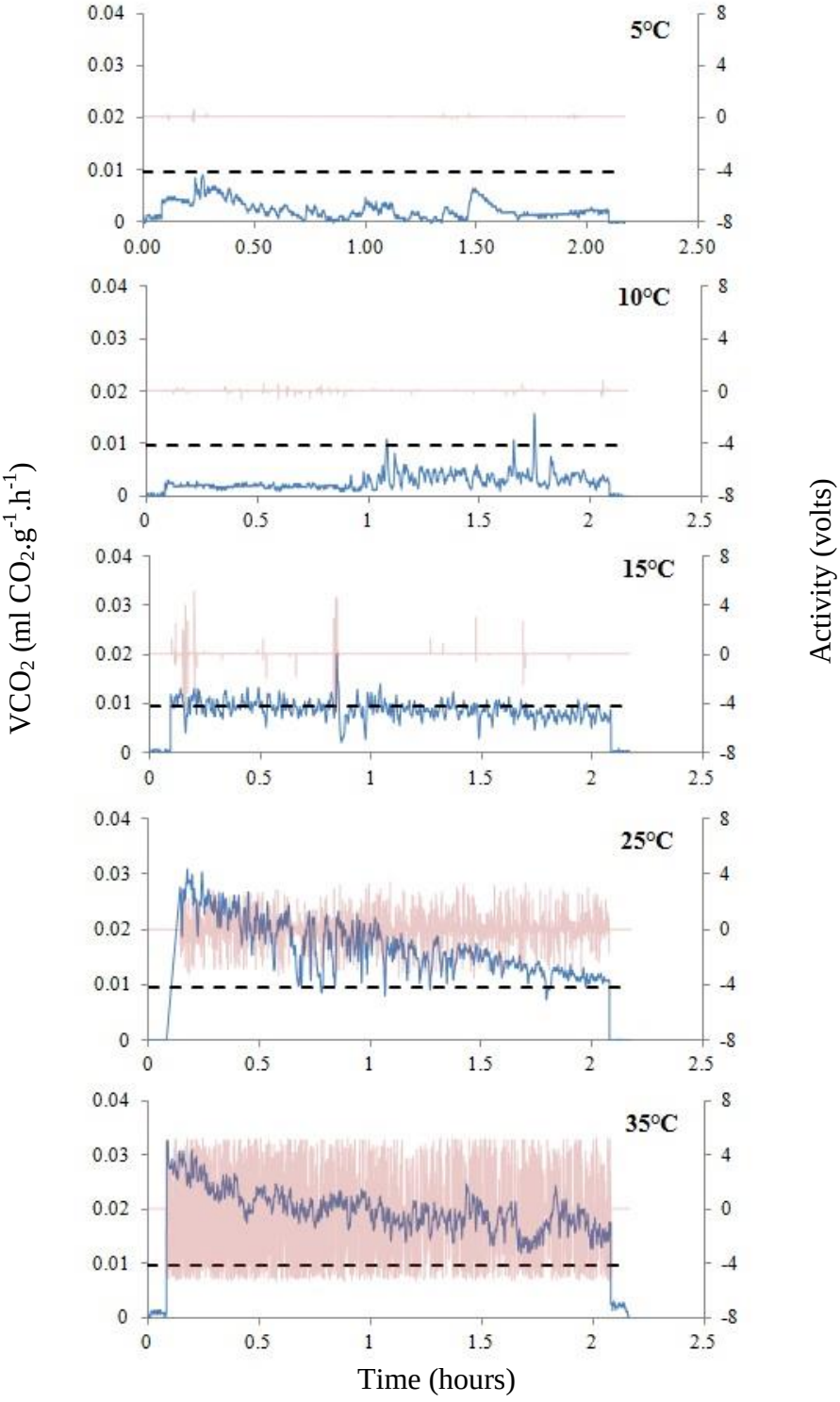


Figure 5.3: A comparison of CO_2 traces recorded under different temperatures. The blue series shows VCO_2, the red series shows activity and the black dotted line represents the cut-off threshold for spiracular closure.

5.3.3 Mass Loss

The mean mass loss experienced over a period of two hours by major workers at 5 °C, 10 °C, 15 °C, 25 °C and 35 °C are $2.98e^{-4}$ g, $3.33e^{-6}$ g, $4.89e^{-4}$ g, 0.003 g and 0.007 g respectively. As expected, termites at higher temperatures experience greater mass loss over the same period of time (Fig. 5.4). Termites at 5 °C, 10 °C, 15 °C appear to lose an equivalent amount of mass. After 15 °C mass loss appears to increase exponentially (Fig. 5.4). A Kruskal Wallis test was performed and the groups were found to differ significantly (chi-squared=20.391, p=$4.18e^{-4}$). A post-hoc test was run which confirmed the observations of the box plot, i.e. 5 °C, 10 °C, 15 °C shared similar means, while 25 °C and 35 °C differed significantly from all other groups (Table 5.2).

ANCOVA was performed to test the influence of various factors on mass loss. Both mass and days since collection were found to have no effect on mass loss ($t_{(28)}$=0.765, p=0.4549 and $t_{(28)}$=-1.370, p=0.189 respectively). Termites exposed to 35 °C were found to be significant in both ANCOVAs. This was thought to be a consequence of the vast difference in mass loss compared with the other temperatures.

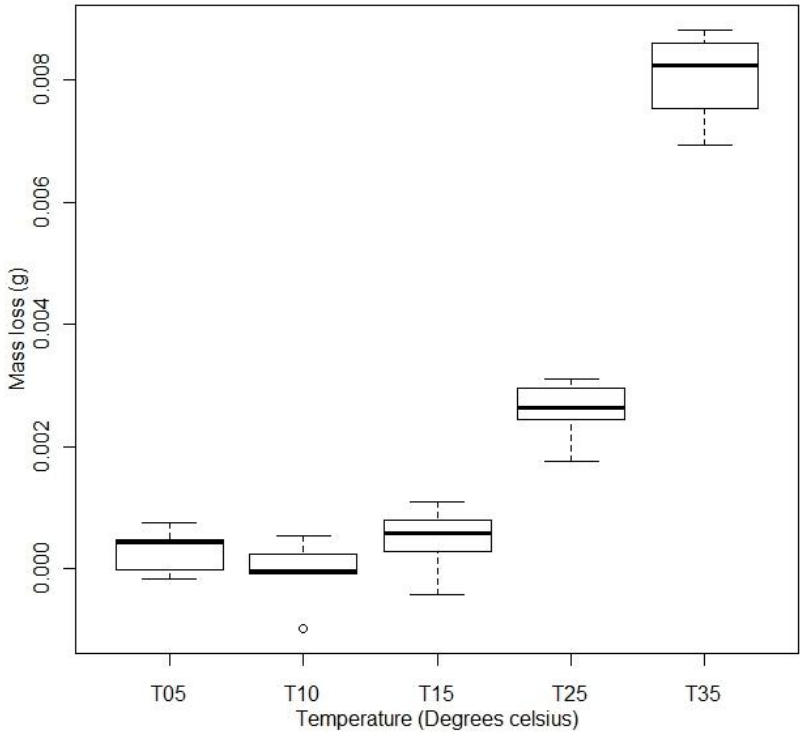


Figure 5.4: The medians and variance of mass loss as a function of temperature, experienced over a period of two hours, by major workers using a box plot. The open circle represents an outlier in the data.

Table 5.2: Table showing the p-values of coefficient of variation, as influenced by temperature, calculated from the Tukey post-hoc test. Significant values are indicated in bold.

	5 °C	10 °C	15 °C	25 °C	35 °C
5 °C		0.834	0.974	**$4.9e^{-6}$**	**<<0.05**
10 °C			0.441	**$0.5e^{-6}$**	**<<0.05**
15 °C				**$0.51e^{-5}$**	**<<0.05**
25 °C					**<<0.05**
35 °C					

5.4 Discussion

As expected, elevated temperature resulted in an elevated rate of CO_2 emission. As temperature increases, the rate of chemical and biological processes within the insect increase resulting in a concomitant increase in O_2 consumption in order to

meet metabolic demands and consequently greater CO_2 emission (Neven, 2000; Gillooly *et al*., 2001; Basson & Terblanche, 2011). VCO_2 or metabolic rate increased significantly after 15 °C. This increase in MR is likely a result of workers preparing for foraging. The lower foraging limit of *H. mossambicus* ranges between 9.5 °C and 12 °C. As ambient environmental temperature increases, termite MR increases to a level where they are capable of foraging, which is a highly energetically expensive endeavor (Mitchell *et al*., 1993). This confirms the observations of this experiment where termites were seen foraging and collected between the temperatures of 21.2 °C and 37.8 °C. This also explains the increased range of VCO_2 experienced at 15 °C, this temperature appears to be a set point for increasing metabolic rate.

Major workers were seen to transition from DGC to continuous gas exchange as a response to increasing temperature. It is worth noting here that the DGC displayed here is not a true DGC as observed in ants. Not all three phases are present and the CV values they produce are not high enough to qualify as true DGC (Lighton, 1998). At 5 °C and 10 °C, workers employed DGC (Fig. 5.3). As the temperature increased to 15 °C, the pattern of gas exchange was highly variable, going between DGC and continuous gas exchange. As temperature increased to 25 °C and 35 °C, continuous gas exchange was observed and the burst or open phase increased in frequency and large amounts of pumping was observed (Fig. 5.3). This shortening of respiratory cycles with increased metabolic rate has been observed in moth pupae (Schneiderman & Williams, 1955). The increased volume of CO_2 emitted is indicative of higher ventilatory efficiency. As the demand for O_2 increases as a result of increased temperature or activity, active ventilation replaces diffusion through rhythmic pumping movements of the body (Basson & Terblanche, 2011). Contreras and Bradley (2010) observed the same transitions in individuals of *Rhodnius prolixus* and *Gromphadorhina portentosa*. At low temperatures both species displayed long periods of spiracular closure indicative of DGC. As MR increased, periods of spiracular closure decreased producing cyclic gas exchange and eventually, as MR increased further, continuous gas exchange was observed. Other studies have also described similar observations

(Chappell & Rogowitz, 2000; Gibbs & Johnson, 2004; Bradley, 2007; Basson & Terblanche, 2011; Contreras & Bradley, 2011). All these studies agree that there exists a strong correlation between temperature and the proportion of time an insect closes its spiracles, and that changes in MR influence the type of gas exchange pattern an insect displays at any given time.

Increased temperature also resulted in increased termite activity, with the greatest level of activity seen at 35 °C. This pattern was observed in bumblebees, *B. terrestris* (Karise *et al*., 2009). When exposed to low temperatures their body movements become less pronounced and the transition from one respiratory pattern to another was associated with the change from an active to a resting state. The respiratory pattern changed from cyclic gas exchange to DGC as the bumblebees became less active (Karise *et al*., 2009). Major workers of *H. mossambicus* have an upper critical thermal limit (CTMax) of 47.27 ± 0.799 °C and a lower limit (CTMin) of 7.13 ± 0.352 °C (Mitchell *et al*., 1993). This CTMin is higher than the lowest temperature we tested, thus minimal termite activity at this temperature is to be expected. Activity levels are still low at 10 °C but as discussed earlier this temperature falls within the lower foraging limit (Mitchell *et al*., 1993), so potentially workers have no need to be active at this temperature.

There are two main factors influencing gas exchange patterns in insects, that is metabolic rate and the capacity of the respiratory system to deliver O_2 to metabolically active tissue (Bradley, 2007; Contreras & Bradley, 2010). Essentially this means that respiratory pattern is determined by the relationship between O_2 supply and O_2 demand. When workers are at rest, the demand for O_2 is low and consequently O_2 supply is decreased by closing the spiracles (Contreras & Bradley, 2011). The activity level begins to increase at 15 °C and this is a result of the concomitant changes in MR as discussed earlier. At 25 °C activity increases substantially and reaches its highest observed level at 35 °C. This increase in activity can be explained by the increase in MR but also by the increase in temperature. Studies have shown that insects are most active at temperatures marginally lower than peak fitness. Activity levels should taper off before

reaching maximum MR in order to avoid the detrimental side effects associated with high temperatures (Martin & Huey, 2008). As workers begin to reach their critical thermal limit, they might be attempting to escape from the respiratory chamber especially considering the length of time that they are exposed to such high temperatures. In field conditions, major workers rarely forage for more than 20 minutes at a time before returning to their underground nests (Duncan & Hewitt, 1989). Their subterranean nests are located on average at a depth of 1-1.5 m, at those depths soil temperatures are no longer subject to diurnal variations in temperature and therefore the termites are buffered from the extremes of ambient temperature (Mitchell *et al*., 1993; Chappell & Rogowitz, 2000). Thus it appears likely that the high levels of activity could partly be a result of workers seeking the thermal refuge of their nests. The increased activity could also be a response to water stress experienced at high temperatures (Tomlinson & Phillips, 2012).

Water loss in insects tends to follow an exponential pattern of increase with increasing temperature (Hadley, 1994). Which was also observed in this experiment (Fig. 5.4), mass loss was similar for termites at 5 °C, 10 °C and 15 °C but it increased significantly and exponentially at 25 °C and 35 °C. Net water loss at any given temperature is a function of both metabolism and how well water is being conserved (Hadley, 1994). The increase in water loss after 15 °C is likely a result of the increased respiration following increased MR and activity, the products of increasing temperature (Tomlinson & Phillips, 2012). Workers with high MR do not appear to be doing anything to conserve water. If the DGC evolved to reduce respiratory water loss as the hygric hypothesis states, one would expect to see DGC at high temperatures where increased respiration is resulting in high respiratory water loss rates. Yet, this is not the case. DGC is only observed at low temperatures where both MR and respiration is low. Therefore, it is unlikely that DGC will be observed in foragers; a consequence of having to increase MR for the activity of foraging is that the spiracles cannot remain closed as a result of the high oxygen demand.

The results of this study conform to the expectations of the emergent-property hypothesis. As metabolic rate increases the gas exchange patterns of workers of *H. mossambicus* transition from discontinuous to cyclic to continuous.

CHAPTER 6: The Oxidative-Damage Hypothesis

6.1 Introduction

Oxygen is a source of reactive oxygen species that can cause oxidative damage even at relatively low concentrations, yet it is also essential for oxidative metabolism. Thus, it is necessary for all insects to regulate the internal partial pressure of O_2 within a fairly narrow range to maintain aerobic metabolism whilst avoiding oxygen toxicity (Hetz & Bradley, 2005; Bradley, 2006; Harrison *et al*., 2006).

In an ideal respiratory system O_2 would enter at the same rate as CO_2 exits, however, this is not the case. O_2 and CO_2 move in opposite directions along an identical pathway. As a consequence of their differing gradients and very slight differences in their rate of diffusion (due to differences in molecular weight), O_2 will always enter the insect more rapidly than CO_2 leaves (Hetz & Bradley, 2005). The direct outcome of this is that when the spiracles open to release CO_2, O_2 rapidly enters the tracheae and quickly reaches an unsafe level (Lighton & Ottesen, 2005). Some insects respond to this short-term exposure to near-atmospheric levels of O_2 with compensatory changes in spiracular opening (Harrison *et al*., 2006).

The spiracles control the rate of oxygen delivery and act as the main barrier to oxygen. When PO_2 is low, the spiracles open longer and more frequently, while the opposite is true when PO_2 is high (Chown & Holter, 2000; Harrison *et al*., 2006). This spiracular restriction serves to regulate PO_2 around the tissues. A consequence of spiracular restriction is the build-up of CO_2 to a critical level, which eventually requires release in the form of an open phase. When CO_2 reaches a safe level the spiracles close to reduce O_2 levels. PO_2 is regulated through spiracular fluttering (Hetz & Bradley, 2005; Lighton & Ottesen, 2005; Forster & Hetz, 2010). This cycling of spiracular opening and closing comes as a

consequence of the O_2 and CO_2 set points. The gas exchange pattern observed is thus a result of the interaction between the amount of oxygen present at the beginning of the closed phase and the insect's metabolic rate (Contreras & Bradley, 2011). Insects with a low metabolic rate, i.e. insects at rest, will display long periods of spiracular closure, as the rate of removal of O_2 is slow. As metabolic rate is ramped up, O_2 is being consumed faster and the closed phase becomes shorter until it eventually disappears altogether (Hetz & Bradley, 2005; Bradley, 2007; Contreras & Bradley, 2010; Contreras & Bradley, 2011).

The oxidative damage hypothesis states that insects employ DGC to guard tissues against long-term oxidative damage. Thus, when insects are exposed to hyperoxia, in theory, they should exhibit DGC. Past studies exposing insects to hyperoxic conditions, i.e. a gas stream of 100 % O_2, have shown that insects respond by constricting spiracular area but DGC was not observed (Lighton *et al*., 2004; Lighton & Ottesen, 2005). These changes in spiracular area reflect active control over internal PO_2 but without the periodic flooding of the tracheal system with O_2 as in the DGC. This may be an adaptation to protect oxygen sensitive symbiotic microorganisms (Lighton & Ottesen, 2005). More recent studies on *Samia cynthia*, have established that DGC was and is maintained at hyperoxic conditions thus failing to reject the predictions of the oxidative damage hypothesis (Terblanche *et al*., 2008).

6.2 Aims and Research Objectives

This experiment aimed to answer two questions. Firstly, do termites of *H. mossambicus* exhibit discontinuous gas exchange under hyperoxic conditions? And, secondly how does relative humidity affect the metabolic rate, gas exchange pattern and water loss of major workers in hyperoxic conditions?

6.3 Results

6.3.1 Hyperoxia and normoxia

As seen in Table 6.1, major workers under hyperoxia (n=8) and normoxia (n=6) share similar means and distributions for VCO_2, CV and mass loss $t_{(12)}$ =-1.972, p=0.072; $t_{(12)}$ =1.452, p=0.172 and $t_{(12)}$ =0.409, p=0.69 respectively).

Table 6.1: Table showing the comparison of metabolic variables obtained for major workers in wet air exposed to hyperoxia and normoxia. Values are displayed as means ± standard deviations and significant values are indicated in bold.

	Hyperoxic	Normoxic	p-value
Mass (g)	0.065 ± 0.01	0.047 ± 0.01	-
VCO2 (ml $CO_2g^{-1}h^{-1}$)	0.25 ± 0.124	0.385 ± 0.131	0.072
CV	0.33 ± 0.14	0.24 ± 0.08	0.172
Mass Loss (g)	0.002 ± 0.001	0.003 ± 0.0	0.69

Workers under hyperoxia have a tendency to display cyclic gas exchange (Fig. 6.1), out of the eight trials run, six displayed cyclic gas exchange. VCO_2 does not go down to zero and the trace rarely falls below the cut-off threshold. This pattern differs from workers under normoxia at the same temperature, which display continuous gas exchange. There is also a difference in the activity levels between the two treatments. Workers under hyperoxia have a very low activity level compared to that of workers under normoxia (Fig. 6.1). Although the activity line for hyperoxia looks flat, the termites were never completely inactive.

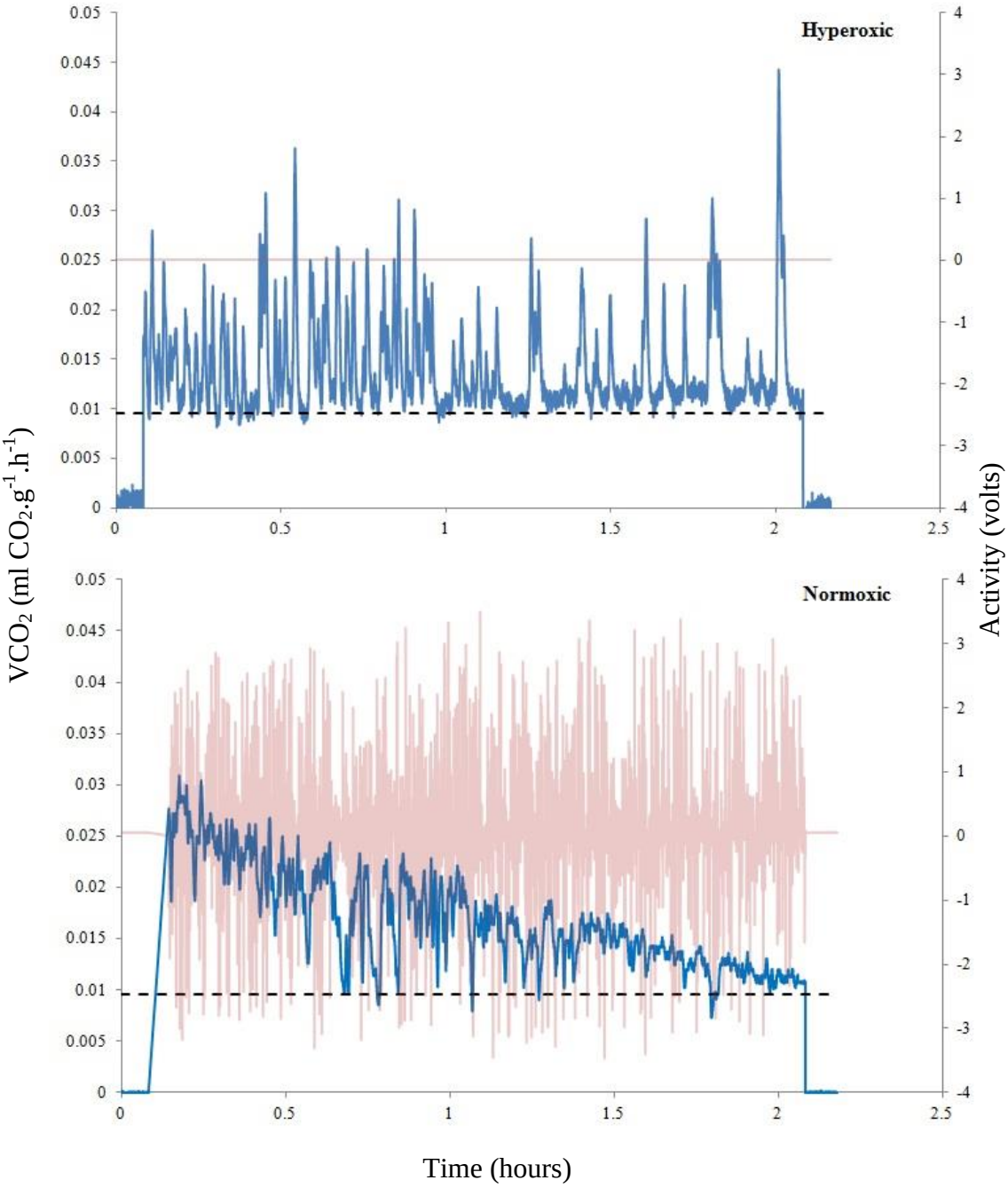


Figure 6.1: Typical CO_2 traces of major workers in hyperoxic and normoxic conditions at 25 °C. The blue series shows VCO_2, the red series shows activity and the black dotted line represents the cut-off threshold for spiracular closure.

6.3.2 Humidity

As seen in Table 6.2, major workers at 0 % RH (n=9) and 100 % RH (n=8), under hyperoxic conditions, share similar means and distributions for VCO_2 and CV ($t_{(15)}$ =0.691, p=0.5 and $t_{(15)}$ =-0.459, p=0.653 respectively). The only significant difference between the two treatments was the amount of mass lost over a period of two hours ($t_{(15)}$ =5.739, p<0.0001). In the water loss experiment, the water loss results for major workers, after a period of two hours, were 0.008 ± 0.002 g (Chapter 3, Fig. 3.3). Major workers at 0 % RH (Table 6.2) lost an equivalent amount, while workers at 100 % RH showed a significantly lower value (p<<0.05).

Table 6.2: Table showing the comparison of metabolic variables obtained for major workers exposed to hyperoxia at 100 % and 0 % relative humidity. Values are displayed as means ± standard deviations and significant values are indicated in bold.

	Dry Air	Wet Air	p-value
Mass (g)	0.061 ± 0.015	0.065 ± 0.01	-
VCO2 (ml $CO_2g^{-1}h^{-1}$)	0.305 ± 0.192	0.25 ± 0.124	0.5
CV	0.30 ± 0.13	0.33 ± 0.14	0.653
Mass Loss (g)	0.007 ± 0.001	0.002 ± 0.001	**<<0.05**

6.4 Discussion

Following from the results of Chapter 4, a change in relative humidity when all other variables are kept constant, especially temperature, results in a difference in mass loss rates only. VCO_2 and CV were similar for both treatments. Termites of *H. mossambicus* do not appear to actively control water loss through a change in respiratory pattern when relative humidity changes. The pattern of gas exchange was cyclic under both humidity regimes. The mass loss experienced in dry, flowing air under hyperoxia is equivalent to that experienced in the water loss experiment over the same time period (Fig. 3.3 and Section 4.4).

This is the first experiment to investigate the reaction of termites to 100 % O_2 for an extended time period. Workers of *H. mossambicus* appear to be particularly resilient to high levels of O_2 and are more prone to desiccation than to oxidative stress. When exposed to 100 % O_2 termites switch from continuous gas exchange to cyclic gas exchange. Termites exposed to hyperoxic conditions did not exhibit DGC but responded by actively increasing spiracular control and constriction. Thus it appears that termites of *H. mossambicus* are actively regulating their interior from the toxic effects of O_2. This corresponds to the results of Lighton and Ottesen, (2005) and Lighton *et al*., (2004). Both of these studies found a substantial reduction in CO_2 output by switching ambient air from 21 % to 100 % O_2. However, this was not the case in our experiment as the rate of CO_2 emission was similar for both hyperoxia and normoxia. DGC was not observed in *Zootermopsis nevadensis* in hyperoxia either but this was thought to be a result of the need to protect the oxygen-sensitive symbionts residing within their guts (Lighton & Ottesen, 2005). Workers of *H. mossambicus* do not possess these intestinal microbes. As seen in Chapter 6, a transition to a lower temperature results in a transition to a more discontinuous pattern. A lower temperature might have lead to prolonged spiracular closure and the expression of DGC.

Mass loss was similar for both oxygen environments, which was expected, as water loss is greatly dependent on temperature and humidity, which were kept constant (Nel, 1968). One might expect slightly less water loss in hyperoxic conditions as a result of the increased spiracular control and this is seen in Table 6.2, however, this difference is not significant. This has implications for the hygric hypothesis. It appears less likely that water savings are the underlying mechanism behind DGC and more likely that water savings are just an added benefit to increased spiracular control.

The activity level between the two oxygen environments differs substantially. This is unexpected, the termites in both environments share similar metabolic rates and are exposed to the same high temperature. We might also expect the termites in hyperoxia to have higher activity levels, expressing their need to

escape. Insects are not passive recipients to changes in the environment. Hyperoxia represents an extremely toxic oxygen environment; a behavioural response might be to move to a more favourable one (Chown *et al*., 2011). However, this is not the case. Workers of *H. mossambicus* appear to be quite resilient to the effects of hyperoxia. The low activity could be a consequence of the pattern of gas exchange. A common feature of insects that display DGC is reduced activity (Lighton, 1998; Matthews & White, 2011; Chown, 2011). This reduced activity is associated with reduced or absent brain activity. Thus, DGC is a consequence of energy-saving, metabolic downregulation. As the brain shuts down, control of respiration is relinquished and the segmental ganglia take over. The O_2 and CO_2 thresholds are sensed at the segmental ganglia and the interaction of these set points result in the discontinuous gas exchange pattern (Matthews & White, 2011; Chown, 2011). Thus quiescence, or a reduced activity, might be a consequence of the transition between respiratory patterns. Although, usually a transition in respiratory pattern is consequence of changing metabolic rate. Perhaps the reduced activity serves as a means to reduce oxygen intake. Increased activity results in an increased respiratory rate and therefore increased oxygen intake (Neven, 2000; Contreras & Bradley, 2010).

CHAPTER 7: Conclusion

Some insects display periodic breathing, an intermittent pattern of gas exchange commonly referred to as discontinuous gas exchange (DGC). This pattern of gas exchange has long been the subject of intense scrutiny, research and debate (Lighton, 1998; Chown *et al*., 2006; Lighton, 2007; Chown, 2011). Discontinuous gas exchange represents an enigma and the question remains, what is the evolutionary genesis and current adaptive significance of this perplexing gas exchange pattern? Given that DGCs appear to have evolved independently at least five times (Marais *et al*., 2005), there are potentially up to five different explanations.

Most current research into the origins and maintenance of DGC is driven by three adaptive hypotheses and one non-adaptive hypothesis. The adaptive hypotheses propose that DGC evolved or is currently maintained in order to reduce respiratory water loss, the hygric hypothesis (Schneiderman, 1960; Chown *et al*., 2006); enhance gas exchange in hypoxic and hypercapnic environments, the chthonic hypothesis (Lighton, 1998; Shelton & Appel, 2001; Gibbs & Johnson, 2004); and reduce the toxic effects of oxygen at the tissue level, the oxidative-damage hypothesis (Hetz & Bradley, 2005; Bradley, 2006). The non-adaptive hypothesis proposes that DGC is merely a consequence of the interaction between the O_2 and CO_2 set points that govern spiracular opening and closing, the emergent-property hypothesis (Chown & Holter, 2000; Chown *et al*., 2006; Bradley, 2007; White *et al*., 2007). This mechanistic hypothesis has been made adaptive with the inclusion of new data showing that energetic considerations promote the downregulation of metabolic rate, resulting in reduced brain activity and control of the respiratory system is then relinquished to the segmental ganglia of the thorax and abdomen. At this point, the interaction between segmental PO_2 sensors and spiracle-based PCO_2 sensors comes into effect and DGC arises as a

consequence of this interaction, the neural hypothesis (Matthews & White, 2011; Chown, 2011).

From their results of a strong-inference and phylogenetic study, White *et al*., (2007) concluded that reducing water loss while ensuring adequate gas exchange is the predominant feature selecting for or maintaining DGCs. This provides evidence that discontinuous gas exchange is maintained for adaptive reasons but does not elucidate the underlying mechanism driving the display of DGCs. With this in mind this study concentrated on three of the aforementioned hypotheses, specifically the hygric, the oxidative-damage and the emergent-property hypotheses.

The insect chosen as the model was workers of the harvester termite, *Hodotermes mossambicus*. This termite is a subterranean species and workers are the only caste to leave the underground nests and galleries to forage above ground during the day, exposed to desiccating ambient conditions (Coaton & Sheasby, 1975; Myburgh, 1989; Uys, 2002). If they exhibit DGC in response to these foraging conditions this would provide further support to the hygric hypothesis. When not foraging, galleries leading to the soil surface are plugged with mud (Coaton & Sheasby, 1975; Myburgh, 1989; Uys, 2002). Workers escaping to the refuge of the underground burrows are likely operating under the selective pressures of hypoxia and hypercapnia as a result of their colony depth and lack of direct O_2 exchange. Therefore, they have the potential to display DGC in accordance with the chthonic hypothesis. However, an initial study (Inder, 2010) found that workers of *H. mossambicus* did not display DGC in response to hypoxia. A study on the damp-wood termite *Zootermopsis nevadensis* (Lighton & Ottesen, 2005), found that individuals exposed to 100 % O_2 did not express DGC but did however restrict their spiracles. This species possess essential intestinal microorganisms that are highly anaerobic. As such full DGC expression, with an O phase, would result in the flushing of these microbes with near ambient levels of O_2 (Lighton & Ottesen, 2005). Workers of *H. mossambicus* do not have these anaerobic microbes and hence provide a more suitable subject to test the oxidative-damage

hypothesis. In an initial study (Inder, 2010), the respiratory pattern exhibited by workers of *H. mossambicus* was found to be cyclic when recorded at 25 °C. If they exhibit DGC at lower temperatures and continuous gas exchange at higher temperatures, this would provide support for the emergent-property hypothesis.

Initially, the respiratory patterns of both major and minor workers were to be investigated, however, after the water loss (Chapter 3) and relative humidity (Chapter 4) experiments were completed the inclusion of minor workers was considered unnecessary for the following reasons; the majority of foraging workers observed in the field were major workers, minor workers were less resilient to experimental manipulations than major workers and had a tendency to display continuous gas exchange despite changes in the environmental conditions. Thus, major workers were both available for collection in the field and presented more suitable subjects to test the hypotheses of DGC.

If the hygric hypothesis selects for and is responsible for the maintenance of DGC the following predictions or assumptions can be made. Firstly, insects displaying DGC should have lower water loss than insects displaying other patterns and secondly, DGC should be present in insects experiencing desiccating conditions such as low humidities and/or high temperatures. Workers of *H. mossambicus* are consistently exposed to the extremes of the external environment (Nel & Hewitt, 1969), where the average foraging conditions were found to be 26.8 °C and 32.6 % RH. Water balance characteristics reflect the environmental conditions that an insect is continually exposed to as well as their physiological condition (Wharton, 1985; Hadley, 1994; Schimpf *et al*., 2009). Insects from habitats of varying water availability show variation in their tolerance to desiccation, resistance to desiccation and their initial body water contents (Gray & Chown, 2008). The workers of *H. mossambicus* have initial body water content of approximately 70 % of their body weight. Their critical hydration level falls between 42 % and 48 %, and their rate of water loss falls below 0.003 $ml.h^{-1}$. These characteristics show that termite workers have quite a high tolerance to desiccation, workers are able to lose almost 60 % of their body water before reaching a point of loss of

coordination but possess no particular ability to resist desiccation, their body water content is average (Hadley, 1994) and their water loss rate is not particularly low. Major and minor workers share similar water balance characteristics despite the difference in their mass. Minor workers generally spend less than 260 s above ground, while the longest observed foraging time for a major worker was 1158 s (Duncan & Hewitt, 1989). A physiological tolerance to desiccation might well be unnecessary in this species if only brief periods are spent on the surface.

The relative humidity experiments (Chapter 4 and 6) were dissimilar in that one was completed in normoxia under varying temperature (Chapter 4), while the other was completed in hyperoxia at a constant temperature of 25 °C (Chapter 6). Yet despite these differences, both experiments shared two common features; workers at 0 % RH lost significantly more water that workers at 100 % RH and discontinuous gas exchange was not observed either in dry air or in wet air. The greater loss of water at 0 % RH is not unexpected as no water savings mechanisms can be completely effective. However, termites at 0 % RH (Chapter 4) did lose less water over the same time period than termites in the water loss experiments in Chapter 3. This indicates that termites at 0 % RH are doing something to curtail water loss. Discontinuous gas exchange was not observed and therefore was not responsible for the lower water loss. In Chapter 4, termites under different humidity regimes displayed different patterns of gas exchange. Major workers at 100 % RH displayed cyclic gas exchange while those at 0 % RH displayed continuous gas exchange. Minor workers had a tendency to breathe continuously despite changes in relative humidity. The continuous pattern observed possessed high levels of pumping or fluttering. This pumping/fluttering is a mechanism considered to reduce respiratory water loss (Duncan *et al*., 2002) and therefore could be responsible for the lower water loss observed compared to the results of Chapter 3. In Chapter 6, major workers displayed similar patterns of gas exchange and cyclic gas exchange was observed at both humidity regimes. The mass loss observed at 0 % RH was not significantly different than that observed over the same time period in Chapter 3. This difference between the

results of the different chapters was attributed to the temperature of the experiments. In Chapter 6, temperature was kept constant at 25 °C, while in Chapter 4, temperature fell between 22 °C and 30 °C (Fig. 4.5). Thus, it appears that a change in ambient humidity has no impact on VCO_2, CV or the pattern of gas exchange but only influences the amount of mass lost.

The temperature experiment (Chapter 5) showed that when temperature was increased above 15 °C, mass loss increased exponentially. DGC was not observed at high temperatures. A change in respiratory pattern, which indicates a change in the spiracular control regime, does seem to influence the level of water loss. Respiratory water loss is the fundamental cost of gas exchange and therefore all insects are under selection pressure to reduce this cost (Lighton, 2007; Woods & Smith, 2010). Thus, any spiracular control regimen should be selected to conserve water, including DGC. The results of the above studies indicate that workers of *H. mossambicus* do not actively control or regulate water loss through use of the DGC. However, this does not preclude that the exhibition of DGC does not result in a reduction in respiratory water loss.

The oxidative-damage hypothesis predicts that insects exposed to high oxygen concentrations will display DGC (Lighton *et al*., 2004; Lighton & Ottesen, 2005). Major workers exposed to 100 % O_2 did not display discontinuous gas exchange. The rate of CO_2 emission, CV and mass loss was not significantly different from that of workers exposed to ambient oxygen conditions (21 % O_2). However, the respiratory pattern was found to differ between the two oxygen environments. Workers exposed to normoxic conditions at 25 °C displayed a continuous gas exchange pattern while workers exposed to 100 % O_2 at the same temperature displayed cyclic gas exchange. The patterns of gas exchange displayed within the two oxygen environments were highly variable, and as a result of this variance, the CVs were not found to significantly different. The spiracles act as the main barrier to O_2 entry (Chown & Holter, 2000; Harrison *et al*., 2006). In response to an increase in O_2 workers actively increase spiracular control and constriction, therefore, decreasing the amount of O_2 entering the tracheal system. Thus it

appears that termites are actively engaged in protecting their interior against the toxic effects of O_2. Yet, against expectations they do not use the DGC to do so. There are several possible reasons for this. A common feature of insects that display DGC is that they display reduced or absent brain activity, i.e. they are at rest (Matthews & White, 2011; Chown, 2011). Although workers exposed to 100 % O_2 have reduced activity compared to that of workers at 21 % O_2, they were not inactive. In fact, termites were active to some degree under all experimental conditions. Activities such as walking, running or flying can increase metabolic rate 10-100 fold (Terblanche *et al*., 2008). This increased activity level might well be a consequence of the temperature of the experiment. Studies performing the same experiment did so under reduced temperature, between 10 °C and 15 °C (Lighton & Ottesen, 2005; Contreras & Bradley, 2010; Contreras & Bradley, 2011). Reduced temperature was shown to reduce both metabolic rate and activity level in termite workers (Chapter 5). Another possible explanation for the lack of DGC at 100 % O_2 is the O phase of DGC. This represents the fundamental flaw or weakness in the oxidative-damage hypothesis. Although the closed and flutter phases reduce and stabilize the level of O_2 around the tissues, the open phase floods the tracheal system with near-ambient levels of O_2 (Lighton, 2007). A cyclic pattern of gas exchange, however, never experiences a period where the spiracles are fully dilated. The results of the above experiment give credence to the oxidative-damage hypothesis. Termites actively guard their interior from excessive O_2 and experimental conditions that decrease termite activity and metabolic rate might very well result in the expression of DGC in hyperoxic conditions.

An implication of the emergent-property hypothesis is that as metabolic rate increases, oxygen is consumed faster thereby reducing the closed phase such that the gas exchange pattern displayed by the insect transitions from discontinuous to cyclic to continuous (Bradley, 2007; Contreras & Bradley, 2010). The results of the temperature experiment (Chapter 5) confirm this. As temperature increased, metabolic rate increased and the gas exchange pattern changed. At low temperatures (5 °C and 10 °C) periods of spiracular closure were observed. A true

DGC, defined as showing all three phases and a CV value close to 2 (Lighton, 1998) was not observed. At 15 °C, the gas exchange pattern was highly variable ranging from cyclic to continuous. Above 15 °C, the pattern of gas exchange was continuous. Activity also increased with temperature and metabolic rate but again termites were never inactive or at rest. This again could explain the lack of DGC expression. Their metabolic rates are too high to sustain a discontinuous pattern of gas exchange. Basson and Terblanche, (2011), observed the same pattern in *Messer* worker ants. When active they did not display DGC.

A common misconception or assumption regarding DGC is that insects that display this pattern do so for the same reason and receive similar benefits from its expression. Yet, as discussed previously this pattern evolved independently five times and considering the wide range of environments that these insects inhabit, their dissimilar reactions to environmental change and their diverse life strategies, this seems unlikely (Marais *et al*., 2005; Terblanche *et al*., 2008; Matthews & White, 2011). There are potentially one to five explanations for the origin of this pattern and many more explanations for its continued existence. It is possible that several factors select for DGC or perhaps DGC can be pressed into service for multiple purposes (Matthews & White, 2011; Chown, 2011). Recent studies seem to have reached a consensus as to what the underlying mechanism driving the exhibition of discontinuous gas exchange might be and the results of the temperature experiment (Chapter 5) have confirmed their conclusions. Respiratory patterns, DGCs included, are a consequence of the interaction between the O_2 and CO_2 set points that govern spiracular control. In this way, O_2 supply is matched to O_2 demand and the respiratory pattern displayed at any given time reflects this balance. Therefore, discontinuous gas exchange is not distinct but represents one endpoint of a continuum of patterns (Bradley, 2007).

A true discontinuous gas exchange cycle was not observed under any of the experimental conditions in the workers of *H. mossambicus.* However, given that workers of this species were never inactive and the expression of DGC requires an insect to be at rest with low metabolic demand (Matthews & White, 2011),

perhaps the conditions for DGC are never met in this species. Workers of *H. mossambicus* do, however, change their respiratory pattern with a change in oxygen concentration and temperature.

The hypotheses for the origin and current adaptive significance of DGC need not be mutually exclusive. The fact that all of the proposed hypotheses receive equivocal support gives credence to the possibility that several factors might select for or maintain DGC. The workers of *H. mossambicus* spend only brief periods above ground before returning to the refuge of their underground nests and galleries and as such there is probably little selective advantage to the DGC for limiting respiratory water loss. The results of this study support the oxidative-damage and emergent-property hypotheses but not the hygric hypothesis. I conclude that DGCs of insects are an emergent property of the insects' nervous and respiratory systems and spiracular control also serves to limit oxidative damage.

REFERENCES

Addo-Bediako, A., Chown, S.L., and Gaston, K.J. (2001). Revisiting water loss in insects: a large scale view. *Journal of Insect Physiology*, 47: 1377-1388.

Basson, C.H. and Terblanche, J.S. (2011). Respiratory pattern transitions in three species of *Glossina* (Diptera, Glossinidae). *Journal of Insect Physiology*, 57: 433-443.

Bazinet, A.L., Marshall, K.E., MacMillan, H.A., Williams, C.M., and Sinclair, B.J. (2010). Rapid changes in desiccation resistance in *Drosophila melanogaster* are facilitated by changes in cuticular permeability. *Journal of Insect Physiology*, 56: 2006-2012.

Bosch, M., Chown, S.L., and Scholtz, C.H. (2000). Discontinuous gas exchange and water loss in the keratin beetle *Omorgus radula*: further evidence against the water conservation hypothesis? *Physiological Entomology*, 25: 309-314.

Bradley, T.J. (2006). Discontinuous ventilation in insects: Protecting tissues from O_2. *Respiratory Physiology and Neurobiology*, 154: 30-36.

Bradley, T.J. (2007). Control of the respiratory pattern in insects. *Advances in Experimental Medicine and Biology*, 618: 211-220.

Chappell, M.A. and Rogowitz, G.L. (2000). Mass, temperature and metabolic effects on discontinuous gas exchange in Eucalyptus-boring beetles (Coleoptera: Cerambycidae). *Journal of Experimental Biology*, 203: 3809-3820.

Chown, S.L. (2002). Respiratory water loss in insects. *Comparative Biochemistry and Physiology Part A,* 133: 791-804.

Chown, S.L. (2011). Discontinuous gas exchange: new perspectives on evolutionary origins and ecological implications. *Functional Ecology*, 25: 1163-1168.

Chown, S.L. and Holter, P. (2000). Discontinuous gas exchange cycles in *Aphodius fossor* (Scarabaeidae): a test of hypotheses concerning origins and mechanisms. *Journal of Experimental Biology*, 203: 397-403.

Chown, S.L., and Nicolson, S.W. (2004). *Insect physiological ecology: mechanisms and patterns*. Oxford University Press, Oxford.

Chown, S.L., Gibbs, A.G., Hetz, S.K., Klok, C.J., Lighton, J.R.B., and Marais, E. (2006). Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. *Physiological and Biochemical Zoology*, 79: 333-343.

Chown, S.L., Sorensen, J.G., and Terblanche, J.S. (2011). Water loss in insects: An environmental change perspective. *Journal of Insect Physiology*, 57: 1070-1084.

Coaton, W.G.H. and Sheasby, J.L. (1975). National survey of the isopteran of Southern Africa 10. The genus *Hodotermes* Hagen (Hodotermitidae). *Cimbebasia*, 3: 105-138.

Contreras, H.L. and Bradley, T.J. (2010). Transitions in insect respiratory patterns are controlled by changes in metabolic rate. *The Journal of Insect Physiology*, 56: 522-528.

Contreras, H.L. and Bradley, T.J. (2011). The effect of ambient humidity and metabolic rate on the gas-exchange pattern of the semi-aquatic insect *Aquarius remigis*. *The Journal of Experimental Biology*, 214: 1086-1091.

Duncan, F.D. and Hewitt, P.H. (1989). Observations on the foraging behavior of the harvester termite, *Hodotermes mossambicus* (Hagen) (Isoptera: Hodotermitidae). *Bulletin of Entomological Research*, 79: 631-642.

Duncan, F.D. and Lighton, J.R.B. (1994). Water relations in nocturnal and diurnal foragers of the Desert Honeypot Ant *Myrecocystus*: Implications for colony-level selection. *The Journal of Experimental Zoology*, 270: 350-359.

Duncan, F.D. and Byrne, M.J. (2000). Discontinuous gas exchange in dung beetles: patterns and ecological implications. *Oecologica*, 122: 452-458.

Duncan, F.D., Krasnov, B. and McMaster, M. (2002). Metabolic rate and respiratory gas-exchange patterns in tenebrionid beetles from the Negev Highlands, Israel. *The Journal of Experimental Biology*, 205: 791-798.

Edney, E.B. (1957). *The water relations of terrestrial arthropods*. Cambridge University Press, London.

Edney, E.B. (1977). *Water balance in land arthropods*. Springer, Berlin.

Feener, D.H., and Lighton, J.R.B. (1991). Is foraging in the desert ant, *Messor pergandei* (Hymenoptera: Formicidae), limited by water? *Ecological Entomology*, 16: 183-191.

Fielden, L.J. and Lighton, J.R.B. (1996). Effects of water stress and relative humidity on ventilation in the tick *Dermacentor andersoni* (Acari: Ixodidae). *Physiological Zoology*, 69: 599-617.

Forster, T.D. and Hetz, S.K. (2010). Spiracle activity in moth pupae-The role of oxygen and carbon dioxide revisited. *Journal of Insect Physiology*, 56: 492-501.

Gibbs, A.G. and Johnson, R.A. (2004). The role of discontinuous gas exchange in insects: the chthonic hypothesis does not hold water. *Journal of Experimental Biology*, 207: 3477-3482.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., and Charnov, E.L. (2001). Effects of size and temperature on metabolic rate. *Science*, 293: 2248-2251.

Gray, E.M., and Chown, S.L. (2008). Bias, precision and accuracy in the estimation of cuticular and respiratory water loss: A case study from a highly variable cockroach, *Perisphaeria* sp. *Journal of Insect Physiology*, 54: 169-179.

Hadley, N.F. (1994). *Water relations of terrestrial arthropods*. Academic Press, San Diego.

Hadley, N.F. and Quinlan, M.C. (1993). Discontinuous carbon dioxide release in the eastern lubber grasshopper *Romalea guttata* and its effect on respiratory transpiration. *Journal of Experimental Biology*, 177: 169-180.

Harrison, J., Frazier, M.R., Henry, J.R., Kaiser, A., Klok, C.J., and Rascon, B. (2006). Responses of terrestrial insects to hypoxia or hyperoxia. *Respiratory Physiology and Neurobiology*, 154: 4-17.

Harrison, J.F., Woods, H.A., and Roberts, S.P. (2012). *Ecological and environmental physiology of insects.* Oxford University Press, Oxford.

Hetz, S.K. and Bradley, T.J. (2005). Insects breathe discontinuously to avoid oxygen toxicity. *Nature*, 433: 516-519.

Hu, J., Neoh, K., Appel, A.G., and Lee, C. (2012). Subterranean termite open-air foraging and tolerance to desiccation: Comparative water relation of two

sympatric *Macrotermes* spp. (Blattodea: Termitidae). *Comparative Biochemistry and Physiology Part A*, 161: 201-207.

Hustert, R. (1975). Neuromuscular coordination and proprioceptive control of rhythmical abdominal ventilation in intact *Locusta migratoria migratorioides*. *The Journal of Comparative Physiology*, 97: 159-179.

Inder, I.M. (2010). Respiration in the termite *Hodotermes mossambicus* (Hagen). Unpublished honours research report, University of the Witwatersrand.

Karise, R., Kuusik, A., Mand, M., Metspalu, L., Williams, I.H., Hiiesaar, K., Luik, A., Muljar, R., and Liiv, K. (2010). Gas exchange patterns of bumble bee foragers before and after exposing to lowered temperature. *Journal of Insect Physiology*, 56: 529-535.

Krishna, K. and Weesner, F.M. (1969). *Biology of termites*. Volume 1. Academic Press, New York.

Lease, H.M. and Wolf, B.O. (2010). Exoskeletal chitin scales isometrically with body size in terrestrial insects. *Journal of Morphology*, 271: 759-768.

Levy, R.I. and Schneiderman, H.A. (1966). Discontinuous respiration in insects II: the direct measurement and significance of changes in tracheal gas composition during the respiratory cycle of silkworm pupae. *Journal of Insect Physiology*, 12: 83-104.

Lighton, J.R.B. (1990). Slow discontinuous ventilation in the Namib dune-sea ant, *Camponotus detritus* (Hymenoptera, Formicidae). *Journal of Experimental Biology*, 151: 71-82.

Lighton, J.R.B. (1998). Notes from the Underground: towards ultimate hypotheses of cyclic, discontinuous gas-exchange in tracheate arthropods. *American Zoologist*, 38: 483-491.

Lighton, J.R.B. (2007). Respiratory biology: Why insects evolved discontinuous gas exchange. *Current Biology*, 17: 645-647.

Lighton, J.R.B., and Feener, D.H. (1989). Water-loss rate and cuticular permeability in foragers of the desert ant *Pogonomyrmex rugosus*. *Physiological Zoology*, 62 (6): 1232-1256.

Lighton, J.R.B., Garrigan, D.A., Duncan, F.D., and Johnson, R.A. (1993). Spiracular control of respiratory water loss in female alates of the harvester ant Pogonomyrmex rugosus. *The Journal of Experimental Biology*, 179: 233-244.

Lighton, J.R.B., Schilman, P.E., and Holway, D.A. (2004). The hyperoxic switch: assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. *The Journal of Experimental Biology*, 207: 4463-4471.

Lighton, J.R.B., and Ottesen, E.A. (2005). To DGC or not to DGC: oxygen guarding in the termites *Zootermopsis nevadensis* (Isoptera: Termopsidae). *Journal of Experimental Biology*, 208: 4671- 4678.

Maina, J.N. (2002). Structure, function and evolution of the gas exchangers: comparative perspectives. *The Journal of Anatomy*, 201: 281-304.

Marais, E., Klok, C.J., Terblanche, J.S., and Chown, S.L. (2005). Insect gas exchange patterns: a phylogenetic perspective. *The Journal of Experimental Biology*, 208: 4495-4507.

Martin, T.L. and Huey, R.B. (2008). Why 'suboptimal' is optimal: Jensen's inequality and ectotherm thermal preferences. *American Naturalist*, 171: E102-E118.

Matthews, P.G.D. and White, C.R. (2011). Discontinuous gas exchange in insects: is it all in their heads? *American Naturalist*, 177: 130-134.

McKechnie, A.E., and Wolf, B.O. (2004). Partitioning of evaporative water loss in white-winged doves: plasticity in response to short-term thermal acclimation. *The Journal of Experimental Biology*, 207: 203-210.

Mitchell, J.D., Hewitt, P.H., and Van Der Linde, T.C.De K. (1993). Critical thermal limits and temperature tolerance in the harvester termite *Hodotermes mossambicus* (Hagen). *The Journal of Insect Physiology*, 39: 523-528.

Moerbitz, C. and Hetz, S.K. (2010). Tradeoffs between metabolic rate and spiracular conductance in discontinuous gas exchange of *Samia cynthia* (Lepidoptera, Saturniidae). *The Journal of Insect Physiology*, 56: 536-542.

Myburgh, A.C. (1989). *Crop pests in Southern Africa Vol.4 Field crops and pastures*. Department of Agriculture and Water supply, Pretoria.

Nel, J.J.C. (1968). Die invloed van temperatuur en relatiewe humiditeit op die gewigsverlies en oorlewing van die grasdraertermiete *Hodotermes mossambicus*

(Hagen) en *Trinervitermes trinervoides* (Sjostedt). *Suid Afrikaanse Tydskrif Landbouwetenskap*, 11:389-402.

Nel, J.J.C., and Hewitt, P.H. (1969). Effect of solar radiation on the harvester termite, Hodotermes mossambicus (Hagen). *Nature*, 223: 862-863.

Neven, L.G. (2000). Physiological responses of insects to heat. *Postharvest Biology and Technology*, 21: 103-111.

Nicolson, S.W. (1980). Water balance and osmoregulation in *Onymacris plana*, a tenebrionid beetle from the Namib Desert. *Journal of Insect Physiology*, 26: 315-320.

Nicolson, S.W. (2009). Water homeostasis in bees, with the emphasis on sociality. *The Journal of Experimental Biology*, 212: 429- 434.

Roberts, S.P., Quinlan, M.C., and Hadley, N.F. (1994). Interactive effects of humidity and temperature on water loss in the lubber grasshopper *Romalea guttata*. *Comparative Biochemistry and Physiology*, 109: 627-631.

Schimpf, N.G., Matthews, P.G.D., Wilson, R.S., and White, C.R. (2009). Cockroaches breathe discontinuously to reduce respiratory water loss. *The Journal of Experimental Biology*, 212: 2773-2780.

Schneiderman, H.A. (1960). Discontinuous respiration in insects: role of spiracles. *Biological Bulletin*, 119: 494-528.

Schneiderman, H.A. and Williams, C.M. (1955). An experimental analysis of the discontinuous respiration of the Cecropia silkworm. *Biological Bulletin*, 109: 123-143.

Shelton, T.G., and Appel, A.G. (2000). Cyclic CO_2 release and water loss in the Western drywood termite (Isoptera: Kalotermitidae). *Annals of the Entomological Society of America*, 93: 1300-1307.

Shelton, T.G. and Appel, A.G. (2001). An Overview of the CO_2 release patterns of lower termites (Isoptera: Termopsidae, Kalotermitidae, and Rhinotermitidae). *Sociobiology*, 37: 193-219.

Sponsler, R.C., and Appel, A.G. (1990). Aspects of the water relations of the Formosan and Eastern subterranean termites (Isoptera: Rhinotermitidae). *Environmental Entomology*, 19: 15-20.

Terblanche, J.S., Marais, E., Hetz, S.K., and Chown, S.L. (2008). Control of discontinuous gas exchange in *Samia cynthia*: effects of atmospheric oxygen, carbon dioxide and moisture. *The Journal of Experimental Biology*, 211: 3272-3280.

Tomlinson, S. and Phillips, R.D. (2012). Metabolic rate, evaporative water loss and field activity in response to temperature in an ichneumonid wasp. *Journal of Zoology*, 287: 81-90.

Uys, V. (2002). *A guide to the termite genera of Southern Africa*. Agricultural Research Council, Pretoria.

Van der Linde, T.C., Hewitt, P.H., Van Der Westhuizen, M.C., and Mitchell, J. (1989). The use of ^{131}I, ^{125}I and aggressive behavior to determine the foraging area of Hodotermes mossambicus (Hagen) (Isoptera: Hodotermitidae). *Bulletin of Entomological Research*, 79: 537-544.

Watson, J.A.L. (1973). The worker caste of the Hodotermitid harvester termites. *Insectes Sociaux*, 20: 1-20.

Wharton, G.W. (1985). Water balance in insects. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (eds. G.A. Kerkut and L.I. Gilbert), 4: 565-601. Pergamon Press, Oxford.

White, C.R., Blackburn, T.M., Terblanche, J.S., Marais, E., Gibernau, M., and Chown, S.L. (2007). Evolutionary responses of discontinuous gas exchange in insects. *Proceedings of the National Academy of Sciences*, 104: 8357-8361.

Williams, C.M., Pelini, S.L., Hellmann, J.J. and Sinclair, B.J. (2010). Intra-individual variation allows an explicit test of the hygric hypothesis for discontinuous gas exchange in insects. *Biology Letters*, 6: 274-277.

Woods, H.A. and Smith, J.N. (2010). Universal model for water costs of gas exchange by animals and plants. *Proceedings of the National Academy of Sciences*, 107: 8469-8474.

Yoder, J.A., Ark, J.T., Benoit, J.B., Bellinger, E.J., and Gribbins, K.M. (2006). Water balance characteristics in adults of terrestrial red mite *Balaustium* sp. (Acarina: Erythraeidae). *Arthropod Biology*, 99: 560-566.

Zachariassen, K.E., Andersen, J., Kamau, J.M.Z., and Maloiy, G.M.O. (1988). Water loss in insects from arid and humid habitats in East Africa. *Acta Entomologica Bohemoslovaca*, 85: 81-93.

Zachariassen, K.E. (1991). Routes of transpiratory water loss in a dry-habitat tenebrionid beetle. *The Journal of Experimental Biology*, 157: 425-437.

Zachariassen, K.E. (1996). The water conserving compromise of desert insects. *The European Journal of Entomology*, 93: 359-367.