Chapter 1: General Introduction

1.1 Predicted impacts of climate change

Climate change is a major current and future threat to agroecosystems. It is already modifying, and will continue to modify, species' geographical ranges, structure of community and ecosystems; as well as increase the range of pest species and potentially will reduce natural enemy effectiveness (Andrew and Hill 2017). To properly assess the direct and indirect effects of climate change, critical observational and experimental data on both populations and species needs to be collected, and then be embedded into predictive models to assess responses of species to rapid changes into the future (McCarty 2001).

For Australia, recent evidence of climate change is compelling: there have been no other warmer periods than post-1950 in the last 1000 years (Gergis and Members 2012) and the most recent decade (2000-09) was the hottest on record. In addition, 2015 was hottest year on record in Australia (Braganza and Church 2011; CSIRO-ABM. 2014; Karoly and Black, 2015).

Prolonged heat exposure can have extremely detrimental impacts on animal populations. For example, during 2010 in Australia, a two-week heat wave led to the death of thousands of birds in the wild: this happened when environmental temperatures exceeded the maximum critical thermal limits of these birds (Miller and Stillman 2012; Bozinovic et al. 2013). In the wild, ectotherms are continuously exposed to several short-term variations in environmental conditions. In this context, the study of the impact of thermal conditions on the performance of individuals and their plastic responses to it, such as lethal and sub-lethal effects, are key to

understanding the responses of biota to the environment and to climate change (Pörtner et al. 2006; Somero 2011; Bozinovic et al. 2013).

Based on climate change models, it is predicted that heatwaves (extended periods of abnormally hot weather) will be extended, more frequent and severe under continued climate change, there will be increases in mean temperatures over the next century (Parmesan 2006 ; Meehl and Tebaldi 2004; IPCC 2007; Gillespie et al. 2012; IPCC 2007; Ju et al. 2013; IPCC. 2014). With exposure to these extreme heatwave events, particularly when insects are under thermal stress at the warmest period of their life cycles (summer), ectotherms (including insects) may be highly susceptible to deleterious effects of extreme heat exposure (Musolin et al. 2010). The direct impacts of climate change including change in behaviour, physiology and ecology, and indirect effects of climate change including CO_2, O_3, temperature and participation will influence insect herbivore population dynamics, ecosystem function and community structure (Cornelissen 2011). Insects able to successfully adapt to host plant and environmental climatic factor, can complete their life cycle, too. By understanding mechanisms behind the strategies in extreme conditions we can develop hypothesis related to impacts of climate change on insect's life-history and responses to climatic change in the future (Bale et al. 2002).

1.2 Global climate change and insects

Ectotherms are very sensitive to climate change due to vital biological characters being strongly influenced by temperature including development time, number of offspring and their locomotion (Wilson 1992). Ectothermic insects cannot regulate their temperature above and

below ambient temperature relative to endotherms and may consequently be more adversely influenced by climate change (Walther et al. 2002).

All insects are responsive to temperature changes, so under a warming climate we would expect this to impact the lifecycle of an insect and the ecosystems they inhabit. Climate change has substantive impacts on physiological characteristics and habitat use by of insects (Stange and Ayres 2010).

Climate change may have a variety of impacts of biotic and abiotic factors of any ecosystem, as direct and indirect effects on several aspects of the life cycle of insects including the population dynamics, physiology, distribution and abundance. To better understand current and future impacts of climate change on insects we need to consider the changes in climate in relation to ability and plasticity performance traits of species concurrently (Stange and Ayres 2010).

Climatic warming tends to influence (and frequently amplify) population dynamics of insects directly through effects on survival, generation time, fecundity and dispersal (Bale et al. 2002; Andrew 2013). Climatic warming also reduces or increase the risk of death in insect winter populations mortality due to extreme cold (Ayres and Lombardero 2000; Williams et al. 2014).

1.2.1 Insects at high temperature

Abiotic factors such as extreme high and low temperature are major threat to insects, and can change their distribution by influence on development time , number of offspring and life span (Hutchinson and Bale 1994; Colinet et al. 2015).

Thermal mean and variability at local scales interact in a non-additive way to determine physiological performance thermal and thermal tolerance . This occur via change in performance curve parameters which in consequences may produce changes in activity patterns, timing of breeding, and finally may alter synchronization between trophic levels and species community structure (Ashton et al. 2009; Bozinovic et al. 2013).

A thermal 'performance' or 'fitness' curve serves as a convenient descriptor of how a change in body temperature (T_b) influences physiological sensitivity and fitness of ectotherms (Huey and Stevenson 1979; Angilletta 2009). There are two endpoint temperatures for ectotherms in their performance curve. Very low and high T_b reduces an ectotherm's performance and can be lethal in the extreme: these endpoints T_b are called the 'critical temperatures' (Figure 1.1; CT_{max}, CT_{min}). Within those critical limits, performance reaches a maximum at an optimal temperature region (T_o), and then typically plummets at higher T_b. These endpoints experimentally measured by the organism performance (Huey and Stevenson 1979; Gilchrist 1995; Frazier et al. 2006; Martin and Huey 2008) and this character in thermal performance curve can be change by acclimation time, duration and frequency of temperature exposure.

The type of insect reactions and responses to climate change depend on whether a species is a thermal generalist or specialist (Janzen 1967; Huey and Slatkin 1976; Huey and Kingsolver 1993; Gilchrist 1995; Deutsch et al. 2008; Amarasekare and Savage 2012; Huey et al. 2012). For example, a given increase in T_b from warming will usually have a larger impact on a thermal specialist than on a thermal generalist: specialist have narrow performance curve whereas generalists have expanded performance curve.
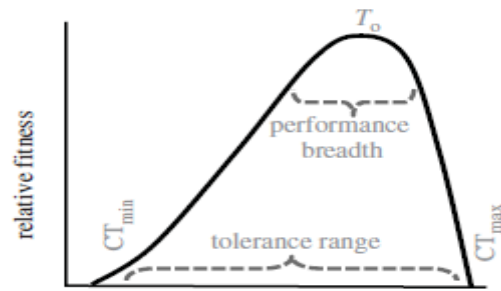
Fig 1.1 Thermal fitness (performance) curve for a hypothetical ectotherm, with key descriptive parameters CT_{max}, CT_{min}, tolerance range, performance breath and optimal temperature (T_o) identified (adapted from Huey et al. (2012)).

Insects use different strategies to survive at unfavourable high temperatures which can be separated into chemical and behavioural responses and these strategies can induce increases in heat tolerance or to prevent exposure to unfavourable conditions). The strategies are outlined below.

1.2.2 Effective compounds in increase tolerance to high temperature

Polyhydroxyl compounds play key roles in unfavourable conditions such as extreme, high and low temperatures, desiccation and toxication of which, trehalose and sucrose are major part of these molecules (Kempf and Bremer 1998; Jain and Roy 2009; Arguelles 2000).

The organic compounds that dominate the plant phloem sap are sugars, commonly sucrose, and amino acids (Wilkinson et al. 2001). Sucrose is the prevailing natural compound in plant phloem sap and is an essential carbon source for phloem feeding insects such as aphids (Fisher 2000; Douglas 2003). Sucrose is the primary respiratory substrate for aphids and gives the carbon skeleton to lipid and protein-amino acid creation (Rhodes et al. 1996; Febvay et al. 1999; Salvucci and Crafts-Brandner 2000; Pescod et al. 2007).

Sucrose is a major and vital source of energy in respiration time in the pea aphids (Febvay et al. 1995; Rhodes et al. 1996). Sucrose can be hydrolysis by the sucrase enzyme to glucose and fructose which can be used in respiration or synthesis of osmolytes compounds such as mannitol in aphids and sorbitol in whiteflies in high temperature (Ehrhardt 1962; Febvay et al. 1995; Rhodes et al. 1996; Hendrix and Salvucci 1998; Ashford et al. 2000).

Higher concentrations of sucrose in the diet delayed high-temperature mortality, possibly a reflection of the high sucrose requirement for sorbitol synthesis in whiteflies (Hendrix et al. 1998). Based on studies phloem tissue of plants are rich in carbohydrates which are necessary and important for aphids and whiteflies performance (Fisher and Gifford 1986; Winter et al. 1992). Artificial studies with radiolabel sucrose have shown that majority of carbohydrate in aphids and whiteflies are used to perform basic metabolic process (Mittler and Meikle 1991; Rhodes et al. 1996; Wilkinson and Ishikawa 1999; Salvucci and Crafts-Brandner 2000).

Osmolytes compounds can stabilise the structure of proteins in high extreme temperature (Back et al. 1979). In addition, first report studies have shown that aphids and whiteflies are the examples of organisms that accumulates polyols compounds at high temperature conditions (Hendrix and Salvucci 1998 ; Henle et al. 1982; Henle and Warters 1982). Since glycerol is known to maintain cells from hyperthermic cell death, induced thermal protection and warming protection may be applied by adjustment of either protein or membranes (Henle et al. 1982).

Trehalose has an important role in heat stress conditions. The level of trehalose has been correlated with thermotolerance (Hottiger et al. 1994), and it has been indicated can inhibit aggregation of proteins after heat, cold desiccation and oxidation as a stress-responsive which helps the cells in remaining stable condition (Jain and Roy 2009).

Apart from trehalose, one of the important factors shaping the nutritional quality of aphids is amino acids and there is a strong correlation between the amount of amino acids in the diet and performance of aphids (Weibull 1988; Kazemi and van Emden 1992; Karley et al. 2002). Amino acids are important for aphids because they are their predominant energy source (Lilewellyn 1972; Dixon 1973; Van hook et al. 1980; Rhodes et al. 1996).

Triacylglycerol's (TG$_s$) serves as a reservoir for fatty acids that can be used for energy production. Very large amounts of TG can occur in aphids, comprising 20-30% of fresh body weight (Strong 1963; Sutherland 1968). These storage lipids are used as a source of metabolic energy for physiological processes, including flight (Itoyama et al. 2000).

One of the most important molecular chaperones that is synthesized after exposure to a variety of unfavourable conditions such as high and low temperature extreme, high concentration of toxically compounds and depletion of cellular reserves is heat shock proteins (Feder and Hofmann 1999). Proteins from Hsp70 family well studied, they have duty of transportation of denatured proteins to lysosomes to prevent aggregation of them or to help re-folding of proteins in stress conditions (Feder and Hofmann 1999; Neven 2000; Sorensen et al. 2003; Bahrndorff et al. 2009).

1.3 Aphids as a model species

Aphids (Hemiptera: Aphididae), with their capacity for rapid population growth and role as a disease vector, are renowned pests of agriculture, horticulture and forestry, classification of aphids can be based on host-alternation during their life cycle which include as monoecious and heteroecious. In monoecious group, aphids remain on the same host plants during the year such as *Sitobion avenae* while in heteroecious aphids alternate between a summer and winter woody

host species such as *Myzus persicae*. In heteroecious groups we can see aphids have a chance to use of diverse nutrition over species than monoecious lifecycle (Shaposhnikov 1987) (Fig 1.2).
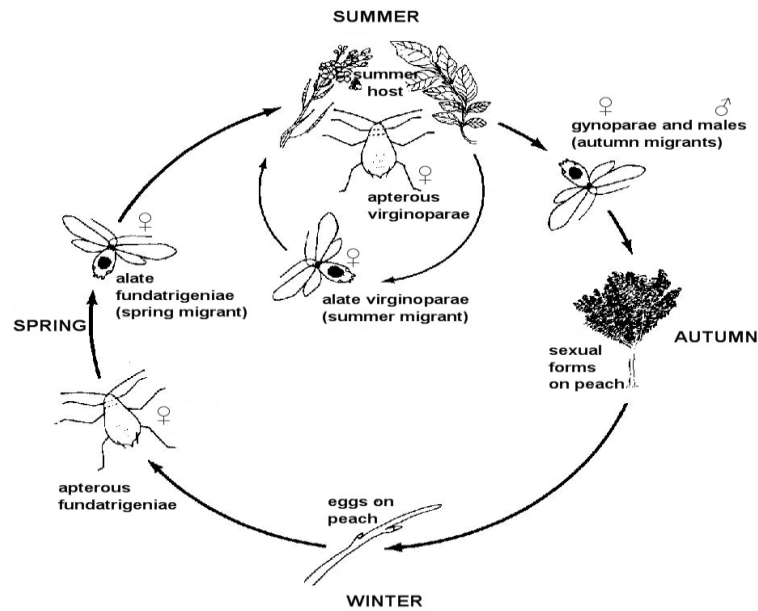

Figure 1.2 the lifecycle of the peach-potato aphid *Myzus persicae* (Blackman and Eastop 2000)

Aphids with high sensitivity, short development time and huge ability to reproduction made them as an indicator in ecosystems and as a model for studying the effects of climate change on pest species (Bezemer et al. 1998; Hulle et al. 2010). Aphids are influenced by direct and indirect factors and also by inter-specific interactions with natural enemies and other herbivores simultaneously when exposed to climate change while the whole outcome of these interactions is often complex (Denno et al. 1995; Thompson and Cunningham 2002; Gomez 2003; Bezemer and van Dam 2005).

Based on studies in green peach aphids lower to higher developmental threshold is from 4.0° to 30.0°C, respectively and optimum survival temperature is at 20°C, they can't produce

offspring above 30°C and higher critical temperatures CT_{max} is 38.5°C (Broadbent and Hollings 1951;Whalon and Smilowitz 1979; Liu and Meng 1999).

Aphids are ubiquitous insects with unique biological features: consequently their abundance and distribution could increase, decrease, or be unaffected by climate change. The density of aphids and *Neophilaenus lineatus* (Hemiptera: Aphrophoridae) in two modelling studies based on insect survey data is tightly linked with temperature and increasing temperature can increase their population (Zhou et al. 1997; Whittaker and Tribe 1998) but in some aphid species based on their thermal requirements and host plant climate change and the increasing temperature could also decrease growth (Bale et al. 2002).

Within Australia, one of the most important threats to pasture and broad acre crops such as oilseeds, cereals and pulses are pest invertebrate species which can cause extensive damage. Climate change is also likely to cause shifts in the geographic distribution and abundance of some pest invertebrate species (Hoffmann et al. 2008). With changes in distribution come changes to phenology and persistence that ultimately lead to pest outbreaks and spread of vector-borne plant pathogens. Obviously, pest species will respond to climate change differently so it is thus important to examine individual species responses through a framework that is broadly applicable and is close to natural condition in the future (Walther et al. 2002).

1.3.1 Biology and Ecology of *Myzus Persicae*

Myzus persicae (Sulzer) (Hemiptera: Aphididae) is a heteroecious aphid that alternates between the primary woody host plant (peach *Prunus persica*) in winter and summer crops that belonging to more than 40 different families: such as agricultural crops : (Blackman and Eastop 1984; Devonshire et al. 1998; Davis et al. 2006; Blackman and Eastop 2000; Jeffs and Leather,

2014). It is also an important pest on Canola in Australia (Davis et al. 2006; Gu et al. 2007) is vector of more than 100 plant viruses (Gu et al. 2007)

Agricultural crops such as canola in Australia is infested by three major aphid pests including cabbage aphid, turnip aphid and the green peach aphid or (*Myzus persicae)* (Gu et al. 2007). However, damage from aphid infestation on plants is strongly dependent to availibility of moisture and perciptation rate in growing season for the agricultiral crops (Lamb 1989). For example, if there is a high chance of precipitation, the host plants have ability to recover from infestation, in contrast in low rainfall areas crops suffer more from damage by aphids in flowering stage (Hertel et al. 2004; Gu et al. 2007).

1.4 Climate change and aphids

1.4.1 Biology

Climatic warming during the past century may have significant effects on the population and reproductive biology of organisms (Archer et al. 2014; Bauerfeind and Fischer 2014; Zhao et al. 2014; Colinet et al. 2015; Ma et al.2015). The effects of temperature change are greatest in above-ground insects whereas on the soil, they experienced buffered environment by denser soil while in above-ground they exposed to the full variability of micro- and macro-climate (Bale et al. 2002). Therefore, these temperature changes may induce more impacts in their voltinism, developmental rate, fecundity, genetic pool and life-cycle characters (Robinet and Roques 2010; Karuppaiah and Sujayanad 2012; Andrew 2013; Dick et al. 2013).

With an elevated in CO_2 and temperatures, aphid's population dynamics are affected. For example in this case, Newman et al (2003) in their paper mentioned that with an elevation in CO_2 and temperatures, aphid's population dynamics are not affected. But Newman (2005) with

his modelling study demonstrated that population of cereal aphids dramatically declined along with climate change by end of this century. In addition, Bezemer et al. (1998) in their paper indicated that with elevated CO_2 and temperatures in the long-term (12-15 generation) increased the abundance of aphids. Main growth section of the aphid lifecycle is in the warmer part of the summer; this optimum temperature for a maximum growth is different between aphid species and is often based on their habitat and climatic range (Bale et al. 2002).

Previous studies assessing the impact of increase temperatures on development and reproductive of aphids have demonstrated that there is negative relationship between temperature and some biological parameters like nymphal period, reproductive period, adult longevity and total longevity in aphids (Satar et al. 2005; Bashir et al. 2012). Chiu et al (2012), who mimicked semi-natural conditions of moderate summer conditions in tropical and sub-tropical regions, demonstrated that with increasing global temperatures we will see a decline in the survival and reproduction of aphids and an increased risk of local extinctions. In addition, Ma et al (2004), demonstrated that high temperature was detrimental for the survival of aphids and that mature aphids had a lower tolerance to high temperatures than nymphs and concluded that their survival would be significantly affected by a warming climate due to an intolerance of increasing temperatures, the duration of exposure and various environmental conditions each life stage was exposed to. Moreover, Mehrparvar and Hatami (2007), it has been demonstrated that the effects of temperature on some biological traits of the rose aphid had an impact on their development at cooler conditions than the Australian population of the same species. This demonstrates that adaptation to local condition is important for different populations to survive a changing climate. For aphids tested on Blue Alfalfa at 15 temperatures, photoperiod regimes indicated that temperature had the most critical role in development and

reproduction of the aphids and there is a significant interaction between temperature/photoperiod on biological traits particularly at low temperatures (Kodet et al. 1982).

1.4.2 Physiology of aphids

Aphid thermal tolerance measurements is very useful index in prediction impacts of climate change but non-lethal thermal threshold is more important than lethal temperatures because is more informative and provide more ecologically relevant information (Macdonald et al. 2004; Hazell et al. 2010a). The study of insect thermal tolerance thresholds is important for several of reasons. Firstly it provides insights into the conditions that have been limiting life of individuals over the course of its evolution and the functional basis for such limits (Terblanche et al. 2011). Secondly, studies of thermal threshold have also long been of interest to ecologists (Broadbent and Hollings 1951; Bowler 1963; Dixon 1973; Hennrson and Banrow 1973; Powell 1974; Powell and Parry. 1976; Strathdee et al. 1995), due to the close original link between ecology and physiology (Huey 1991). A mechanistic understanding of the distribution and abundance of insects requires information about their physiological abilities and the mechanisms underlying those (Gaston et al. 2009).

Knowing the upper and lower temperature thresholds also helps to anticipate the geographical range over which a particular species may spread and become established (Hazell et al. 2010b).

Based on literature review higher thermal tolerance is less flexible than lower thermal tolerance (such as data collected for *M. persicae*, *M. ornatus*, and *M. polaris*) and while most studies in biology and physiology of aphids looked at the impact of increased temperature in

winter in reality increase in frequency of summer heat waves is anticipated to rise in the future (Meehl and Tebaldi 2004; Smith et al. 2009) but consequences of these exposure to increasingly severe and repeated periods of abiotic stress are largely unknown (Dooremalen et al. 2013). Therefore, to develops complete and comprehensive picture of the impacts climate change on aphids we need to considerate effects of increasing summer temperature as well (Addo-Bediako et al. 2000; Hazell et al. 2010a). Therefore, aphid populations might be limited by both extreme high and low temperatures. However, few studies have considered the potential effects of extreme summer temperatures (Ma et al 2015; Jeff et al 2014). Most studies of the thermal tolerance of aphids have assessed the impact of low extreme temperatures (see Table 1.1).

Table 1.1 a summary of thermal tolerance temperature studies of aphid's species. Only studies that measured physiological/biochemical variables are included, while studies that investigated only developmental rates or fecundity are excluded. Abbreviations of variables measured: (CT_{min}: Critical temperature minimum); (CT_{max}: critical temperature maximum); (SCP: supercooling point); (ULT: upper lethal temperature); (LLT: Lower lethal temperature); (S: survival); (RCH: Rapid cold hardening); (Constant: constant temperature); (Plunge: plunge protocol); (Cycling: fluctuation temperature protocol);

Species	protocol	Temperature of exposure	Duration of exposure	metrics	results	references
Lethal T *Myzus persicae*	constant	5/10/20℃	generation	SCP/LLT	SCP -26,-25,-26 5/10/20℃ LLT -17,-17,-7 5/10/20℃	Bale, 1988
Myzus persicae	Plunge	5℃	7Day	SCP	Nymph SCP not change -26℃ Adult from -25 to -15℃	Doherthy,1985
Myzus persicae	Constant	5/10/20℃	III generation	LLT	LLT(birth sequence nymph 1: -15 to -8℃	Clough, 1990
Myzus persicae	constant	10/20/25℃	I-III generation	LLT/ULT	ULT: no change in any condition LLT: (10°CItoIII: -14 t0-16℃) LLT: (25ItoIII: -11to -11℃)	Alford, 2012
Sitobin avenae *Metopolophium dirhodum* *R.padi*	plunge	5/10/28℃	One week	LLT	Positive relationship between LLT50&winter/aphids feeding LLT50 close to their host plants	Griffiths, 1979
Sitobion avenae	constant	10/20℃	6 month	LLT	Adult: 20°C(-9)/10℃(-12) Nymph: 20°C(-8)/10℃(-16) LLT: (10CItoIII: -14 t0-16℃)	Powell,2008
Sitobion avenae	plunge	0/5℃	2,7,14,21,28 Day	SCP	(0℃) -23………died acclimation (5℃) -24……….-24 2…………28D (0℃)-24………….-22 acclimation + starvation (5℃)-23………….died	Knight, 1986
Pemphigus bursarius	10/20℃ Winter &summer form	0℃	0-21Day	SCP/LLT	Summer: SCP-21.1c / LLT: 2.3c winter: -21.69c / LLT: -13.1c	Phillip, 2000
Rhopalosiphum padi *Myzus ascalonicus* *Macrosiphum euphorbiae*	plunge	0/5/10/15℃	10Day	SCP/LLT	LLT50<PFE<SCP	Worland, 2010
Rhopalosiphum .padi *Acyrthosiphon brevicorne* *Acyrthosiphon svalbardicum*	plunge	0/10/-10/-30℃	Week/month	SCP	Around -38to-39℃ in arctic eggs	Strathdee, 1995

Species	protocol	Temperature of exposure	Duration of exposure	metrics	results	references
Sub lethal *Myzus persicae* *Myzus polaris* *Myzus ornatus*	constant	10/15/20/25°C	I-IIIgeneration	CTmin	1….. ……7 tropical 10/15/20/25°C -1………....1 Arctic & temperate	Hazell,2010a
Myzus persicae *Myzus polaris* *Myzus ornatus*	Constant	10/15/20/25°C	I-IIIgeneration	CTmax	10/15/20/25°C 38.5………40.5 in all groups	Hazell,2010b
Myzus persicae	constant	10/20/25°C	I-IIIgeneration	CTmin CTmax	10I (8.8 to 2.5°C) 25I (40.1to 41.1°C)	Alford,2012
Survival *Pemphigus bursarius*	10/20°C Winter &summer form	0°C	0-21Day	S	0 -21 Day (0°C) 85% -0% winter from 0-500min (0Day) 85%-0% summer form	Phillip,2000
Rhopalosiphum .padi *Acyrthosiphon brevicorne* *Acyrthosiphon svalbardicum*	plunge	0/10/-10/-30°C	Week,month	S	*R.padi / A.brevicorne / A. svalbardicum* 0°C 99 78 78 -10°C 93 62 80 -30°C 35 31 80	Strathdee,1995
Myzus persicae	Cycling	5/3/-5°C	1-10 Day	S	Adult Nymph 10°C(-5) 100-0% 100-0% 10(5) 100-90% 100-90% 20°C(-5) 100-0% 100-0% 20(5) 95-100% 90-95%	Howling,1994

Species	protocol	Temperature of exposure	Duration of exposure	metrics	Results					References
Sitobion avenae	**10(3g)**	10to0°C	(1/0.1/0.05rate)	S	A	10%	25% 20%		20%	Powell, 2005
					1	0.1	0.05	rate change	direct plunge	
					N 70%	80%	80%		25%	
Survival	**Cycling**	5c between-5°C(cycling)	18h,6h	S		Adult	Nymph			Howling, 1994
Myzus persicae	**10,20(3g)**				10C(-5)	100-0%	100-0%			
					10(5)	100-90%	100-90%			
					20C(-5)	100-0%	100-0%			
					20(5)	95-100%	90-95%			

Species	protocol	Temperature of exposure	Duration of exposure	metrics	Results		References
Diuraphis noxia	**Seasonality**			SCP	-26…………..27°C	Field	Armstrong, 2000
					May………………Sep	Lab	
					-26…………..-27°C		

Species	protocol	Temperature of exposure	Duration of exposure	metrics	results	references
Lethal *Acyrthosiphon brevicorne*	**Plunge 15(g)**	**-9to-10°C**	**3h**	**LLT**	Viviparae (-9.5°C)/ Males(-10.5°C) Lab(Nymph): -9°C Field(Nymph): -10.7°C	**Bale, 1994**
Acyrthosiphon pisum *Aulacorthum solani* *Megoura Crassicauda*	**Plunge**	**-10°C**	**Several time**	**SCP**	-24°C	**Asai, 2002**
Brevicoryne brassicae	**Plunge**	**-5/-10/-13/-15°C**	**24h**	**SCP** **LLT**	A -20…………-23………………-19°C N -25…………-26………………-24°C Oct……………Jan…………………..May A -6……………-12………………-7°C N -7……………-12…………………-7°C	**Saeidi, 2012**
Soybean aphids	**Plunge**	**3-5h**	**3h**	**SCP**	Eggs : -34°C First instar: -26°C Adult: -25°C	**MacCornack, 2005**
Sublethal *Sitobion avenae*	**Plunge 10, 20g**	**0°C**	**3h(20g)/30min(10g)**	**CT$_{min}$**	-0.9°C as same as in both groups	**Powell,2006**
Survival *Brevicoryne brassicae*	**Plunge**	**-5/-10/-13/-15°C**	**24h**	**S**	-5 -10 -13 -15°C Oct 70% 20% 2% 0% Jan 100% 100% 30% 0% May 70% 40% 0% 0%	**Saeidi, 2012**
Sitobion avenae	**Plunge**	**0Cto -8°C**	**1-5h**	**S**	1h 2h 3h 4h 5 h (0°C) direct plunge 60% 70% 70% 60% 50% 20%	**Powell, 2004**

17

Species	protocol	Temperature of exposure	Duration of exposure	metrics	results	references
Myzus persicae	**Plunge**	**-9°C**	**14h**	**S**	Obligate = Cyclical parthenogenesis	**Vorburger, 2004**
Sitobion avenae	**Plunge 10(g)**	**0°C**	**10min-8h**	**S**	A 55 / 70 /35/30/45/25/0/0 21% -11.5°C(direct) 10min /0.5h /1 / 2 /3 /4 /5 /6 /8 (0°C) N 30…………100……….60% 25%	**Powell, 2005**
Rhopalosiphum .padi *Acyrthosiphon brevicorne* *Acyrthosiphon svalbardicum*	**Plunge**	**Minimum T**	**1min**	**S**	(1min) -38°C…….5% mortality (Control) -38°C……30% mortality	**Strathdee, 1995**
Pemphigus bursarius	**Plunge**	**5/0/-5°C(summer)** **0/-5/-10/-15/-20C(winter)** **0°C**	**1min** **0-500min**	**S**	Winter 90%..............23% 5 /0 /-5 / 10/ -15 /-20°C (1min) Summer 70%..........0%	**Phillips, 2000**
Sub-lethal **Sitobion avenae**	**Ramping** **10, 20°C(6m)**	**10to-10°C**	**(1/0.1/0.5rate)**	CT_{min}	20°C 1.5 1.5 -1.3 1 0.5 0.1 rate change 10°C 0.8 0.8 0.5	**Powell, 2006**
Survival **Sitobion avenae**	**20(3g)**	**10to0°C**	**(1/0.1/0.05rate)**	**S**	A 10% 50% 60% 10-20% 1 0.1 0.05 rate change direct plunge N 60% 65% 70% 10-20%	**Powell, 2004**

The entire animal oxygen consumed is the first process restricted in animals at low and high temperatures, and is connected to the limitation of blood circulation and ventilation (Pörtner 2001). There are few studies that have measured respiration or metabolic rate in the aphids (but see Lamb 1961) who demonstrated that, at constant temperatures, the metabolic rate of the cabbage aphid (*Brevicorynie brassicae* (L.) increased exponentially from 8 to 30°C then declined rapidly. Similar studies have only measured aphid respiration in aphids without considering temperature (Castañeda et al. 2009; SlamaL and Jedlicka 2012).

1.4.3 Important physiological and artificial diet studies

In physiological studies to reach comprehensive view, it is necessary to consider specific performance traits that should be tailored to the ecology of the species being studied (Huey and Stevenson 1979) these traits that have been assessed include development time, fecundity, net energy reserves and their thermal tolerance (Huey and Stevenson 1979; Arnold 1983; Angilletta Jr et al. 2002; Kingsolver et al. 2004; Huey et al. 2012).

Artificial liquid diets have been devised to mimic plant phloem and have been used successfully to rear aphids over multiple generations. Rearing aphids on artificial diets has many benefits: it can be used to standardise food resources for sap-sucking insects whereas plant phloem concentrations can vary among leaves and branches of individual plants, as well as among different environmental conditions (such as temperature and moisture

variation). In artificial diets concentrations of component products will also remain constant regardless of the environmental conditions (Febvay et al. 1999; Wille and Hartman 2008; Clissold et al. 2013; Bouvaine et al, 2012). In addition, in the studies with artificial diets researchers can to rear insects in a medium with constant chemically compositions regardless of changing in environmental conditions and also, this type of studies need less space than plant based research (Wille and Hartman 2008).

At extreme high and low temperatures, insects rely on different strategies and combination of molecular compounds such as polyols, proteins, cryoprotectants and energy reserves to recover from those unfavourable conditions (Back et al. 1979; Wang et al. 2006; Clark and Worland 2008; Tollarová-Borovanská et al. 2009; Teets et al. 2011). Moreover, insects with a reduction in their metabolic rate can be saving energy and water resources as well (Schimpf et al. 2009; Chown et al. 2011; Lalouette et al. 2011; Yocum et al. 2011).

As aphids are known to respond differently to constant versus 'natural' fluctuating temperature regimes, conclusions drawn from constant temperature data may be problematic (Colinet et al. 2015). We suggest future experiments assessing insect responses to thermal stress incorporate a repeated stress and recovery pattern into their methodologies to predictive models and climate change studies.

Also, despite previous work on the cold tolerance of *Myzus persicae* (O'doherty and Bale 1985; Bale et al. 1988; Clough et al. 1990; Howling et al. 1994; Liu and Meng 1999; Vorburger 2004; Alford et al. 2012a, b) and other effects of temperature on this species (Lamb 1961; Stanley and Fenton 2000; Davis et al. 2006; Hazell et al. 2010b; Silva et al. 2012; Jiang et al. 2013; Bouvaine et al. 2014; Jeffs and leather , 2014), there is generally a lack of quantitative data on how their physiology are affected by fluctuating high temperatures.

1.5 Thesis outline

This thesis is based on three research chapters. These chapters are written in manuscript format so there may be overlapping information, particularly in the methods to make each chapter stand-alone in preparation for publication in scientific journals.

The specific questions of each chapter outlined below:

Chapter 2. How does metabolic changes in adult *Myzus persicae* exposed to fluctuating thermal regimes?

The study of environmental stress tolerance in aphids has primarily been at low temperatures. In these cases, and in the rare cases of high temperature tolerance assessments, all exposures had been during a single stress event. In this chapter, I examined the physiological consequences of repeated high temperature exposure with recovery periods between these stress events in *Myzus persicae*. I subjected individuals to either a single prolonged three hour heating event, or three one hour heating events with a recovery time of 24 hours between pulses. Aphids exposed to repeated pulses of high temperatures had more glucose and higher expression of proteins and osmolyte compounds, such as glycerol, compared to the prolonged exposure group. However, aphids exposed to the repeated high temperature treatment had reduced sources of energy such as trehalose and triglyceride compounds than the prolonged exposure group. Recovery time had more costs (based on production of more protein and consumption of more trehalose and triglyceride) and benefits (based on production of more osmolytes) in repeated high temperature treatments. As aphids are known to respond differently to constant versus 'natural' fluctuating temperature regimes, conclusions drawn from constant temperature data may

be problematic. I suggest future experiments assessing insect responses to thermal stress incorporate a repeated stress and recovery pattern into their methodologies.

Chapter 3. How does amino acids and sucrose composition affect tolerance to heat stress and metabolite profile exposed to fluctuating thermal regimes in adult *Myzus persicae*?

Insect nutrition may affect a range of individual life history traits as well as responses to environmental stresses. In this chapter I tested the influence of three different nutritional regimes on green peach aphid (*Myzus persicae*) resistance to heat stress, metabolite production and respiration. Individuals of the 3rd generation lab-reared from birth were used. The three nutritional regimes were: AA150Su1000 (Amino Acid 150 + Sucrose 1000), AA150Su250 (Amino Acid 150 + Sucrose 250) and AA50Su1000 (Amino Acid 50 + Sucrose 1000). Aphids reared on the high amino acid medium had increased heat tolerance and respiration rates compared to aphids reared on the low amino acid medium. Aphids reared on the diet with high amino acid and sucrose concentration had more glucose and higher expression of proteins and osmolyte compounds, such as glycerol, compared to those reared on a lower amino acid and sucrose diet. However, aphids reared on lower amino acid diets had reduced sources of energy such as trehalose and triglyceride compounds compared to high amino acid diets. My study indicates that aphid nutrition has a strong impact on the ability of aphids to resistance high temperatures and plays a critical role in environmental stress responses.

Chapter 4. How do metabolite responses differ during different recovery times from heat injury in the green peach aphid (*Myzus persicae*)?

In pulsed heat exposures, the duration, and frequency of pulses, as well as the recovery time between pulses can change through time. I examined the changing in key important metabolites in different physiological recovery time from the effects of exposure to sub-lethal temperature in adult green peach aphids (*Myzus persicae*) that had one of three recovery regimes: LONG, SHORT and MIXED. Following a stress pulse metabolites were assessed at different time throughout the heating and recovery stage. Among recovery pulses, there were not significant differences in total amount of trehalose post pulses among treatments. However, in the final cycles of treatments there were significant differences after each heating time in most of the metabolites, with negative effects of heating appearing after long recovery time treatment. I also found that trehalose and triglyceride are the most important sources of energy compared to glycogen in adult aphids when exposed to high temperatures. My findings indicate that adult aphids on the short time recovery treatment produce more polyol (glycerol) and protein than other treatments and we founded that, in this species they need more than 11h for recovery for their metabolites.

References

Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2000. Thermal tolerance, climatic variability and latitude. _Proceedings of The Royal Society of London_ B **267**:739-745.

Alford, L., T. M. Blackburn, and J. S. Bale. 2012a. Effect of latitude and acclimation on the lethal temperatures of the peach-potato aphid _Myzus persicae_. _Agricultural and Forest Entomology_ **14**:69-79.

Alford, L., T. M. Blackburn, and J. S. Bale. 2012b. Effects of acclimation and latitude on the activity thresholds of the aphid _Myzus persica_e in Europe. _Journal of Applied Entomology_ **136**:332-346.

Amarasekare, P. and V. Savage. 2012. A framework for elucidating the temperature dependence of fitness. _American Naturalist_ **179**:179-191.

Andrew, N. R. 2013. Population dynamics of insects: impacts of a changing climate. In: The Balance of Nature and Human Impact. (ed Rohde K). Cambridge University Press. pp311-323.

Andrew, N. R. and S. J. Hill. 2017. Effect of climate change on insect pest management. Pest Management within the Environment: Challenges for Agronomists, Ecologists, Economists and Policymakers (eds E. Wajnberg & M. Coll). Wiley.In press.

Angilletta Jr, M. J., T. Hill, and M. A. Robson. 2002. Is physiological performance optimized by thermoregulatory behavior? A case study of the eastern fence lizard, _Sceloporus undulatus_. _Journal of Thermal Biology_ **27**:199-204.

Angilletta, M. J. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford: Oxford University Press.

Archer, C. R., C. W. Pirk, G. A. Wright, and S. W. Nicolson. 2014. Nutrition affects survival in African honeybees exposed to interacting stressors. *Functional Ecology* **28**:913-923.

Arguelles, J. C. 2000. Physiological roles of trehalose in bacteria and yeasts: a comparative analysis. *Archives of Microbiology* **174**:217-224.

Armstrong, J. S. and D. C. Nielson. 2000. Supercooling points for Russian wheat aphid and winter wheat tissue as indicators of clod acclimation. *The southwestern entomologist* **25**:265-272.

Arnold, S. J. 1983 Morphology, performance and fitness. *American Zoologist* **23**:347-361.

Asai, M.,H. Yoshida,K. Honda.and H. Tsumuki. 2002. Cold hardiness of three aphid species, *Acyrthosiphon pisum*, *Megoura crassicauda* and *Aulacorthum solani* (Hemiptera: Aphididae). *Applied Entomology and Zoology* **37**:341-346.

Ashford, D. A., W. A. Smith, and A. E. Douglas. 2000. Living on a high sugar diet: the fate of sucrose ingested by a phloem-feeding insect, the pea aphid *Acyrthosiphon pisum*. *Journal of Insect Physiology* **46**:335–341.

Ashton, S., D. Gutierrez, and R. J. Wilson. 2009. Effects of temperature and elevation on habitat use by a rare mountain butterfly: implications for species responses to climate change. *Ecological Entomology* **34**:437-446.

Ayres, M. P. and M. J. Lombardero. 2000. Assessing the consequences of global change for forest disturbance from herbivores and pathogens. *Science of the Total Environment* **262**:263-286.

Back, J. F., D. Oakenfull, and M. B. Smith. 1979. Increased thermal stability of proteins in the presence of sugars and polyols. *Biochemistry* **18**:5191-5196.

Bahrndorff, S., J. Mariën, V. Loeschcke, and J. Ellers. 2009. Dynamics of heat-induced thermal stress resistance and Hsp 70 expression in the springtail, *Orchesella cincta*. *Functional Ecology* **23**:233-239.

Bale, J. S., R. Harrington, and M. S. Clough. 1988. Low temperature mortality of the peach-potato aphid *Myzus persicae*. *Ecological Entomology* **13**:121-129.

Bale, J. S., A. T. Strathdee. and F. C. Strathdee. 1994. Effects of low temperature on the Arctic aphid *Acyrthosiphon brevicorne*. *Functional Ecology* **8**:621-626.

Bale, J. S., G. J. Masters, I. D. Hodkinson, C. Awmack, T. M. Bezmer, V. Brown, J. Butterfield, A. Buse, J. C. Coulson, J. Farrar, J. G. Good, R. Harrington, S. Ehartley, T. Hefinjones, R. Lindroth, M. Press, I. Rnioudisni, A. Watt, and J. Whittaker. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology* **8**:1_16.

Bashir, F., M. NAzim, and N. Akhter. 2012. Effect of temperature on the biology of *brevicoryne brassicae* L. (Homoptera: Aphididae) on Kale (*brassica oleracea* var.Acephala) under field conditions. *International Journal of Innovations in Bio-Sciences* **2** 44-47.

Bauerfeind, S. S. and K. Fischer. 2014. Simulating climate change: Temperature extremes but not means diminish performance in a widespread butterfly. *Population Ecology* **56**:239-250.

Bezemer, T. M., T. H. Jones, and K. J. Knight. 1998. Long-term effects of elevated CO_2 and temperature on populations of the peach potato aphid *Myzus persicae* and its parasitoid *Aphidius matricariae*. *Oecologia* **116**:128 -135.

Bezemer, T. M. and N. M. van Dam. 2005. Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology and Evolution* **20**:617-624.

Blackman, R. L. and V. F. Eastop. 2000. Aphids on the world's crops: An identification and information guide. John Wiley & Sons Ltd., Chichester.

Bouvaine, S., M. L. Faure, R. J. Gerbenok, S. T. Behmer, and A. E. Douglas. 2014. A dietary test of putative deleterious sterols for the aphid *Myzus Persicae*. _PLoS ONE_ **9**:e86256.

Bowler, K. 1963. A study of the factors involved in acclimatization to temperature and death at high temperatures in Astacus pallipes. II Experiments at the tissue level. _Journal of Cellular and Comperative Physiology_ **62**:133-146.

Bozinovic, F., T. P. Catalan, S. A. Estay, and P. Sabat. 2013. Acclimation to daily thermal variability drives the metabolic performance curve. _Evolutionary Ecology Research_ **15**:579-587.

Braganza, K. and J. A. Church. 2011. Observations of global and Australian climate. In: Climate Change: Science and Solutions for Australia (Eds Cleugh, H., Smith, M. S., Battaglia, M. & Graham, P.).Australia. CSIRO Publishing.

Broadbent, L. and M. Hollings. 1951. The influence of heat on some aphids. _Annals of Applied Biology_ **38**.

Castañeda, L. E., C. C. Figueroa, E. Fuentes-Contreras, H. M. Niemeyer, and R. F. Nespolo. 2009. Energetic costs of detoxification systems in herbivores feeding on chemically defended host plants: a correlational study in the grain aphid, *Sitobion avenae*. _The Journal of Experimental Biology_ **212**:1185-1190.

Chiu, M. C., Y. H. Chen, and M. H. Kuo. 2012. The effect of experimental warming on a low-latitude aphid, *Myzus varians*. _Entomologia Experimentalis et Applicata_ **142**:216-222.

Chown, S. L., J. G. Sorensen, and J. S. Terblanche. 2011. Water loss in insects: an environmental change perspective. _Journal of Insect Physiology_ **57**:1070-1084.

Clark, M. S. and M. R. Worland. 2008. How insects survive the cold: molecular mechanisms-a review. *Journal of Comperative Physiology* B **178**:917-933.

Clissold, F. J., N. Coggan. and S. J. Simpson .2013. Insect herbivores can choose microclimates to achieve nutritional homeostasis. *Journal of Experimental Biology.* **216**:2089-2096.

Clough, M. S., J. S. Bale, and R. Harrington. 1990. Differential cold hardiness in adults and nymphs of the peach-potato aphid *Myzus persicae*. *Annals of Applied Biology* **116**:1-9.

Colinet, H., B. J. Sinclair, P. Vernon, and D. Renault. 2015. Insects in Fluctuating Thermal Environments. *Annual Review of Entomology* **60**:7.1-7.18.

Cornelissen, T. 2011. Climate change and its fffects on terrestrial insects and herbivory patterns. *Neotropical Entomology* **40**:155-163.

CSIRO-ABM. 2014. State of the Climate 2014. CSIRO and the Australian Bureau of Meteorology, Canberra.

Davis, J. A., E. B. Radcliffe, and D. W. Ragsdale. 2006. Effects of High and Fluctuating Temperatures on *Myzus persicae* (Hemiptera: Aphididae). *Environmental Entomology* **35**:1461-1468.

Denno, F. R., M. S. McClure, and J. R. Ott. 1995. Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annual Review of Entomology* **40**:297±331.

Deutsch, C. A., J. J. Tewksbury, H. B., K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedingd of the National Academy of Science* **105**:6668-6672.

Dick, C. A., N. E. Rank, M. McCarthy, S. McWeeney, D. Hollis, and E. P. Dahlhoff. 2013. Effects of temperature variation on male behavior and mating success in a montane beetle. *Physiological and Biochemical Zoology* **86**:432-440.

Dixon, A. F. G. 1973. Metabolic acclimatization to seasonal changes in temperature in the sycamore aphid, *Drepanosiphum platanoides* (Schr.) and the lime aphid, *Eucallipterus tiliae L*. *Oecologia* **13**:205-210.

Dooremalen, C. V., M. P. Berg, and J. Ellers. 2013. Acclimation responses to temperature vary with vertical stratification: implications for vulnerability of soildwelling species to extreme temperature events. *Global Change Biology* **19**:975-984.

Ehrhardt, P. 1962. Untersuchungen zur Stoffwechselphysiologie von *Megoura viciae* Buckt., einer phloemsaugenden Aphide. *Zeitschrift Vergleiche Physiologie* **46**:169-211.

Febvay, G., I. Liadouze, J. Guillaud, and G. Bonnot. 1995. Analysis of energetic amino acid metabolism in *Acyrthosiphon pisum*: a multidimensional approach to amino acid metabolism in aphids. *Archives of Insect Biochemistry and Physiology* **29**:45-69.

Feder, M. E. and G. E. Hofmann. 1999. Heat shock proteins, molecular chaperones, an dthe stress response: evolutionary and ecologicaly physiology. *Annual Review of Plant Biology* **61**:243-282.

Fisher, D. B. and R. M. Gifford. 1986. Accumulation and conversion of sugars by developing wheat grains. VI. Gradients along the transport pathway from the peduncle to the endosperm cavity during grain filling. *Plant Physiology* **82**:1024-1030.

Frazier, M. R., R. B. Huey, and D. Berrigan. 2006. Thermodynamics constrains the evolution of insect population growth rates: ''Warmer is better. *The American Naturalist* **168**:512-520.

Gaston, K. J., S. L. Chown, P. Calosi, J. Bernardo, D. T. Bilton, A. Clarke, S. Clusella-Trullas, C. K. Ghalambor, M. Konarzewski, and L. S. e. a. Peck. 2009. Macrophysiology: a conceptual reunification. *The American Naturalist* **174**:595-612.

Gergis, J., Neukom, R., Phipps, S. J., Gallant, A. J. E., Karoly, D. J. & PAGES and A. K. P. Members. 2012. Evidence of unusual late 20th century warming from an Australasian temperature reconstruction spanning the lastmillennium. *Journal of Climate.*

Gilchrist, G. W. 1995. Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. *The American Naturalist* **146**:252-270.

Gillespie, D. R., A. Nasreen, C. E. Moffat, P. Clarke, and B. D. Roitber. 2012. Effects of simulated heat waves on an experimental community of pepper plants, green peach aphids and two parasitoid species. *Oikos* **121**:149-159.

Gomez, J. M. 2003. Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. *American Naturalist* **162**:242-256.

Griffiths, E. and S. D. Wratten. 1979. Intra and inter- specific differences in cereal aphid low-temperature tolerance. *Entomologia Experimentalis et Applicata* **.26**:161--167.

Gu, H., G. P. Fitt, and G. H. Baker. 2007. invertebrate pests of canola and their managemant in Australia: a review. *Australian Journal of Ento*mology **46**:231-243.

Hazell, S. P., C. Groutides, B. P. Neve, T. M. Blackburn, and J. S. Bale. 2010a. A comparison of low temperature tolerance traits between closely related aphids from the tropics, temperate zone, and Arctic. *Journal of Insect Physiology* **56**:115-122.

Hazell, S. P., B. P. Neve, C. Groutides, A. E. Douglas, T. M. Blackburn, and J. S. Bale. 2010b. Hyperthermic aphids: insights into behaviour and mortality. *Journal of Insect Physiology* **56**:123-131.

Hendrix, D. L. and M. E. Salvucci. 1998. Polyol metabolism in Homopterans at high temperature: accumulation of mannitol in aphids (Aphididae: Homoptera) and sorbitol in whiteflies (Aleyrodidae: Homoptera). *Comparative Biochemistry and Physiology and zoology* **120**:487-494.

Henle, K. J., W. A. Nagle, A. J. Moss Jr, and T. S. Herman. 1982. Polyhydroxy compounds and thermotolerance: a proposed concatenation. *Radiation Research* **92**:445-451.

HennrsoN, J. R. and C. A. Banrow. 1973. Survival of the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididaed), at extreme temperatures. *The Canadian Entomologist*. **105**:1513-1518.

Hertel, K., M. Schwinghamer, and R. Bambach. 2004. Virus Diseases in Canola and Mustard. Agnote DPI 495, NSW DPI-NSW Agriculture, Dubbo, Australia.

Hoffmann, A. A., A. Weeks, M. Nash, P. Mangano, and P. A. Umina. 2008. The changing status of invertebrate pests and the future of pest management in the Australian grains industry. *Australian Journal of Experimental Agriculture* **48**:1481-1493.

Hottiger, T., C. de Virgilio, M. N. Hall, T. Boller, and A. Wiemken. 1994. The role of trehalose synthesis for the acquisition of thermotolerance in yeast. II. Physiological concentrations of trehalose increase the thermal stability of proteins in vitro. *Eurupean Journal of Biochemistry* **219**:187-193.

Howling, G. G., J. S. Bale, and R. Harrington. 1994. Effects of extended and repeated exposures to low temperature on mortality of the peach-potato aphid *Myzus persicae*. *Ecological Entomology* **19**:361 -366.

Huey, R. B. 1991. Physiological consequences of habitat selection. Am. Nat **137**:S91- S115.

Huey, R. B., M. R. Kearney, A. Krockenberger, J. A. Holtum, M. Jess, and S. E. Williams. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological* **367**:1665-1679.

Huey, R. B. and J. G. Kingsolver. 1993. Evolution of resistance to high temperature in ectotherms. *American Naturalist* **142**:S21-S46.

Huey, R. B. and M. Slatkin. 1976. Costs and benefits of lizard thermoregulation. *Quarterly Review of Biology* **51**:363-384.

Huey, R. B. and R. D. Stevenson. 1979. Integrating thermal physiology and ecology of ectotherms: Discussion of approaches. *American Zoologist* **19**:357-366.

Hulle, M., A. C. Acier, S. Bankhead-Dronne, and R. Harrington. 2010. Aphids in the face of global changes. *Comptes Rendus Biologies* **333** 497-503.

Hutchinson, L. A. and J. S. Bale. 1994. Effects of sublethal cold stress on the aphid *Rhopalosiphum-Padi*. *Journal of Applied Ecology* **31**:102-108.

IPCC. 2007. Climate Change 2007: The Physical Science Basis: Summary for Policy Makers. Cambridge Univ. Press, Cambridge, UK.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the

Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Itoyama, K., S. Tojo, T. Yanagita, and J. Hardie. 2000. Lipid composition in long-day and short-day forms of the black bean aphid, *Aphis fabae*. *Journal of Insect Physiology* ***46:119-125.***

Jain, N. K. and I. Roy. 2009. Effect of trehalose on protein structure. *Protein Science* **18**:24-36.

Janzen, D. H. 1967. Why mountain passes are higher in tropics. *American Naturalist* **101**:233–249.

Jeffs, C. T, and S. R. Leather .2014. Effects of extreme, fluctuating temperature events on life history traits of the grain aphid,*Sitobion avenae*. *Entomologia Experimentalis et Applicata*. **150**:240-249.

Jiang, Z., D. H. Jones, S. Khuri, N. F. Tsinoremas, T. Wyss, G. Jander, and A. C. C. Wilson. 2013. Comparative analysis of genome sequences from four strains of the *Buchnera aphidicola* Mp endosymbiont of the green peach aphid, *Myzus persicae*. *BMC Genomics* **14**:917.

Ju, R. T., L. Gao, X. H. Zhou, and B. Li. 2013. Tolerance to high temperature extremes in an invasive Lace Bug, *Corythucha ciliata* (Hemiptera: Tingidae), in subtropical China. *PLoS ONE* **8**:e54372.

Karley, A. J., A. E. Douglas, and W. E. Parker. 2002. Amino acid composition and nutritional quality of potato leaf phloem sap for aphids. *The Journal of Experimental Biology* **205**:3009–3018.

Karoly, D. and M. Black. 2015. It's been Australia's hottest ever October, and that's no coincidence, The Conversation, 29 October, Accessed at https://theconversation.com/ its-been-australias-hottest-ever-october-and-thats-nocoincidence-49941.

Karuppaiah, V. and G. K. Sujayanad. 2012. Impact of Climate Change on Population Dynamics of Insect Pests. *World Journal of Agricultural Sciences* **8**:240-246.

Kazemi, M. H. and H. F. van Emden. 1992. Partial antibiosis to *Rhopalosiphum padi* in wheat and some phytochemical correlations. *Annals of Applied Biology* **121**:1-9.

Kempf, B. and E. Bremer. 1998. Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Archives of Microbiology* **170**:319-330.

Kingsolver, J. G., G. J. Ragland, and J. G. Shlichta. 2004. Quantitative genetics of continuous reaction norms: thermal sensitivity of caterpillar growth rates. *Evolution* **65**: 65-72. **58**:1521-1529.

Knight, J. D. and J. S. Bale. 1986. Cold hardiness and overwintering of the grain aphid *Sitobion avenue*. *Ecological Entomology* **11**:189-197.

Kodet, R. T., M. W. Nielson, and R. O. Kuehl. 1982. Effect of temperature and photoperiod on the biology of blue alfalfa aphid, *Acyrthosiphon kondoi Shinji*. *Technical Bulletin* Number 1660, :10p.

Lalouette, L., C. M. Williams, F. Hervant, B. J. Sinclair, and D. Renault. 2011. Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations. *Comparative Biochemistry and Physiology A* **158**:229-234.

Lamb, K. P. 1961. Some effects of fluctuating temperatures on metabolism, development, and rate of population growth in the Cabbage Aphid, *Brevicoryne Brassica*. *Ecology* **42**:740-745.

Lamb, R. J. 1989. Entomology of oilseed Brassica crops. *Annual Review of Entomology* **34**:211-229.

Lilewellyn, M. 1972. The effects of the lime aphid, *Eucallipterus tiliae L*. (Aphididae) on the growth of the lime Tilia 3 vulgaris Hayne. I. Energy requirements of the aphid population. *Journal of Applied Ecology* **9**:261-282.

Liu , S. S. and X. D. Meng. 1999. Modelling development time of *Myzus persicae* (Hemiptera: Aphididae) at constant and natural temperatures. *Bulletin of Entomological Research* **89**:53–63.

Liu, S. S. and X. D. Meng. 1999. Modelling development time of *Myzus persicae* (Hemiptera: Aphididae) at constant and natural temperatures. *Bulletin of Entomological Research* **89**:53-63.

Ma, C. S., B. Hau , and H. M. Poehling. 2004. The effect of heat stress on the survival of the rose grain aphid, *Metopolophium dirhodum* (Hemiptera: Aphididae). *European Journal of Entomology*. **101**:327-331.

Ma, G., A. A. Hoffmann,and C. S. Ma. 2015. Daily temperature extremes play an important role in predicting thermal effects. *Journal of Experimental Biology* **218**:2289-2296.

Macdonald, S. S., L. Rako, P. Batterham, and A. A. Hoffmann. 2004. Dissecting chill coma recovery as a measure of cold resistance: evidence for a biphasic response in *Drosophila melanogaster*. *Journal of Insect Physiology* **50**:695-700.

McCornack, B. P.,M. A. Carrillo, R. C. Venette. and D. W. Ragsdale**.** 2005. Physiological Constraints on the Overwintering Potential of the Soybean Aphid (Homoptera: Aphididae). *Environmental Entomology* **34**:235-240.

Martin, T. L. and R. B. Huey. 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. *The American Naturalist* **171**:E102-118.

McCarty, J. P. 2001. Ecological consequences of recent climate change. *Conservation Biology* **15**:320–331.

Meehl, G. A. and C. Tebaldi. 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. *Science of the Total Environment* **305**:994-997.

Mehrparvar, M. and B. Hatami 2007. Effect of temperature on some biological parameters of an Iranian population of the Rose Aphid, *Macrosiphum rosae* (Hemiptera: Aphididae). *European Journal of Entomology* **104**:631-634.

Miller, N. A. and J. H. Stillman. 2012. Physiological optima and critical limits. *Nature Education Knowledge* **3**:1.

Mittler, T. E. and T. Meikle. 1991. Effects of dietary sucrose concentration on aphid honeydew carbohydrate levels and rates of excretion. *Entomologia Experimentalis et Applicata* **59**:1-7.

Musolin, D. L., D. Tougou, and K. Fujisaki. 2010. Too hot to handle? Phenological and life-history responses to simulated climate change of the southern green stink bug Nezara viridula (Heteroptera: Pentatomidae). *Global Change Biology* **16**:73-87.

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

Newman, J. A. 2003. Climate change and cereal aphids: the relative effects of increasing CO_2 and temperature on aphid population dynamics. *Global Change Biology* **10**:5-15.

Newman, J. A. 2005. Climate change and the fate of cereal aphids in Southern Britain. *Global Change Biology* **11**:940-944.

O'doherty, R. and J. S. Bale. 1985. Factors affecting the cold hardiness of the peach-potato aphid *Myzus persicae*. *Annals of Applied Biology* **106**:219-228.

Parmesan, C. 2006. Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology Evolution and Systematics* **37**:637-669.

Phillips, W. S.,J, S. Bale. and G. M. Tatchell. 2000. Overwintering adaptations in the lettuce root aphid *Pemphigus bursarius* (L.). *Journal of Insect Physiology* **46**:353-363.

Portner, H. O. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**:137-146.

Pörtner, H. O., A. F. Bennett, F. Bozinovic, A. Clarke, M. A. Lardies, and R. E. Lenski, et al. 2006. Trade-offs in thermal adaptation: in need of a molecular to ecological integration. *Physiological and Biochemical Zoology* **79**:295-313.

Powell, W. 1974. Supercooling and the low - temperature survival of the green spruce aphid *Elatobiurn abietinum*. *Annals of Applied Biology* **78**:27-37.

Powell, W. and W. H. Parry. 1976. Effect of temperature on overwintering populations of the green spruce aphid *Elatobium abietinum*. *Annales of Applied Biology* **82**:209–219.

Powell, S. J. and J. S. Bale. 2004. Cold shock injury and ecological costs of rapid cold hardening in the grain aphid *Sitobion avenae* (Hemiptera: Aphididae). *Journal of Insect Physiology* **50**:277-284.

Powell, S. J. and J. S. Bale. 2005. Low temperature acclimated populations of the grain aphid *Sitobion avenae* retain ability to rapidly cold harden with enhanced fitness. *The Journal of Experimental Biology* **208**: 2615-2620.

Powell, S. J. and J. S. Bale. 2006. Effect of long-term and rapid cold hardening on the cold torpor temperature of an aphid. *Physiological Entomology*. **31**: 348-352.

Powell, S. J. and J. S. Bale. 2008. Intergenerational acclimation in aphid overwintering. *Ecological Entomology* **33**:95-100.

Rhodes, J. D., P. C. Croghan, and A. F. G. Dixon. 1996. Uptake, excretion and respiration of sucrose and amino acids by the pea aphid *Acyrthosiphon Pisum*. *The Journal of Experimental Biology* **199**:1269-1276.

Robinet, C. and A. Roques. 2010. Direct impacts of recent climate warming on insect populations. *Integrative Zoology* **5**:132-142.

Saeidi, F.,S. Moharramipour. and M. Barzegar. 2012. Seasonal Patterns of Cold Hardiness and Cryoprotectant Profiles in *Brevicoryne brassicae* (Hemiptera: Aphididae). *Environmental Entomology* **41**:1638-1643.

Salvucci, M. E. and S. J. Crafts-Brandner. 2000. Effects of temperature and dietary sucrose concentration on respiration in the silverleaf whitefly, *Bemisia argentifolii*. *Journal of Insect Physiology* **46**:1461-1467.

Satar, S., U. Kersting, and M. R. Ulusoy. 2005. Temperature dependent life history traits of *Brevicoryne brassicae (L.)* (Hom., Aphididae) on White Cabbage. *Turkish Journal of Agriculture & Forestry* **29**:341-346.

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

Shaposhnikov, G. C. 1987. Evolution of aphids in relation to evolution of plants. In MINKS, A. K. & HARREWIJN, P. (Eds.) World crop pests, 2A, Aphids, their biology, . natural enemies and control. Elsevier, Amsterdam.

Silva, A. X., G. Jander, H. Samaniego, J. S. Ramsey, and C. C. Figueroa. 2012. Insecticide Resistance Mechanisms in the Green Peach Aphid *Myzus Persicae* (Hemiptera: Aphididae) I: A Transcriptomic survey. *Plose one* **7**:e36366.

SlamaL, K. and P. Jedlicka. 2012. Respiratory metabolism of the pea aphid, Acyrthosiphon pisum (Hemiptera: Aphididae). *European Journal of Entomology* **109**:491-502.

Smith, J. B., S. H. Schneider, and M. e. a. Oppenheimer. 2009. Assessing dangerous climate change through an update of the Intergovernmental Panel on Climate Change (IPCC)

''reasons for concern''. *Proceedings of the National Academy of Sciences* **106**:4133-4137.

Somero, G. N. 2011. Comparative physiology: a 'crystal ball' for predicting consequences of global change. *American Journal Physiology: Regultory, Integrative and Comperative Physiology* **302**:R1-R14.

Sorensen, J. G., T. N. Kristensen, and V. Loeschcke. 2003. The evolutionary and ecological role of heat shock proteins. *Ecology Letters* **6**:1025-1037.

Stange, E. E. and M. P. Ayres. 2010. Climate Change Impacts: Insects. *Encyclopedia of Life Sciences*.

Stanley, K. and B. Fenton. 2000. A member of the Hsp60 gene family from the peach potato aphid, *Myzus persicae* (sulzer.). *Insect Molecular Biology* **9**:211-215.

Strathdee, A. T., G. G. Howling, and J. S. Bale. 1995. Cold Hardiness of Overwintering Aphid Eggs. *Jouenal of Insect Physiology* **41**: 653_657.

Strong, F. E. 1963. Studies on lipids in some homopterous insects. Hilgardia **34**:43-61.

Sutherland, O. R. W. 1968. Dormancy and lipid storage in the pemphigine aphid *Thecabious affinis*. *Entomologia Experimentalis et Applicata* **11**:348-354.

Teets, N. M., Y. Kawarasaki, R. E. Lee Jr., and D. L. Denlinger. 2011. Survival and energetic costs of repeated cold exposure in the Antarctic midge, *Belgica Antarctica*: a omparison between frozen and supercooled larvae. *Journal of Experimental Biology* **214**:806-814.

Terblanche, J. S., A. A. Hoffmann, K. A. Mitchell, L. Rako, P. C. le Roux, and S. L. Chown. 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. *The Journal of Experimental Biology* **214**:3713-3725.

Thompson, J. N. and B. M. Cunningham. 2002. Geographic structure and dynamics of coevolutionary selection. *Nature* **417**:735-738.

Tollarová-Borovanská, M., L. Lalouette, and V. Koštál. 2009. Insect cold tolerance and repair of chill-injury at fluctating thermal regimes: Role of 70 kDa heat shock protein experssion. *CryoLetters* **30**:312-319.

Van hook, R. I., M. G. Nielesen, and H. H. Shugart. 1980. Energy and nitrogen relations for a *Macrosiphum liriodendri* (Homoptera: Aphididae) population in an East Tennessee Liriodendron tulipifera stand. *Ecology* **61**:960-975.

Vorburger, C. 2004. Cold tolerance in obligate and cyclical parthenogens of the peach-potato aphid, *Myzus persicae*. *Ecological Entomology* **29**:-505.

Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. M. Fromentin, O. Hoegh-guldberg, and F. Birlein. 2002. Ecological responses to recent climate change. *Nature* **416**:389-395.

Wang, H.-S., C.-S. Zhou, W. Gho, and L. Kang. 2006. Thermoperiodic acclimations enhance cold hardiness of the eggs of the migratory locust. *Cryobiology* **53**:206-217.

Weibull, J. H. W. 1988. Free amino acids in the phloem sap from oats and barley resistant to *Rhopalosiphum padi*. *Phytochem* **27**:2069-2072.

Whalon, M. E. and Z. Smilowitz. 1979. Temperature-dependent model for predicting Þeld populations of green peach aphid, *Myzus persicae* (Homoptera: Aphididae). *The Canadian Entomologist* **111**:1025-1032.

Whittaker, J. B. and N. P. Tribe. 1998. Predicting numbers of an insect (*Neophilaenus lineat*us: Homoptera) in a changing climate. *Journal of Animal Ecology* **67**:987-991.

Wilkinson, T. L. and H. Ishikawa. 1999. The assimilation and allocation of nutrients by symbiotic and aposymbiotic pea aphids, *Acyrthosiphon pisum*. *Entomologia Experimentalis et Applicata* **91**:195-201.

Wille, B. D. and G. L. Hartman. 2008. Evaluation of artificial diets for rearing *Aphis glycines* (Hemiptera: Aphididae). *Journal of Economic Entomology* **101**:1228-1232

Williams, C. M., H. A. L. Henry, and B. J. Sinclair. 2014. Cold truths: how winter drives responses of terrestrial organisms to climate change. *Biology Reivew* **90**:214-235.

Wilson, E. O. 1992. The Diversity of Life. Harvard Univ Press, Cambridge, MA.

Winter, H., G. Lohaus, and H. W. Heldt. 1992. Phloem transport of amino acids in relation to their cytosolic levels in barley leaves. *Plant Physiology* **99**:996-1004.

Yocum, G. D., K. J. Greenlee, J. P. Rinehart, M. M. Bennett, and W. P. Kemp. 2011. Cyclic CO_2 emissions during the high temperature pulse of fluctuating thermal regime in eye-pigmented pupae of *Megachile rotundata*. *Comparative Biochemistry and Physiology A* **160**:480-485.

Zhao, F., W. Zhang, A. A. Hoffmann, and C. S. Ma. 2014. Night warming on hot days produces novel impacts on development, survival and reproduction in a small arthropod. *Journal of Animal Ecology* **83**:769-778.

Zhou, X., J. N. Perry, I. P. Woiwod, R. Harrington, J. S. Bale, and S. J. Clark. 1997. Temperature change and complex dynamics. *Oecologia* **112**:543-550.

Chapter 2: The physiological consequences of varied heat exposure events in adult *Myzus persicae*: a single prolonged exposure compared to repeated shorter exposures

This chapter is written as a standalone manuscript, a modified version of this manuscript has been published in PeerJ journal

Chapter 2: The physiological consequences of varied heat exposure events in adult *Myzus persicae*: a single prolonged exposure compared to repeated shorter exposures

2.1 Abstract

The study of environmental stress tolerance in aphids has primarily been at low temperatures. In these cases, and in the rare cases of high temperature tolerance assessments, all exposures had been during a single stress event. In the present study, we examined the physiological consequences of repeated high temperature exposure with recovery periods between these stress events in *Myzus persicae*. We subjected individuals to either a single prolonged three hour heating event, or three one hour heating events with a recovery time of 24 hours between pulses. Aphids exposed to repeated pulses of high temperatures had more glucose and higher expression of proteins and osmolyte compounds, such as glycerol, compared to the prolonged exposure group. However, aphids exposed to the repeated high temperature treatment had reduced sources of energy such as trehalose and triglyceride compounds than the prolonged exposure group. Recovery time had more costs (based on production of more protein and consumption of more trehalose and triglyceride) and benefits (based on production of more osmolytes) in repeated high temperature treatments. As aphids are known to respond differently to constant versus 'natural' fluctuating temperature regimes, conclusions drawn from constant temperature data may be problematic. We suggest future experiments assessing insect responses to thermal stress incorporate a repeated stress and recovery pattern into their methodologies.

Keywords: Metabolite, heat exposure, aphid, thermal tolerance, repeated heating

2.2 Introduction

Climate change is one of the most critical threats to biodiversity (Dawson et al., 2011; IPCC., 2014). Human-induced climate change is predicted to increase the frequency of climatic extremes (e.g. heat waves and severe droughts or floods), climatic variability (Lean and Rind, 2008; Rahmstorf and Coumou, 2011) and mean of the thermal environment in regions around the world (Niehaus et al., 2012). Global mean temperature has risen by 0.85°C from 1880 to 2012 and all of the warmest 20 years on record have occurred since 1990 (CSIRO-ABM, 2014).

Exposure to different thermal means and variability can have a substantive influence on animal physiological performance (Huey et al., 2012). This may include a modification of performance curve parameters including two critical fitness-influencing components: the upper critical temperature and the thermal optimum (Angilletta, 2009). All insects keep their physiological performance within a specific range of temperatures, and many of their physiological functions may be reduced when exposed to extreme temperatures (Mironidis and Savopoulou-Soultani, 2010). An important aspect of habitat quality is minimal exposure to extreme thermal stress (Huey, 1991). In terrestrial habitats, climate change is strengthening fluctuations and amplitude of temperature variation, which will lead to prolonged adverse temperature exposure in terresterial habitats (Sinclair et al., 2006; Andrew, 2013; Vasseur et al., 2014).

Critical temperature responses can shift somewhat based on an animal's environmental thermal experience (Somero, 2010). As insects are ectotherms, their survival, population dynamics, and distribution are influenced by temperature (Chown et al., 2010; Bauerfeind and Fischer, 2014). Therefore investigating impacts of temperature variation on

an individual's performance and understanding these plastic responses of populations to temperature is critical (Pörtner et al., 2006) and may have implications for their potential responses to climate change.

Some aphid species, including *M. persicae*, are distributed widely across the globe. The cabbage aphid, *Brevicoryne brassicae* (L.), the turnip aphid, *Lipaphis erysimi* Kalt, and the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphidae) are the three major aphid pests infesting canola in Australia (Gu et al., 2007; Gia and Andrew, 2015). *Myzus persicae* (green peach aphid) is known to transmit over 100 phytopathogenic viruses among 50 different plant families. Many of its hosts include major crops (e.g. sugar beet, beans, brassicas, potatoes, citrus) and on a world wide scale this species is regarded as the most important aphid pest (Blackman and Eastop, 1984). Green peach aphid host alternating between the primary peach *Prunus persica* host in winter and various herbaceous hosts, belonging to 50 different families, which include brassicas, potatoes and sugar beet, in summer (Blackman and Eastop, 2000)

Extreme climatic events such as heat waves and daily fluctuations can impact aphids in various ways: reducing fecundity and population growth; slowing development; and can also affect community structure and interrupt trophic cascades through effects on performance of individual species and through changes in the strength of interactions between them (Davis et al., 2006; Gillespie et al., 2012; Jeffs and Leather, 2014; Zhao et al. 2014; Colinet et al. 2015; Ma et a;, 2015). Aphids are more sensitive to acute changes in temperature rather than the duration of extreme temperature exposure, and nymphs do not experience diapause (Davis et al., 2006; Jeffs and Leather, 2014), making them a suitable test organism for our study. Studying the effects of climate change on aphids is complex.

Many organisms that live in fluctuating thermal environments, such as aphids, display a high degree of thermal plasticity in their response to changing conditions, and have a greater resilience in their ability to recover from ecological perturbations (Davis et al., 2006; Jeffs and Leather, 2014). In order to understand how aphids perform in a warming climate it is necessary to consider both exposure temperature and exposure duration with or without recovery time.

In responding to extreme environmental conditions, insects rely on a combination of different factors: such as molecular processes (gene expression, heat shock proteins, and enzymes); changes in membrane structure; and osmolyte compounds; to survive and recover from unfavourable conditions (Back et al., 1979; Henle et al., 1983; Lin et al., 1984; Kim and Lee, 1993; Meng et al., 2004; Wang et al., 2006; Clark and Worland, 2008; Tollarová-Borovanská et al., 2009). Aphids and whiteflies were one of the first insect taxa reported to accumulate polyols in response to high temperatures and also reveal that these compounds could stabilize proteins structure against thermal denaturation (Back et al., 1979). Whole-animal respiration will change when exposed to repeated stress (Lalouette et al., 2011; Yocum et al., 2011) and understanding the implications of this is critical as this will impact on animal survival and body maintenance as changes occur in energy and water usage (Schimpf et al., 2009; Chown et al., 2011). In addition, the upper critical temperature threshold at which an animal loses muscular control (CT_{max}) is a metric of interest as it is useful in predicting phenotypic effects of warming (Huey et al., 2012). Using laboratory estimates of thermal tolerance enables the seasonal abundance and geographic distribution of organisms to be determined (Ju et al., 2013).

It has become clear that constant temperatures are not always useful for studying the performance thermal responses of organisms as they do not mimic the fluctuating temperature conditions which occur within the natural environment (Lamb, 1961; Davis et al., 2006; Jeffs and Leather, 2014). Constant temperature conditions also underestimate thermal thresholds of individuals and become less accurate compared to fluctuating temperature regimes (Davis et al., 2006; Niehaus et al., 2012; Jeffs and Leather, 2014). This implies that fluctuating temperature experiments ought to be "normal" while constant temperature insect development studies were "abnormal" experimental conditions (Cloudsley-Thompson, 1953). Fluctuating temperatures enhanced resistance of leaf beetles and fruit-flies (Casagrande and Haynes, 1976; Meats, 1976) and that fitness could be more significant in repeated temperatures (Beardmore and Levine, 1963; Fischer et al. 2011).

In previous studies much attention has focused on the cold tolerance of *Myzus persicae* (O'doherty and Bale, 1985; Bale et al., 1988; Clough et al., 1990; Howling et al., 1994; Bezemer et al., 1998; Vorburger, 2004) as well as assessments of the effects of temperature on physiological parameters including their thermal tolerance at different latitudes and altitudes (Bezemer et al., 1998; Alford et al., 2012a). There is a paucity of knowledge regarding how aphid physiology is affected by repeated exposure to high temperatures. Here, we assessed aphid metabolic rates after two different heating regimes (prolonged exposure and repeated exposure with recovery time) using flow-through respirometry; we also assessed the upper critical temperature threshold, energy reserves and their osmolyte compound profile, as indicators of aphid stress response after exposure to the different heating regimes.

We predict that multiple high temperature exposure will increase adult aphid thermal tolerance, as recovery time between heat stress pulses will enable metabolic and cellular repairs to occur (Storey and Storey, 2004). We also predict that aphids will accumulate polyols and sugars in response to high temperatures (Hendrix and Salvucci, 1998) which will result in an increased CT_{max}.

2.3 Materials and Methods

Aphids and Diet Chamber Construction

Stock colonies of *Myzus persicae* (green peach aphid), were collected from canola plants at the Laureldale Farm property (University of New England, Armidale, NSW Australia) in 2013.

The lab colony were established from an isofemale line (BG_13-001) and maintained on canola variety 'Thunder Bay' plants in a glasshouse with 25±0.5°C temperatures, 65% relative humidity, under a 16:8 h (L: D) photoperiod provided by fluorescent lamp for two years to ensured continuous apomictic parthenogenesis.

Since aphids have parthenogenetic embryogenesis (Dixon, 2005) we bred three generations of aphids in the lab on the artificial diets in the incubators to reduce maternal and grand maternal affects: we removed the wingless female adult aphids after three days, then the resulting nymphs were left for seven days and we continued this process for three generations. The third generation resulting nymphs were housed at 25°C, 65% relative humidity, under a 16:8 h (L: D) in a Thermoline incubator (TRH-300) prior to experimental manipulation.

Diet cage preparation

To rear our aphids on artificial diets we used diet cages. Diet cages were constructed from rigid clear PVC plastic tubing with a 12cm diameter (Fig 2.1). A 7cm x 7cm square of parafilm was extended by force across the top of a cage, and 2 ml of artificial diet was pipetted on top of the parafilm layer. A second 7cm x 7cm sheet of parafilm was then extended over the first sheet, forcing diet across the top surface of the cage, but avoiding leakage over the edge. Then by using a paintbrush, aphids were placed on the underside of the parafilm. The diet cages where then put into a closed transparent box (30×30cm) and put into the incubator prior to the application of treatments.
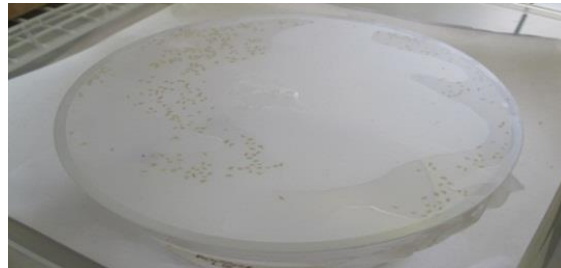
Fig 2.1 Diet cages were constructed from rigid clear PVC plastic tubes, 12cm in diameter (aphid feeding) and covered with a 7 x 7cm sheet of Parafilm laboratory film.

Artificial Diet

The artificial diet used to rear the aphids was based on work by Kunkel (1976) and consisted of specific concentrations of phosphate, vitamins, and minerals (Table 1). In the experiments, four replicate groups of 200 larval aphids were maintained on the test diet for three generation in the incubators. The specified amounts of each component (see Table 1) were prepared with distilled deionized water to the total volume of 10ml in a glass container. The pH of the solution was 7.0-7.5. The diet solution was divided into 2ml aliquots, and stored at 4°C s for less than 1 week or at – 20°C for less than three months. In all experiments, distilled deionized water was used in all of the solutions and the diets of the aphids were changed twice a week for the duration of our study.

Table 2.1 Concentrations and composition of the artificial diet used to rear *Myzus persicae*. Molecular weight (MW); molar Mass (mM) and mole percent (Mol%).

Amino Acid	MW	Mol%	150mM	Sucrose mix	1000 mM
Alanine	89.09	3.8	50.8mg	ascorbic acid	10mg
Asparagine	150.1	9.5	213.9mg	citric acid	1mg
Aspartate/Aspartic Acid	133.1	9.5	189.7mg	$MgSO_4$	11mg
Cysteine	157.6	1.8	42.5mg	Sucrose	3400mg
Glutamic Acid	147.13	5.6	123.6mg	**Mineral stock**	
Glutamine	146.1	11	241.1mg	$FeCl_3$	13.1mg
Glycine	75.07	0.8	9.0mg	$CuCl_2. 2H_2O$	1.7mg
Proline	115.1	3.8	65.6mg	$MnCl_2.4H_2O$	4mg
Serine	105.09	3.8	59.9mg	$ZnCl_2$	13.6mg
Tyrosine	181.2	0.4	10.9mg	**Vitamin stock**	
Arginine	210.66	9.5	300.2mg	Biotin	0.1 mg
Histidine	209.6	5.8	182.4mg	Pantothenate	5mg
Isoleucine	131.18	5.8	114.1mg	folic acid	2mg
Leucine	131.18	5.8	114.1mg	nicotinic acid	10mg
Lysine	182.6	5.8	158.9mg	Pyridoxine	2.5mg
Methionine	149.2	1.9	42.5mg	Thiamine	2.5mg
Phenylalanine	165.2	1.9	47.1mg	Choline	50mg
Threonine	119.1	5.8	103.6mg	myo-inositol	50mg
Tryptophan	204.2	1.9	58.2mg	**Phosphate**	
Valine	117.1	5.8	101.9mg	$K_2PO_4.3H_2O$	150mg

Experimental Conditions

To investigate the effects of fluctuating thermal regimes (FTR), the experimental design was a simplified version of the experimental protocol of Marshall and Sinclair (2011) and Zhang et al. (2011). For the repeated heat stress exposure treatment, adult aphids (7days aged) were exposed to one to three diurnal cycles of 1h at 38°C (we didn't control RH) followed by 24h 65% relative humidity (RH) at 25°C, .Adult aphids (seven day old individuals) for the three generation reared on the artificial diet were used (rearing aphids for three generation on artificial diet at 25°C, 65% relative humidity, under a 16:8 h (L: D) in a

Thermoline incubator (TRH-300). For the single prolonged heat exposure treatment, separate groups of adults were exposed to 38°C for 3h, because after three cycles of repeated heat stress, adults have accumulated 3h at 38°C. Post treatment, this group were also given a 24h-period of recovery at 25°C, 65% RH. Meanwhile, control animals were held at 25°C, 65% RH for the duration of the study.

Estimates for the upper critical temperature threshold limits for *M. persicae* have previously found to range from 38.5°C (Broadbent and Hollings, 1951) to 42°C (Hazell et al., 2010). *Myzus persicae* are known to survive 1h per day above their CT_{max} of 38.5°C (Davis et al., 2006) and so the FTR was chosen to fluctuate around a value close to their CT_{max}. In general, upper temperatures are difficult to experiment with compared to lower temperatures because as their performance curve optima is very close to CT_{max}. A test temperature of 38°C was chosen as there was 60-70% survival following the prolonged thermal exposure for 3 hours.
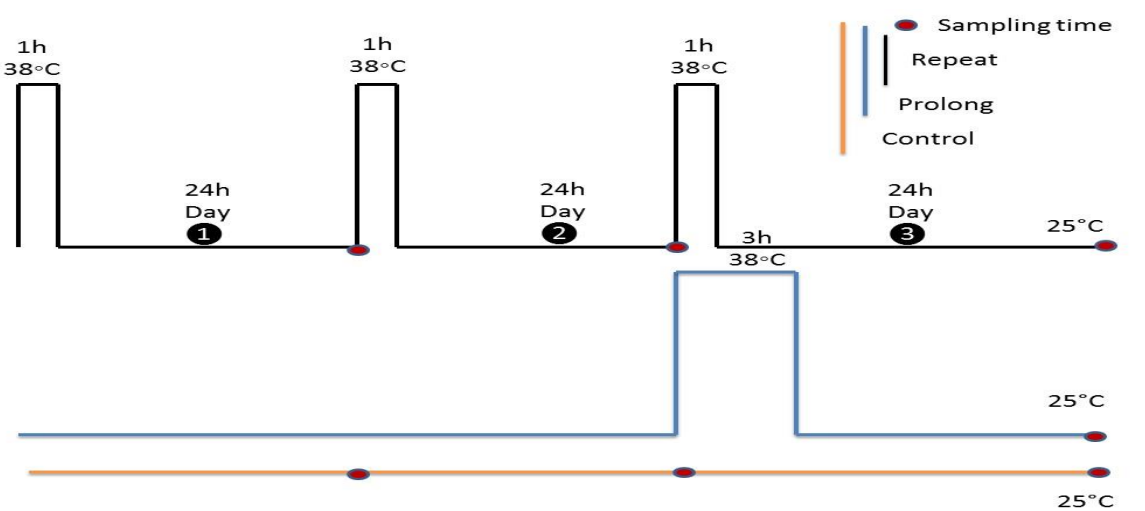
Fig 2.2 Experimental design, all experiments were performed on adult aphids that were 6 days old on the first experimental day. Each black rectangle represents a 1h exposure to 38°C. The blue rectangle represents a 3h exposure to 38°C. Adults were kept at 25°C as the control group (yellow line). Red dots indicate sampling points. All samples were collected 24h after final treatments.

Quantification of Metabolic Reserves

Twenty-four hours after a heat exposure (Figure 2.2), four replicate groups of adult aphids were weighed to 0.001mg on a Mettler Toledo XP2U (Switzerland) electronic balance, then homogenized in 80 µl extraction buffer (35mM Tris, 25 mM KCl, 10 mM MgCl2, pH 7.5) with 0.1% (v/v) Triton-X-100 after centrifuging for 1 minute at 13,000 rpm at 4°C. Supernatant was then removed and placed in a new tube which was then stored at -20°C until all assays were conducted.

The assay kits used included the Sigma triglyceride and glycerol assay kit (Sigma Triglyceride (Sigma:T2449), Free glycerol reagent (Sigma:F6428), Glycerol stock G7793-5 ml at 2.5 mg/ml)); the BioRad Coomassie Brilliant Blue microassay method (500-0201), with bovine serum albumin as standard (40–480 mg protein) for protein; and the Sigma glucose assay kit for glucose (Product Code GAGO-20, contains 500 units of glucose oxidase, O-Dianisidine Reagent (Product Code D 2679)) and following trehalose measurement (Porcine Kidney Trehalase (Sigma:T8778) 1 unit per mg of protein, 3.7 mg per ml and 0.2 M sodium citrate (5.882 g/100ml), 1 mM Sodium EDTA (37.22 mg/100 ml), D+Trehalose dihydrate (Sigma:T0167)).

For these assays, we measured glucose, triglyceride, glycerol, trehalose and total protein content in triplicate and we used a visible wavelength spectrophotometer (Epoch: Microplate Spectrophotometer) with absorbance at 544 nm, 540 nm and 750 nm and 96 well plates, with volumes scaled down from the manufacturer protocols (Ridley et al., 2012).

Active Metabolic Rate Measurement (AMR)

Flow-through CO_2-based respirometry was used to record $\dot{V}CO_2$, with a similar experimental setup as described by Terblanche *et al*. (2007). A HiBlow HP40 air pump was used to feed atmospheric air into sodalime (VWR with indicator AnalaR NORMAPUR analytical reagent) and Drierite (W.A. Hammond Drierite Company) scrubber columns, to remove CO_2 and water vapour from the air stream, respectively. The flow rate of the airstream was regulated at 80 ml min^{-1} by a flow control valve (Model 840, Sierra Side-Trak, Sierra Instruments Inc., Monterey, USA), connected to a mass flow controller (Sable MFC-2). Thereafter, air flowed through the zero channel (cell A) of a calibrated (to 6 ppm CO_2 in air) infrared CO_2-H_2O analyzer (Li-7000, Li-Cor, Lincoln, NE, USA). The airstream then flowed over the test animal in the 5ml glass cuvette, which was placed in a programmable water bath (Grant, GP200-R4), programmed using LABWISE software with increasing temperature of 0.25°C min^{-1} (see Basson & Terblanche, 2010). The air leaving the cuvette then entered the analyser through another channel which recorded the difference in CO_2 concentration of the air before and after it flowed through the cuvette, at 1s intervals. Changes in animal position (activity) were recorded using an infrared activity detector (AD-1, Sable Systems, Las Vegas, NV, USA). Aluminium foil was placed around this cuvette to restrict light exposure and to ensure high quality activity recordings (MacMillan et al., 2012).

Four aphids per replicate were weighed to 0.001mg on an electronic microbalance before and after the experiment and mean mass used as a covariate in statistical analyses. The output from the CO_2 analyzer and activity data were recorded with the LiCor 7000 software and analysed using Expedata V1.25 software (Sable Systems International, Las Vegas, NV, USA). Volumes of CO_2 in ppm were corrected for baseline drift and then

converted to μl CO_2 h^{-1} using Expedata software. Rates of CO_2 production (in μl CO_2 h^{-1}) were calculated from the whole record by transforming ppm concentration of CO_2 to CO_2 fraction and then multiplying by the flow rate (80 ml min^{-1}). The area under the curve (integral of ml CO_2 min^{-1} *vs* min) was calculated. This area was equal to the volume of CO_2 produced by each replicate in the cuvette, and this volume was divided by the total period of measurement (2.30 h), multiplied by 1000 to give μl $CO_2 h^{-1}$ to give the metabolic rate per aphid per hour (Castañeda et al., 2009). Metabolic rate were measured four replicate after the final exposure in all experimental groups.

Measuring Upper Critical Thermal Limits (CT_{max})

Critical thermal limits were determined by subjecting aphids to a regime of increasing temperatures and monitoring their ability to control their movement: loss of muscular controls to determine their CT_{max} threshold. Forty adult aphids were each placed within an individual 5ml plastic tube at a pre-set temperature (25°C). A programmable water bath was set to increase the temperature from 25°C to 35°C with a rate of 0.5°C min^{-1} then the temperature was increased to 45°C with a rate of 0.1°C min^{-1} to minimize the hardening response across a broad range of upper critical temperatures and to determine the temperature at which ceased walking and succumbed to uncontrollable spasm (Hazell et al., 2008; Alford et al., 2012b). Upper critical temperature was measured for 40 adult aphids for each of the three experimental treatments after final heat exposure in all experiments group.

Statistical Analysis

All data were analysed for normality and tested for homogeneity of variances for treatment means using the Levene's Test, in the HOVTEST option of GLM procedure within SAS software (2008). A completely randomized design was employed. One-way analysis of variance was performed using the GLM procedure. Tukey post hoc tests were used to compare means ($P< 0.05$).

2.4 Results

Glucose Content

The glucose content of the control group exhibited no significant differences over the course of the experiment (Day 1: 1.61±0.02 to Day 3: 1.96±0.06 µg glucose mg^{-1}(Fig 2.3A). However, adult aphids in both the prolonged and repeated exposure groups experienced a significant increase in glucose content. After a single cycle of 38°C for 1h and 25°C for 24h, glucose content increased significantly, nearly one and half fold for aphids in the repeated exposure group ($P<0.0001$). For the duration of the experiment, the glucose content of aphids in the repeated exposure group peaked at 3.26±0.02 µg glucose mg^{-1} (Fig 2.3A). For adult aphids continuously exposed to 38°C for 3h, glucose content significantly increased compared to the control group (3.01±0.02 µg glucose mg^{-1}).

Trehalose content

The trehalose content of aphids exposed to repeated heating events significantly decreased from 0.44±0.01 to 0.27±0.01 µg trehalose mg^{-1} after the second heating exposure (Fig 2.3B). After three cycles of heating, trehalose content of aphids was

significantly lower than the control group on the third day of the experiment (P <0.001). For aphids exposed to 38°C for 3h, the trehalose content did not significantly differ (P =0.329) from that of the control group.

Protein content

Similar to glucose and glycerol content, protein content of aphids that were heated during repeated exposures was significantly higher than in the control group and their prolonged exposure counterparts (P <0.001) (Fig 2.3C). After one cycle of 1h at 38°C and 24h at 25°C, the protein content of aphids was 17.16±0.06 µg protein mg^{-1}, but this value steadily increased every cycle, and by the end of third cycle protein content was 19.79±0.1 µg protein mg^{-1} (Fig 2.3C). Protein content in aphids heated at 38°C for 3h did not significantly differ from the control (Fig 2.3C).

Glycerol and triglyceride content

Triglyceride content in aphids did not significantly differ between control and the prolonged exposure groups. However there was a single drop of triglyceride content in aphids after three cycles of repeated heating in the repeated exposure group: triglyceride content was less than half that of control (0.659±0.01) and prolonged (0.597±0.01) aphids and reached 0.324±0.02 µg triglyceride mg^{-1} (P<0.002)(Fig 2.3E).

Glycerol content was significantly higher (P<0.0001) in aphids of the repeated exposure group increasing from 1.53±0.01 to 1.96±0.02 µg triglyceride mg^{-1} after three cycles of heating. There was also significantly higher glycerol content in aphids of the prolonged exposure group compared to the control group (P =0.0001) (Fig 2.3D).

Thermal Tolerance

There was no significant difference in aphid CT_{max} between the thermal treatments (Fig 2.4).

Active metabolic rate measurements

There was no significant difference in metabolic rate 24h after heating among treatments (P =0.6; Fig 2.5A). Aphids from the repeated heating and prolonged heating treatments exhibited significant water loss compared to the control group (P =0.04) (Fig 2.5b).
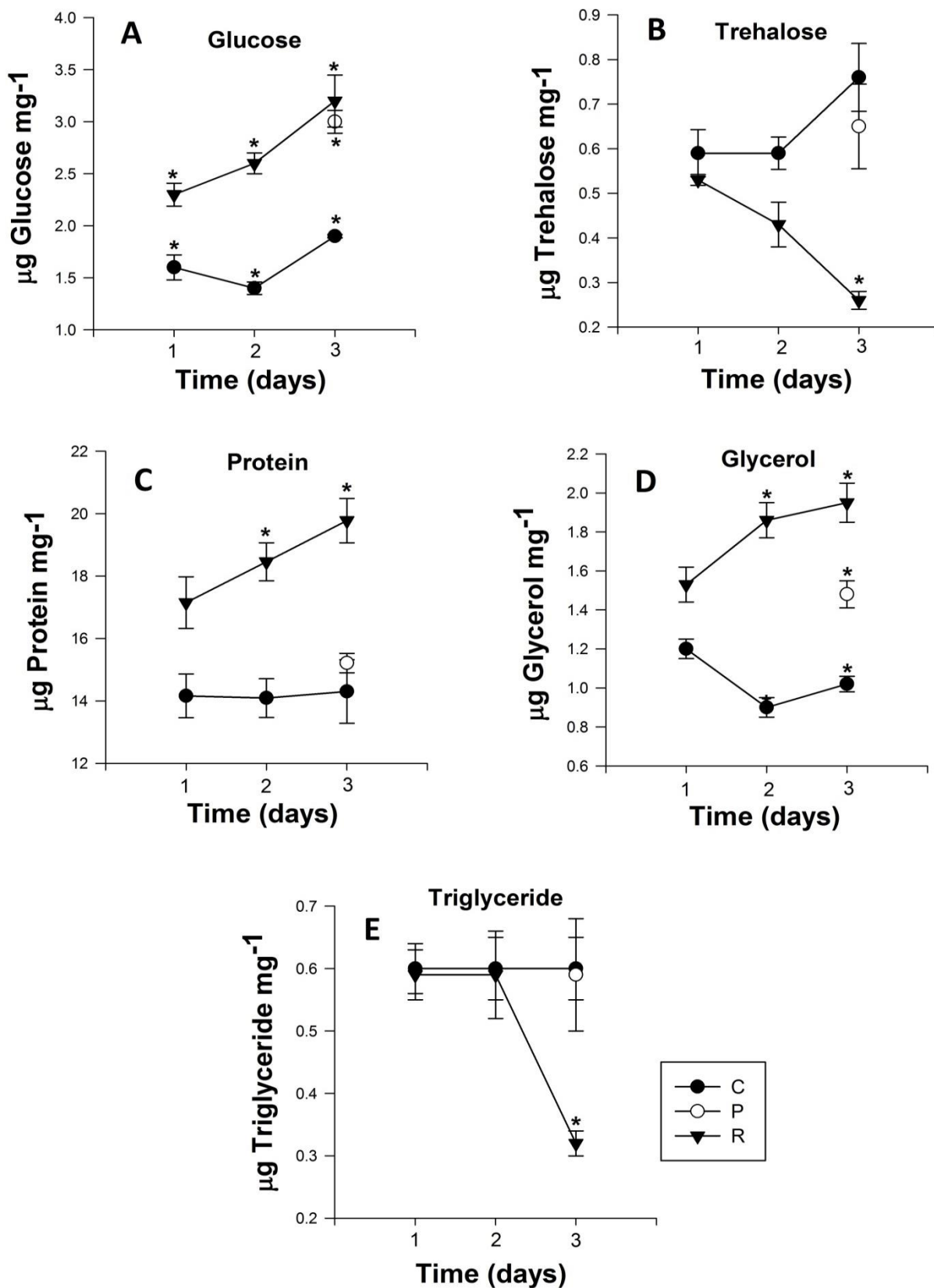
Fig 2.3 Mean (± s.e.m.) (A) Glucose, (B) Trehalose, (C) Protein, (D) glycerol and (E) Triglyceride content of *Myzus persicae* during repeated (R1, R2, R3), prolonged (P) and control state (C1, C2, C3). The metabolite content is based on the mean of four replicates, two aphids per replicate for the all metabolites (ANOVA, $P<0.05$). Post-hoc pairwise differences among treatments indicated by star above each Dot plot.
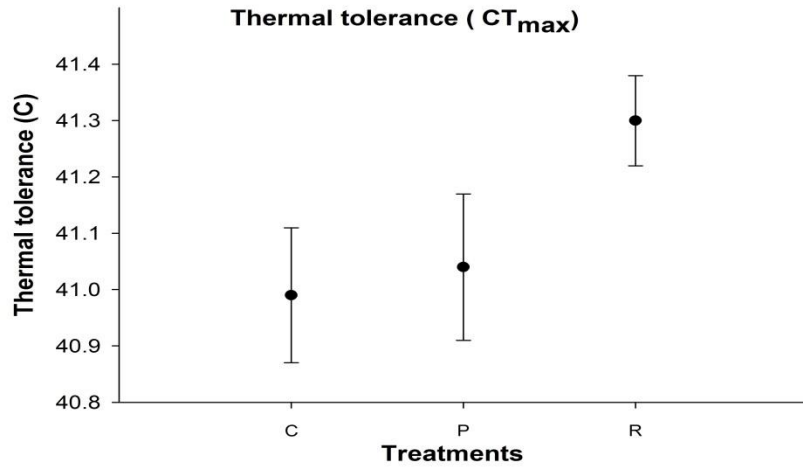
Fig 2.4 Mean (± s.e.m.) thermal tolerance point (CT_{max}) of aphids among control (C), prolonged (P) and repeated (R) exposure treatments.
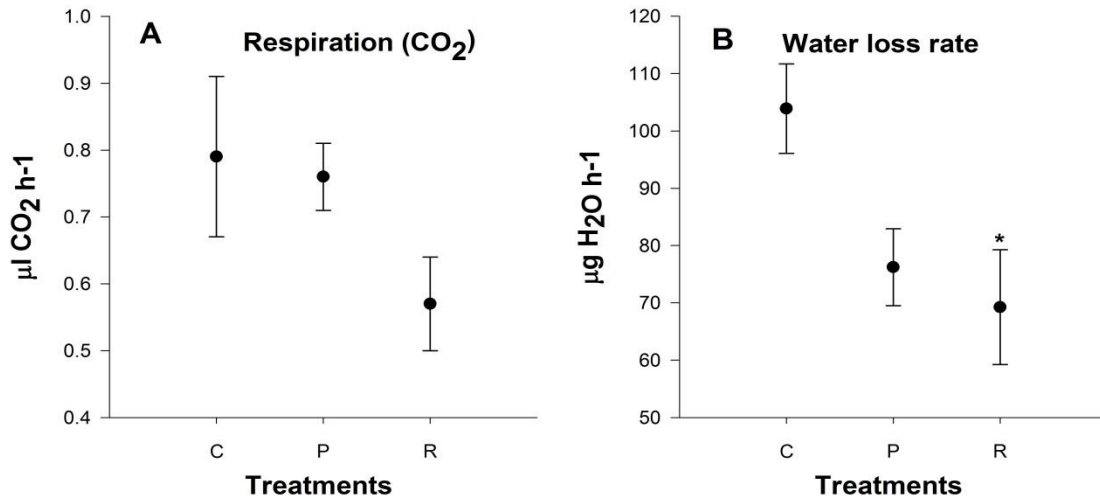

Fig 2.5 Mean (± s.e.m.) (A) CO_2 production (µl CO_2 h⁻1) and (B) H_2O output rates (µg H_2O h⁻¹) of aphids among control (C), prolonged (P) and repeated (R) exposure treatments.

Table 2.2 Energy reserves, osmolytes concentration and protein mass of *Myzus persicae* in three treatment groups: maintained at 25°C (control), heated for a single pulse of 3h at 38°C (1×3h) and heated for three pulses of 1h at 38°C (3×1h). Significant values in bold.

Treatments	Treatments			Contrasts											
				C_3-R_3			C_3-P			P-R_3			C_3-R_3-P		
Measurements	D.F	F Value	P-value	D.F	F Value	P-Value	D.F	F Value	P-Value	D.F	F Value	P-Value	D.F	F Value	P-Value
Glucose	6	22.31	**<0.0001**	1	39.71	**<0.0001**	1	25.85	**<0.0001**	1	1.48	0.2262	2	43.22	**<0.0001**
Trehalose	6	4.40	**0.0005**	1	21.06	**<0.0001**	1	0.96	0.3297	1	13.03	**0.0005**	2	10.34	**0.0017**
Protein	6	15.85	**<0.0001**	1	84.09	**<0.0001**	1	32.54	0.3719	1	12.01	**<0.0001**	2	73.75	**0.0004**
Glycerol	6	23.24	**<0.0001**	1	67.76	**<0.0001**	1	16.08	**0.0001**	1	17.82	**<0.0001**	2	49.96	**<0.0001**
Triglyceride	6	3.57	**0.0029**	1	16.02	**0.0001**	1	0.55	0.4614	1	10.65	**0.0015**	2	7.49	**0.0073**

Measurements	Contrasts											
	C_3-P-R_3			C_3-P			C_3-R_3			P-R_3		
	D.F	F- Value	P-Value	D.F	F- Value	P-Value	D.F	F- Value	P-Value	D.F	F- Value	P-Value
CO_2	2	0.48	0. 62	1	0.07	0.967	1	4.65	0.481	1	3.55	0.312
H_2O	2	3.61	**0.04**	1	11.24	**0.012**	1	17.69	**0.005**	1	0.73	0.872
CT_{max}	2	1.63	0.127	1	3.61	0.759	1	0.09	0.059	1	2.54	0.113

2.5 Discussion

In the present study, we examined the physiological consequences of either being exposed to repeated high temperatures with recovery time periods between them, or prolonged temperature exposure, in the aphid *Myzus persicae* (Figure 2.2)*.* We observed increased costs and benefits during repeated heating exposure: this group of aphids had more glucose and higher expression of proteins and osmolyte compounds such as glycerol compared to the prolonged exposure group. However, the repeated high temperature exposure group also had fewer sources of energy such as trehalose and triglyceride compounds compared to the prolonged exposure group. We found that recovery time had more costs (based on production of more protein and consumption of more trehalose and triglyceride) and benefits (based on production of more osmolytes) for repeated high temperature exposure group, but interestingly we saw no changes in thermal tolerance and metabolic rate across treatments.

Impacts of repeated high temperature on thermal tolerance of aphids

Fluctuating temperature studies are better at predicting the thermal tolerance of aphids than constant temperature studies, even when the mean temperatures are the same between constant and fluctuating regimes (Lamb, 1961); constant temperature studies also underestimate critical temperature thresholds as it is known that fluctuating temperatures develop threshold limits (Casagrande and Haynes, 1976; Meats, 1976). The upper critical temperatures in *M. persicae* start from 38.5°C (Broadbent and Hollings, 1951) to 42°C (Hazell et al., 2010). To our knowledge just three studies (Davis et al. 2006, Gillespie et al.

2012; Jeffs and Leather, 2014) have assessed fluctuating high temperatures in aphids*:* but our study is the first to assess physiology of aphids at high temperatures. Davis et al. (2006) examined the effect of high and fluctuating temperatures on the development of *M. persicae*. They demonstrated that under fluctuating temperatures, *M. persicae* had greater fertility and faster development and had the capacity to survive 1h every day above the CT_{max} of 38.5°C (Davis et al., 2006). These results are in contrast to Gillespie et al. (2012) and Jeffs and Leather (2014) who found that under heat wave conditions, the growing population in aphids was lower when exposed to heat waves than weather with periodic hot days. So differences in their results may be due to other factors such as changes in mean temperatures.

Extreme temperature exposure in short pulses often increase survival compared to prolonged exposure. At optimum conditions, injury repair can occur by restoring ion homeostasis (Kostal et al., 2007) and replenishing energy levels. In *Drosophila melanogaster,* Krebs and Loeschcke (1994) found similar results for effects of heating in adult female flies by exposing them to 36°C 1–3 times with 48h rest between heating exposure. Their results showed that exposure to pulses of stress increased survival temperature to 39°C.

Furthermore, recovery time at optimum temperatures may have permitted aphids to recover from the negative impacts of high temperatures (Hazell et al., 2010). In our study, it appears that recovery times between high temperatures can repair injuries, as aphids exhibited higher levels protein and osmolyte in the repeated exposure treatment compared to the prolonged exposure treatment group: but there was no significant difference in their CT_{max} between treatments. One possible explanation is that the proteins and osmolytes

were upregulated during the recovery time, but upregulation was not great enough to change their thermal threshold between treatments (Tammariello et al., 1999). One further reason for conservatism of physiological resistance to heat is due to upper thermal tolerance being largely uncorrelated to estimates of natural temperature (Grigg and Buckley, 2013) as a high number of terrestrial organisms are unlikely to evolve physiological resistance to increased heat (Kellermann et al., 2012; Hoffmann et al., 2013). In such cases, development of physiological resistances will be weakened.

Changes in Energy Reserves at Fluctuating Temperatures

Acclimation impacts the structure of lipid layers (Hazel, 1995), sugar or polyol amount (Hendrix and Salvucci, 1998) and metabolic rate (Hoffmann and Parsons, 1997): all of which can influence temperature resistance (Andersen et al., 2010). Whiteflies and aphids appear to be the first reported organisms that collected polyols in response to high temperatures (Hendrix and Salvucci, 1998). Sugars and polyols balance out and stabilize the natural structure of proteins, protecting them from warm denaturation (Back et al., 1979).

The osmolyte compounds in aphids heated continuously for 3h were less than those aphids exposed to three cycles of repeated high temperatures. Adaptations to surviving high temperatures, such as stress protein production and the synthesis of resources like glucose and trehalose (Hottiger et al., 1994; Jain and Roy, 2009). Accordingly, we anticipated that repeated high temperatures would result in the consumption of vital energy reserves.

Our results indicate that repeated high temperature exposure is costly for aphids compared to prolonged high temperature exposure, as there is a significantly lower amount of trehalose and triglyceride production after repeated high temperatures. Trehalose and proteins play important roles in stress responses (Parsell and Lindquist, 1993; Feder and Hofmann, 1999; Salvucci et al., 2000; Jain and Roy, 2009; Smith et al., 2012). Induction of thermal tolerance by trehalose is also inferred from the fact that the level of trehalose is correlated with thermal tolerance (Hottiger et al., 1994): with this in mind we measured the content of these two known compounds in *M. persicae*. Protein content was higher in aphids of the repeated high temperature treatment group, which has a well-defined role as a protective material at high temperatures (Jain and Roy, 2009) and this result is in agreement with previous studies (Huang et al., 2007; Tollarová-Borovanská et al., 2009; Zhang et al., 2011). We hypothesise that in repeated high temperature exposure, protein production is triggered once the aphids are returned to their recovery temperature: a trigger that is not available to aphids continually kept at prolonged high temperatures.

An unusual result in our study was that the amount of trehalose decreased in aphids exposed to repeated high temperatures compared to the aphids exposed to prolonged high temperatures. This contradicts the findings of previous studies assessing trehalose after exposure to high temperatures (Hottiger et al., 1994; Jain and Roy, 2009) but does support the findings of Teets et al., (2010). Trehalose may be used to produce an osmolyte, such as mannitol, in aphids which is important at high temperatures (Hendrix and Salvucci, 1998). Trehalose can also function as a reserve carbohydrate in others, which acts as a protectant. For example it has been noted that trehalose accumulates under conditions of environmental stress, such as desiccation and is rapidly metabolized in rehydration. A

reserve function could be inferred. Trehalose can also accumulate in response to other stresses, such as heat or osmotic shock (Newman et al., 1993), for example Lalouette et al. (2007) demonstrated that trehalose can be changed to glycogen therefore is related to energy storage functions.

Triacylglycerols constitute a large part of the aphid lipid assemblage (Itoyama et al. 2000) and serve as a reservoir for fatty acids that can be used for energy production. Very large amounts of triacylglycerol can occur in aphids, comprising 20-30% of fresh body weight (Strong, 1963; Sutherland, 1968). According to expectations, we observed lower amount of triglyceride content after repeated high temperatures compared to the prolonged treatment aphids. One explanation is, these storage lipids are used as a source of metabolite energy for physiological processes: many insects use lipids as an energy source, and fatty acids are stored in the fat body in the form of triacylglycerol (Blacklock and Ryan, 1994; Itoyama et al., 2000). Since glycerol is known to maintain cells from hyperthermic cell death, induced thermal protection and warming protection may be applied by adjustment of either protein or membranes (Henle and Warters, 1982). Our results demonstrated that glycerol in aphids exposed to repeated extreme heat exposure (by the end of the third cycle) is higher than the levels in aphids exposed to prolonged thermal extremes; and this is in accordance with the role of this compound during heat stress (Benoit et al., 2007).

Aphids are very sensitive to acute changes in temperature rather than the duration of stress (Davis et al., 2006; Jeffs and leather, 2014). We found significant changes in metabolite reserves after repeated high temperature exposure compared to the prolonged temperature exposure treatments with the same duration indicating that the number of extreme high temperature events is more important than the duration of extreme temperatures although the CT_{max} of aphids was constant for both temperature regimes.

Impacts of repeated high temperature on metabolic rate of aphids

Respiration is the first process restricted in animals at low and high temperatures, and is connected to the limitation of blood circulation and ventilation (Pörtner, 2001). We observed no significant differences in metabolic rate 24h after heating and this is in contrast to previous studies looking at metabolic rates at upper critical temperature (Klok et al., 2004; Boardman et al., 2013). Elevated standard metabolic rate after stressful conditions is a repair cost (Boardman et al., 2013). We measured active metabolic rate with increasing temperatures not resting metabolic rate or standard metabolic rate at 25°C. In addition, we also found that there was a lower rate of water loss after repeated heating: this may be due to aphids closing their spiracles to reduce water loss after repeated heating events, and possibly a strategy for heat resistance.

Perspectives

To date, the key assessment of environmental stress tolerance in the aphid, *M. persicae* has been at low temperatures and in most known studies; the aphids have been exposed to a single stress event. In natural conditions, *M. persicae* is exposed to repeated pulses of high temperature punctuated by periods of recovery. Furthermore, because aphids are known to respond differently to consistent versus more "normal" fluctuating temperatures, conclusions drawn from constant temperature studies may be unreliable. We suggest future experiments incorporate a repeated stress and recovery pattern into their methodologies to fully assess responses to extreme temperature exposure to reflect the

natural growing conditions of this species of aphid and also, incorporate biological and fitness studies to physiological studies at the same time to reach comprehensive achievements in this filed.

References

Alford, L., Blackburn,T. M. and Bale, J. S.2012a. Effect of latitude and acclimation on the lethal temperatures of the peach-potato aphid *Myzus persicae*. *Agricultural and forest entomology* **14**:69-79.

Alford, L., Blackburn, T. M. and Bale, J. S. 2012b. Effects of acclimation and latitude on the activity thresholds of the aphid *Myzus persicae* in Europe. *Journal of Applied Entomology* **136**:332-346.

Andersen, L. H., Kristensen, T. N., Loeschcke, V., Toft, S. and Mayntz, D. 2010. Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult *Drosophila melanogaster*. *Journal of Insect Physiology* **56**:336-340.

Andrew, N. R. 2013. Population dynamics of insects: impacts of a changing climate. In: The Balance of Nature and Human Impact. (ed Rohde K). Cambridge University Press.311-323.

Angilletta, M. J. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford, UK: Oxford University Press.

Back, J. F., Oakenfull, D. and Smith, M. B.1979. Increased thermal stability of proteins in the presence of sugars and polyols. *Biochemistry* **18**:5191-5196.

Bale, J. S., Harrington, R. and Clough, M. S.1988. Low temperature mortality of the peach-potato aphid *Myzus persicae*. *Ecological Entomology* **13**:121-129.

Basson, C. H. and Terblanche, J. S.2010. Metabolic responses of *Glossina pallidipes* (Diptera: Glossinidae) puparia exposed to oxygen and temperature variation: implications for population dynamics and subterranean life. *Journal of Insect Physiology* **56**:1789-1797.

Bauerfeind, S. S. and Fischer, K..2014. Simulating climate change: Temperature extremes but not means diminish performance in a widespread butterfly. *Population Ecology*. **56**:239-250.

Beardmore, J. A. and Levine, L. 1963. Fitness and environmental variation. I. A study of some polymorphic populations of *Drosophila pseudoobscura*. *Evolution* **17**:121-129.

Benoit, J. B., Lopez-Martinez, G., Michaud, M. R., Elnitsky, M. A., Lee, R. E. and Denlinger, D. L.2007. Mechanisms to reduce dehydration stress in larvae of the Antarctic midge, *Belgica antarctica*. *Journal of Insect physiology* **53**:656-667.

Bezemer, T. M., Jones, T. H. and Knight, K. J.1998. Long-term effects of elevated CO2 and temperature on populations of the peach potato aphid *Myzus persicae* and its parasitoid *Aphidius matricariae*. *Oecologia* **116**:128 -135.

Blacklock, B. J. and Ryan, R. O.1994. Hemolymph lipid transport. *Insect Biochemistry and Molecular Biology* **24**:855-873.

Blackman, R. L. and Eastop, V. F.1984. Aphids on the worlds crops, an identifcation guide. Wiley, New York.

Blackman, R. L. and Eastop, V. F. 2000. Aphids on the world's crops: An identification and information guide. John Wiley & Sons Ltd., Chichester.

Boardman, L., Sorensen, J. G. and Terblanche, J. S .2013. Physiological responses to fluctuating thermal and hydration regimes in the chill susceptible insect, *Thaumatotibia leucotreta*. *Journal of Insect Physiology* **59**:781-794.

Broadbent, L. and Hollings, M. 1951. The influence of heat on some aphids. *Annual Applied Biology* **38**.

Casagrande, R. A. and Haynes, D. L.1976. A predictive model for cereal leaf beetle mortality from sub-freezing temperatures. *Environmental Entomology* **5**:761-769.

Castañeda, L. E., Figueroa, C. C., Fuentes-Contreras, E., Niemeyer, H. M. and Nespolo, R. F.2009. Energetic costs of detoxification systems in herbivores feeding on chemically defended host plants: a correlational study in the grain aphid, *Sitobion avenae*. *Journal of Experimental Biology* **212**:1185-1190.

Chown, S. L., Hoffmann, A. A., Kristensen, T. N., Angilletta, M. J., Stenseth, N. C. and Pertoldi, C.2010. Adapting to climate change: a perspective from evolutionary physiology. *Climate Research* **4**3:3-15.

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.

Clark, M. S. and Worland, M. R.2008. How insects survive the cold: molecular mechanisms-a review. *Journal of Comperative Physiology.* B **178**:917-933.

Cloudsley-Thompson, J. L.1953. The significance of fluctuating temperatures on the physiology and ecology of insects. *Entomologist* **86**:183-189.

Clough, M. S., Bale, J. S. and Harrington, R.1990. Differential cold hardiness in adults and nymphs of the peach-potato aphid *Myzus persicae*. *Annual Applied Biology*. **116**:1-9.

Colinet, H., B. J. Sinclair, P. Vernon, and D. Renault. 2015. Insects in Fluctuating Thermal Environments. *Annual Review of Entomology* **60**:7.1-7.18.

CSIRO-ABM.2014. State of the Climate 2014. CSIRO and the Australian Bureau of Meteorology, Canberra.

Davis, J. A., Radcliffe, E. B. and Ragsdale, D. W. 2006. Effects of High and Fluctuating Temperatures on *Myzus persicae* (Hemiptera: Aphididae). *Environmental Entomology* **35**:1461-1468.

Dawson, T. P., Jackson, S. T., House, J. I., Prentice, I. C. and Mace, G. M. 2011. Beyond predictions: biodiversity conservation in a changing climate. *Science of The Total Environment* **332**:53-58.

Dixon, A. F. G. (2005). Insect Herbivore-Host Dynamics: Tree-Dwelling Aphids. *Cambridge,UK: Cambridge University Press*.

Feder, M. E. and Hofmann, G. E. 1999. Heat shock proteins, molecular chaperones, an dthe stress response: evolutionary and ecologicaly physiology*. Annual Review of Plant Biology* **61**:243-282.

Fischer, K., N. Kölzow, H. Höltje, and I. Karl. 2011. Assay conditions in laboratory experiments: is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? *Oecologia* **166**:23-33.

Gia , M. H. and N. R. Andrew.2015. Performance of the cabbage aphid *Brevicoryne brassicae* (Hemiptera: Aphididae) on canola varieties. *General and Applied Entomology* **43**:1-10.

Gillespie, D. R., Nasreen, A., Moffat, C. E., Clarke, P. and Roitber, B. D. 2012. Effects of simulated heat waves on an experimental community of pepper plants, green peach aphids and two parasitoid species. *Oikos* **121**:149-159.

Grigg, J. W. and Buckley, L. B .2013. Conservatism of lizard thermal tolerances and body temperatures across evolutionary history and geography. *Biology Letters* **9**:20121056.

Gu, H., Fitt, G. P. and Baker, G. H.2007. invertebrate pests of canola and their managemant in Australia: a review. *Australian Journal of Entomology* **46**:231-243.

Hazel, J. R.1995. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annula Review of Physiology* **57**:19-42.

Hazell, S. P., Neve, B. P., Groutides, C., Douglas, A. E., Blackburn, T. M. and Bale, J. S. 2010. Hyperthermic aphids: insights into behaviour and mortality. *Journal of Insect Physiology* **56**:123-131.

Hazell, S. P., Pedersn, B. P., Worland, M. R., Blackburn, T. M. and Bale, J. S.2008. A method for the rapid measurment of thermal tolerance in studies of small insects. *Physiological Entomology* **33**:389-394.

Hendrix, D. L. and Salvucci, M. E.1998. Polyol metabolism in homopterans at high temperature: accumulation of mannitol in aphids (Aphididae: Homoptera) and sorbitol in whiteflies (Aleyrodidae: Homoptera). *Comperative Biochemistry and Physiology of Zoology* **120**:487-494.

Henle, K. J., Peck, W. J. and Higashikubo, R.1983. Protection against Heat-induced Cell Killing by Polyols in Vitro. *Cancer Research* **43**:1624-1627.

Henle, K. J. and Warters, R. L.1982. Heat protection by glycerol in vitro. *Cancer Research* **42**:2171-2176.

Hoffmann, A. A., Chown, S. L., Clusella-Trullas, S. and Fox, C.2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology* .27:934-949.

Hoffmann, A. A. and Parsons, P. A.1997. Extreme environmental change and evolution. Cambridge, UK: Cambridge University Press.

Hottiger, T., de Virgilio, C., Hall, M. N., Boller, T. and Wiemken, A.1994. The role of trehalose synthesis for the acquisition of thermotolerance in yeast. II. Physiological concentrations of trehalose increase the thermal stability of proteins in vitro. *Eurpean Journal of Biochemistry* **219**:187-193.

Howling, G. G., Bale, J. S. and Harrington, R. 1994. Effects of extended and repeated exposures to low temperature on mortality of the peach-potato aphid *Myzus persicae*. _Ecological Entomology_ **19**:361 -366.

Huang, L. H., Chen, B. and Kang, L. 2007. Impact of mild temperature hardening on thermotolerance, fecundity, and Hsp gene expression in *Liriomyza huidobrensis.* _Journal of Insect Physioogy_ **53**:1199-1205.

Huey, R. B. 1991. Physiological consequences of habitat selection. _The American Naturalist_ **137**:S91- S115.

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A., Jess, M. and Williams, S. E.2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. _Philosophical Transactions of the Royal Society B_ **367**:1665-1679.

IPCC, 2014.2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Itoyama, K., Tojo, S., Yanagita, T. and Hardie, J.2000. Lipid composition in long-day and short-day forms of the black bean aphid, *Aphis fabae. Journal of Insect Physiology* **46**:119-125.

Jain, N. K. and Roy, I. 2009. Effect of trehalose on protein structure. *Protein. Sci*. 18:24-36.

Jeffs, C. T, and S. R. Leather .2014. Effects of extreme, fluctuating temperature events on life history traits of the grain aphid,*Sitobion avenae*. _Entomologia Experimentalis et Applicata_ **150**:240-249.

Ju, R. T., Gao, L., Zhou, X. H. and Li, B.2013. Tolerance to High Temperature Extremes in an Invasive Lace Bug, *Corythucha ciliata* (Hemiptera: Tingidae), in Subtropical China. *PLoS ONE* **8**:e54372.

Kellermann, V., Overgaard, J., Hoffmann, A. A., Fløjgaard, C., Svenning, J.C. and Loeschcke, V.2012. Upper thermal limits of Drosophila are linked to species distributions and strongly constrained phylogenetically. *Proceedings of the National Academy of Sciences.* **109**: 16228-16233 .

Kim, D. and Lee, Y. J.1993. Effect of glycerol on protein aggregation: quantitation of thermal aggregation of proteins from CHO cells and analysis of aggregated proteins. *Journal of Thermal Biology* **18**:41-48.

Klok, J. C., Sinclair, B. J. and Chown, S. L.2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *Journal of Experimental Biology* **207**:2361-2370.

Kostal, V., Renault, D., Mehrabianová, A. and Bastl, J.2007. Insect cold tolerance and repair of chill-injury at fluctuating thermal regimes: Role of ion homeostasis. *Comparative Biochemistry and Physioogyl* **147**: 231-238.

Krebs, R. A. and Loeschcke, V.1994. Effects of exposure to short-term heat stress on fitness components in *Drosophila melanogaster*. *Journal of Evoloution Biology* **7**:39-49.

Kunkel, H 1976. Membrane feeding systems in aphid research in *Aphids as Virus Vectors* (eds KF Harris and K Maramorosch), pp. 311-338. Academic Press

Lalouette, L., Kostal, V., Colinet, H., Gagneul, D. and Renault, D.2007. Cold exposure and associated metabolic changes in adult tropical beetles exposed to fluctuating thermal regimes. *FEBS J* **274**:1759-1767.

Lalouette, L., Williams, C. M., Hervant, F., Sinclair, B. J. and Renault, D.2011. Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations. *Comperative Biochemistry Physiology A* **158**:229-234.

Lamb, K. P.1961. Some Effects of Fluctuating Temperatures on Metabolism, Development, and Rate of Population Growth in the Cabbage Aphid, *Brevicoryne Brassica*. *Ecology* **42**:740-745.

Lean, J. L. and Rind, D. H.2008. How natural and anthropogenic influences alter global and regional surface temperatures: 1889 to 2006. *Geophysical Research Letters* **35**:1-6.

Lin, P. S., Hefter, K. and Ho, K. C.1984. Modification of Membrane Function, Protein Synthesis, and Heat Killing Effect. *Cancer Research* **44**:5776-5784.

Ma, G., A. A. Hoffmann,and C. S. Ma. 2015. Daily temperature extremes play an important role in predicting thermal effects. *Journal of Experimental Biology* **218**:2289-2296.

MacMillan, H. A., Williams, C. M., Staples, J. F. and Sinclair, B. J. 2012. Metabolism and energy supply below the critical thermal minimum of a chillsusceptible insect. *Journal of Experimental Biology* **215**:1366-1372.

Marshall, K. E. and B. J. Sinclair. 2011. The sub-lethal effects of repeated freezing in the woolly bear caterpillar *Pyrrharctia isabella*. *The Journal of Experimental Biology*. **214**:1205-1212.

Meats, A. 1976. Developmental and long-term acclimation to cold by the Queensland fruit-fly (*Dacus tryoni*) at constant and fluctuating temperatures. *Journal of Insect physiology* **22**:1013-1019.

Meng, F. G., Hong, Y. K., He, H. W., Lyubarev, A. E., Kurganov, B. I., Yan, Y. B. and Zhou, H. M..2004. Osmophobic effect of glycerol on irreversible thermal denaturation of rabbit creatine kinase. *Biophyicss Journal* **87**:2247-2254.

Mironidis, G. K. and Savopoulou-Soultani, M. 2010. Effect of heat shock on survival and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) adults. *Journal of Thermal Biology* **35**:59-69.

Newman, Y. M., Ring, S. G. and Colaco, C.1993. The Role of Trehalose and Other Carbohydrates in Biopreservation. *Biotechnology & Genetic Engineering Reviews* **11**:263-294.

Niehaus, A. C., Angilletta, M. J., Jr., Sears, M. W., Franklin, C. E. and Wilson, R. S. 2012. Predicting the physiological performance of ectotherms in fluctuating thermal environments. *Journal of Experimental Biology* **215**:694-701.

O'doherty, R. and Bale, J. S.1985. Factors affecting the cold hardiness of the peach-potato aphid *Myzus persicae*. *Annual Applied Biology* **106**:219-228.

Parsell, D. A. and Lindquist, S.1993. The function of heat shock proteins in stress tolerance: Degradation and reactivation of dameged proteins. *Annual Review of Genetics* **27**:437-496.

Portner, H. O.2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**:137-146.

Pörtner, H. O., Bennett, A. F., Bozinovic, F., Clarke, A., Lardies, M. A. and Lenski, R. E. et al.2006. Trade-offs in thermal adaptation: in need of a molecular to ecological integration. *Physiological and Biochemical Zoology* **79**:295-313.

Rahmstorf, S. and Coumou, D. 2011. Increase of extreme events in a warming world. *Proceedings of the National Academy of Sciences* **108**:17905-17909.

Ridley, E., Wong, A. C.-N., Westmiller, S. and Douglas, A. E.2012. Impact of the resident microbiota on the nutritional phenotype of *Derosophila Melanogaster*. *PLoS ONE* **7**:e36765.

Salvucci, M. E., Stecher, D. S. and Henneberry, T. J.2000. Heat shock proteins in whiteflies, an insect that accumulates sorbitol in response to heat stress. *Journal of Thermal Biology* **25**:363-371.

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

Sinclair, B. J., Terblanche, J. S., Scott, M. B., Blatch, G. L., Klok, C. J. and Chown, S. L.2006. Environmental physiology of three species of Collembola at CapeHallett, North Victoria Land, Antarctica. *Journal of Insect physiology* **52**:29-50.

Smith, H. A., Burns, A. R., Shearer, T. L. and Snell, T. W.2012. Three heat shock proteins are essential for rotifer thermotolerance. *Journal of Experimental Marine Biology and Ecology* **413**:1-6.

Somero, G. N.2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology* **213**:912-920.

Storey, K. B. and Storey, J. M. 2004. Metabolic rate depression in animals: transcriptional and translational controls. *Biological Reviews* **79**:207-233.

Strong, F. E. (1963). Studies on lipids in some homopterous insects. *Hilgardia* .34:43-61.

Sutherland, O. R. W.1968. Dormancy and lipid storage in the pemphigine aphid *Thecabious affinis*. *Entomologia Experimentalis et Applicata* **11**:348-354.

Tammariello, S. P., Rinehart, J. P. and Denlinger, D. L.1999. Desiccation elicits heat shock protein transcription in the flesh fly, *Sarcophaga crassipalpis*, but does not enhance tolerance to high orlow temperatures. *Journal of Insect Physiology* **45**:933-938.

Teets, N. M., Kawarasaki, Y., Lee, R. E., Jr. and Denlinger, D. L.2010. Survival and energetic costs of repeated cold exposure in the Antarctic midge, *Belgica abtarctica*: a comparison between frozen and supercooled larvae. *Journal of Experimental Biology* **214**:806-814.

Terblanche, J. and Chown, S. 2007. The effects of temperature, body mass and feeding on metabolic rate in the tsetse fly *Glossina morsitans centralis*. *Physiological Entomology* **32**:175-180.

Tollarová-Borovanská, M., Lalouette, L. and Koštál, V.2009. Insect cold tolerance and repair of chill-injury at fluctating thermal regimes: Role of 70 kDa heat shock protein experssion. *CryoLetters* **30**:312-319.

Vasseur, D. A., DeLong, J. P., Gilbert, B., Greig, H. S., Harley, C. D., McCann, K. S., Savage, V., Tunney T. D. and O'Connor, M. I.2014. Increased temperature variation poses a greater risk to species than climate warming. *Proceeding Biology Society* **281**:20132612.

Vorburger, C.2004. Cold tolerance in obligate and cyclical parthenogens of the peach-potato aphid, *Myzus persicae*. *Ecological Entomology* **29**:498-505.

Wang, H.S., Zhou, C.S., Gho, W. and Kang, L.2006. Thermoperiodic acclimations enhance cold hardiness of the eggs of the migratory locust. *Cryobiology* **53**:206-217.

Yocum, G. D., Greenlee, K. J., Rinehart, J. P., Bennett, M. M. and Kemp, W. P. 2011. Cyclic CO_2 emissions during the high temperature pulse of fluctuating thermal regime in

eye-pigmented pupae of *Megachile rotundata*. *Comparative Biochemistry and Physiology A* **160**:480-485.

Zhang, J., Marshall, K. E., Westwood, J. T., Clark, M. S. and Sinclair, B. J.2011. Divergent transcriptomic responses to repeated and single cold exposures in *Drosophila melanogaster*. *Journal of Experimental Biology* **214**:4021-4029.

Zhao, F., W. Zhang, A. A. Hoffmann, and C. S. Ma. 2014. Night warming on hot days produces novel impacts on development, survival and reproduction in a small arthropod. *Journal of Animal Ecology* **83**:769-778.

Chapter 3:

Aphid metabolite profiles and tolerance to fluctuating thermal regimes shaped by amino acid and sucrose quality.

This chapter is written as a standalone manuscript, a modified version of this manuscript is intended for journal submission

Chapter 4: Changes in the metabolites of the green peach aphid (*Myzus persicae*) during recovery from heat stress

This chapter is written as a standalone manuscript, a modified version of this manuscript is intended for journal submission

Chapter 5: General Discussion

5-1 Overview

It is critical to understand the impacts that global climate change will have on the thermal tolerance, fecundity, development time, abundance and distribution of aphid species. The research that I have carried out as part of my PhD is investigating how aphid physiology is affected by high temperatures and nutrition. Indeed, recent reviews of insect physiological patterns have once again highlighted the lack of information on physiological responses by aphids at repeated high temperature due to climate change (Lamb 1961; Powell 1974; Griffiths and Wratten 1979; O'doherty and Bale 1985; Davis et al. 2006; Powell and Bale 2006; Dunbar et al. 2007; Mehrparvar and Hatami 2007; Hazell et al. 2010; Hulle et al. 2010; Alford et al. 2012a, b; Chiu et al. 2012; Gillespie et al. 2012; Ma and Ma 2012; Jeffs and Leather, 2014; Zhao et al. 2014; Colinet et al. 2015; Ma et al,2015)

In this thesis, I assessed climate change responses for green peach aphid, *M. persicae,* species growing in Australia. I have generated new and important information relating to aphid responses to prolonged and repeated high temperature exposure (Chapter 2), impacts of repeated high temperature experiment on nutrition (Chapter 3), and impacts of different recovery time between repeated heat stresses (Chapter 4) in *M. persicae* in Australia. This work has gathered novel data on the influence of variation temperature and nutrition regimes on the thermal tolerance, metabolites and metabolic rate of *M. persicae* populations from Armidale in NSW, Australia in the context of predicted climate change.

Physiological variables of *M. persicae* populations were examined under high temperature extremes, with particular emphasis on discriminating critical thermal limits (CTL) and the effect of temperature acclimation on metabolites and respiration in the

laboratory. The observations made have given a greater insight into how these insects respond to different recovery time and nutrition in the environments and to temperature stresses.

5-2 Plasticity of thermal tolerance traits

Aphids with their capacity for rapid population growth quickly respond to acute changes in temperature (within just one generation), is considered to be of much greater importance than long term acclimation (Powell and Bale 2008). Based on different predictions, in general, the elevated surface and air temperatures, are anticipated to occur with climate change in future (Cannon, 1998; CSIRO-ABM. 2014; IPCC. 2014), which in consequence, lead to reduction in the thermal tolerance range of *M. persicae* resulting in a greater susceptibly of aphids to unpredictable pulse of extreme weather such as frosts and heat waves.

Based on results from Chapters 2 and 4 it appears that recovery times between high temperatures can repair injuries, as aphids exhibited higher levels protein and osmolyte: but there was no significant difference in their CT_{max} between treatments. One possible explanation is that the proteins and osmoloytes were upregulated during the recovery time, but upregulation was not great enough to change their thermal threshold between treatments (Tammariello et al., 1999); or the upper critical thermal temperatures threshold is very conservative in this species. I found that aphid nutrition had important key roles in *Myzus persicae* to cope with stress conditions (Chapter 3): Aphids reared on the high amino acids medium increased heat tolerance compared to aphids reared on the low amino acids medium.

The aphids reared on the amino acids-enriched medium showed an elevation in heat knockdown tolerance and the physiological basis responsible for this increase is not well known. One possible mechanism may be explained on the basis of management strategies of several stress conditions in aphids which involves the induction of heat shock proteins (Sorensen et al. 2003; Sinclair et al. 2007; Schmidt and Paaby 2008). However, further testing of this hypothesis is also recommended. Also, Archer et al. (2014) in their study mentioned that only bees fed a high protein diet could tolerate a combination of stressors without experiencing reduced survival.

5-3 Relationship between indices

Studies related to the relationships between different metabolites and upper thermal tolerance in aphid species is limited to a small number of studies (Hendrix and Salvucci 1998), none of them consider the number of indices of thermal tolerance used in the current study at the same time.

In Chapters 2, 3 and 4, I addressed the metabolite change after heat stress in *M. persicae*. In chapter 2 and 4, I used metabolite assays to survey biochemical changes accompanying heat stress responses at different recovery time and number of repetition of heat stress. Whilst in Chapter 3, I used different concentration of amino acids and sucrose in the diet experiments to test the role of these two vital biochemical elements in phloem sap during heat exposure.

While repeated heat stress failed to significantly alter the thermal tolerance and respiration in *M. persicae*. Taken together, the results indicated that repeated heat stress in *M. persicae* stimulated production of glucose, glycerol as a osmolytes to manage osmotic pressure: consumption of trehalose and triglyceride is used as a source of energy; and

production of glycogen is a source of water in heating time and aphids produce more protein under heat stress.

Based on results of Chapter 4, speed of recovery is different between different types of metabolites. Indeed, repeated heat stress altered the metabolic signature during recovery from heat stress; aphids exposed to repeated heat stress with long recovery time had a lower degree of metabolic perturbation compared to those with a short recovery time. Heat stress also caused major disruption of metabolism in the short recovery treatment groups. The level of nearly every metabolite measured was significantly altered during recovery from heat stress in long recovery treatment groups. But speed of this process is different between different metabolites.

5-4 Limitations of current study

While the framework used in this study allowed me to predict the mechanisms underlying heat stress responses of *M. persicae*, there are some limitations to the framework. One of the main limitations is that each species is considered in isolation and as a single population. Interactions such as competition, predation and parasitism are all likely to be impacted by climate change, resulting in species composition shifts and biocontrol failures (Gillespie et al. 2012).

While the aphids' response to heat is worthy of investigation regardless of the presence or absence of symbionts, knowing which secondary symbionts the aphids are or are not infected with is an important factor when considering the broader claims made regarding aphid tolerance to heat. Similarly, the primary endosymbionts of aphids (*Buchnera aphidicola*) are highly susceptible to heat stress, and likely have a large impact on

the tolerance or lack thereof in *Myzus persicae*. Unfortunately, because of time and equipment constraints, the presence of secondary symbionts in experimental groups could not be tested for in the current study. However, in a study by Hazell et al. (2010), no secondary symbionts known to confer heat tolerance were detected in clones of *M. persicae*, a finding further supported by Russell et al. (2003).

My study is relevant in view of the global changes that our planet is experiencing, as it can explain aphids resistance or susceptibility to these changes. But without further ecological behavioural information, and measurement of reproductive output, it is a difficult to fully interpret the results. Unfortunately, because of time, development, fecundity and fitness could not be tested in the current study.

There is pseudoreplication in this study as all aphid individuals were generated from a single population, and kept in a single incubator. As it was not possible using of 12 incubators at the same time in one day. I randomised across all treatments to reduce its influence. Also, is not possible measure various physiological characters, such as respiration, for all of samples in a one day. So, in all of the studies we have to separate each replicate in a one day due to limitation of the time and equipment. I do not believe that this is a fatal flaw in the experimental setup, as it was randomised across all treatments.

5-5 Future directions

In this thesis I demonstrated a framework to understand the response of *M. persicae* to repeated heat stress and vital role of nutrition and we found distinctive differences which are critical in understanding the role of diet in modifying stress tolerance and metabolic rate among aphids. The ability of aphids to manage various kinds of challenges such as stress

resistance is directly influenced by their dietary compositions. Thus, mystudy suggests that the nutritional composition of the diet plays vital role in aphid's responses to repeated heat stress.

The methods outlined in chapters of this thesis provided an understanding of different aspects of physiology in *M. persicae*. However, there are several directions for future research in order to understand the response of *M.persicae* to climate change in finer detail and to also make this framework more broadly applicable to other pest invertebrate species. In this final section, I suggest ways to address the limitations described above and discuss how new tools might allow for these processes to be incorporated into this framework.

One of the main future working is that each species is considered in field population with presence interactions such as competition, predation and parasitism because all of them likely to be impacted by climate change, resulting in species composition shifts and biocontrol failures (Gillespie et al. 2012).

As we know the secondary symbionts and primary endosymbionts of aphids (*Buchnera aphidicola*) are highly susceptible to heat stress, and likely have a large impact on the tolerance or lack thereof in *Myzus persicae* attention to them is very useful and suggested in future studies. In addition to the view point of biological working and fitness measurements, these factors will create good indexes to anticipate impacts of climate change in the first steps in aphids in the future research.

References

Alford, L., T. M. Blackburn, and J. S. Bale. 2012a. Effect of latitude and acclimation on the lethal temperatures of the peach-potato aphid *Myzus persicae*. *Agricultural and Forest Entomology* **14**:69-79.

Alford, L., T. M. Blackburn, and J. S. Bale. 2012b. Effects of acclimation and latitude on the activity thresholds of the aphid *Myzus persicae* in Europe. *Journal of Applied Entomology* **136**:332-346.

Archer, C. R., C. W. Pirk, G. A. Wright, and S. W. Nicolson. 2014. Nutrition affects survival in African honeybees exposed to interacting stressors. *Functional Ecology* **28**:913-923.

Cannon, R. J. C. 1998. The implications of predicted climate change in the UK, with emphasis on non-indigenous species. *Global Change Biology* **4**:785-796.

Chiu, M. C., Y. H. Chen, and M. H. Kuo. 2012. The effect of experimental warming on a low-latitude aphid, Myzus varians. *Entomologia Experimentalis et Applicata* **142**:216-222.

CSIRO-ABM. 2014. State of the Climate 2014. CSIRO and the Australian Bureau of Meteorology, Canberra.

Colinet, H., B. J. Sinclair, P. Vernon, and D. Renault. 2015. Insects in fluctuating thermal environments. *Annual Review of Entomology* **60**:123-140.

Davis, J. A., E. B. Radcliffe, and D. W. Ragsdale. 2006. Effects of High and Fluctuating Temperatures on *Myzus persicae* (Hemiptera: Aphididae). *Environmental Entomology* **35**:1461-1468.

Dunbar, H. E., A. C. C. Wilson, N. R. Ferguson, and N. A. Moran. 2007. Aphid Thermal Tolerance Is Governed by a Point Mutation in Bacterial Symbionts. *PLoS Biology*. **5**:e96.

Gillespie, D. R., A. Nasreen, C. E. Moffat, P. Clarke, and B. D. Roitber .2012. Effects of simulated heat waves on an experimental community of pepper plants, green peach aphids and two parasitoid species. *Oikos* **121**:149-159.

Griffiths, E. and S. D. Wratten. 1979. Intra and inter- specific differences in cereal aphid low-temperature tolerance. *Entomologia Experimentalis et Applicata* **26**:161-167.

Hazell, S. P., B. P. Neve, C. Groutides, A. E. Douglas, T. M. Blackburn, and J. S. Bale. 2010. Hyperthermic aphids: insights into behaviour and mortality. *Journal of Insect Physiology* **56**:123-131.

Hendrix, D. L. and M. E. Salvucci. 1998. Polyol metabolism in homopterans at high temperature: accumulation of mannitol in aphids (Aphididae: Homoptera) and sorbitol in whiteflies (Aleyrodidae: Homoptera). *Comparative Biochemistry and Physiology and zoology* **120**:487-494.

Hulle, M., A. C. Acier, S. Bankhead-Dronne, and R. Harrington. 2010. Aphids in the face of global changes. *Comptes Rendus Biologies* **333** 497-503.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jeffs, C. T, and S. R. Leather .2014. Effects of extreme, fluctuating temperature events on life history traits of the grain aphid, *Sitobion avenae*. *Entomologia Experimentalis et Applicata*. **150**:240-249.

Lamb, K. P. 1961. Some Effects of Fluctuating Temperatures on Metabolism, Development, and Rate of Population Growth in the Cabbage Aphid, *Brevicoryne Brassica*. *Ecology* **42**:740-745.

Ma, G. and C. S. Ma. 2012. Climate warming may increase aphids' dropping probabilities in response to high temperatures. *Journal of Insect Physiology* **58**:1456-1462.

Ma, G., A. A. Hoffmann, and C. S. Ma. 2015. Daily temperature extremes play an important role in predicting thermal effects. *Journal of Experimental Biology* **218**:2289-2296.

Mehrparvar, M. and B. Hatami 2007. Effect of temperature on some biological parameters of an Iranian population of the Rose Aphid, *Macrosiphum rosae* (Hemiptera: Aphididae). *European Journal of Entomology* **104**:631-634.

O'doherty, R. and J. S. Bale. 1985. Factors affecting the cold hardiness of the peach-potato aphid *Myzus persicae*. *Annals of Applied Biology* **106**:219-228.

Powell, S. J. and J. S. Bale. 2006. Effect of long-term and rapid cold hardening on the cold torpor temperature of an aphid. *Physiological Entomology* **31**: 348-352.

Powell, S. J. and J. S. Bale. 2008. Intergenerational acclimation in aphid overwintering. *Ecological Entomology* **33**:95-100.

Powell, W. 1974. Supercooling and the low - temperature survival of the green spruce aphid *Elatobiurn abietinum*. *Annals of Applied Biology* **78**:27-37.

Russell, J. A., A. Latorre, B. Sabater-Munoz, A. Moya, and N. A. Moran. 2003. Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. *Molecular Ecology* **12**:1061-1075.

Schmidt, P. S. and A. B. Paaby. 2008. Reproductive diapause and life-history clines in North American populations of *Drosophila melanogaster*. *Evolution* **62**:1204-1215.

Sinclair, B. J., A. G. Gibbs, and S. P. Roberts. 2007. Gene transcription during exposure to, and recovery from, cold and desiccation stress in *Drosophila melanogaster*. *Insect Molecular Biology* **16**:435-443.

Sorensen, J. G., T. N. Kristensen, and V. Loeschcke. 2003. The evolutionary and ecological role of heat shock proteins. *Ecology Letters* **6**:1025-1037.

Zhao, F., W. Zhang, A. A. Hoffmann, and C. S. Ma. 2014. Night warming on hot days produces novel impacts on development, survival and reproduction in a small arthropod. *Journal of Animal Ecology* **83**:769-77

Appendix I

A1.1 Equipment used

A   **B**

Fig 1. (A) HOBO data loggers can measure T, RH and light; (B) Programmable incubator
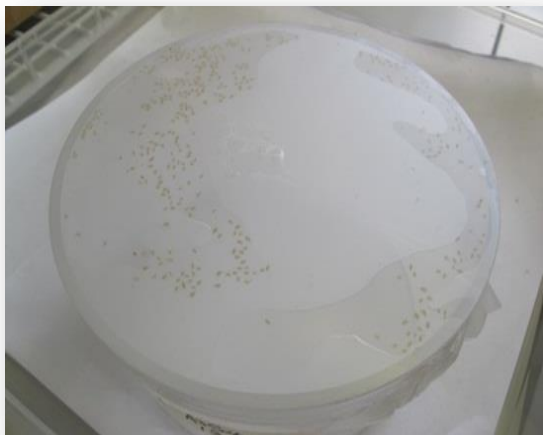

Fig 2.Diet cages were constructed from rigid clear PVC plastic tubes, 12cm in diameter (aphid feeding) and covered with a 7 x 7cm sheet of Parafilm laboratory film.

Fig 3. Vial frames with *Myzus persicae vials* were submerged into liquid in a water bath.

Fig 4. The water bath was connected to a computer which programmed the ramp of temperature at a different rate

Fig 5. Nutritional assay set up (96 well plate and Micropipettes)

Fig 6. Visible wavelength spectrophotometer (Epoch: Microplate Spectrophotometer) with absorbance at 544 nm, 540 nm and 750 nm and 96 well plates
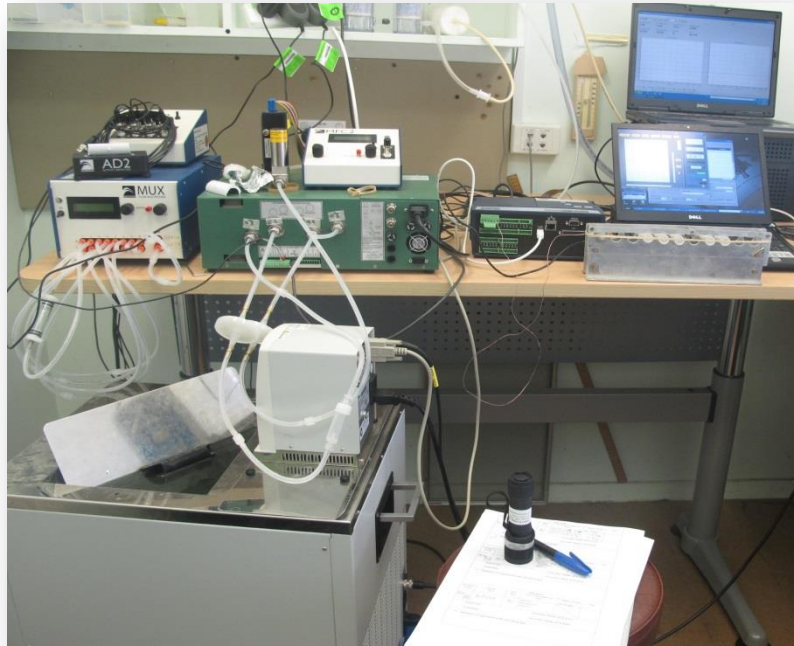
Fig 7. Metabolic Rate setup

Fig 8. Cuvette (5ml) for aphid respiration measurements

Appendix 2

Supplementary results of Chapter 3

Table 1. Proportional hazards analysis on resistance to thermal stress, respiration rate and metabolites with focus on the two factors, nutritional regime (protein or carbohydrate-enriched medium) and temperature. Abbreviations includes: temperature (T); control (C); prolonged (P); multiple (M); AA50SU1000 (1); AA150Su250 (2); AA150Su1000 (3).

Trait	Protein	Glucose	Trehalose	Glycerol	Triglyceride	CT_{max}	CO_2
C	14.4965b	1.2488c	0.86313a	0.78750c	0.61958a	40.3136a	0.57541516a
P	14.7306b	2.3579b	0.79188a	1.1500b	0.53588a	40.3548a	0.61483319a
M	17.4040a	2.8202a	0.35458b	1.49292a	0.28563b	40.5911a	0.57845999a
SEM							
P-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0513	0.2750
Diet							
1	16.4338a	2.7415a	0.56167b	1.48979a	0.52708a	41.1150a	0.69235754a
2	16.9817a	2.0556b	0.56083b	1.04583b	0.38171b	40.8611a	0.73551362a
3	13.2156b	1.6298c	0.88708a	0.89479b	0.53229a	39.2834b	0.34083717b
SEM							
P-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0035**	**<0.0001**	**<0.0001**
T * Diet							
11	14.3019cde	1.9600c	0.7619bc	1.0294cd	0.65938ab	40.9925a	0.74839170a
12	16.9044bc	1.0469d	0.6756dc	0.8044ed	0.37500cde	40.7149a	0.68836864ab
13	12.2831e	0.7394d	1.1519a	0.5288e	0.82438a	39.2334b	0.34493114c
21	15.2138bcd	3.0069a	0.6563cd	1.4819b	0.59750abc	41.0438a	0.74717607a
22	16.2350bc	2.2181bc	0.6362cde	1.0263cd	0.48513bcde	40.9231a	0.79447450a
23	12.7431ed	1.8488c	1.0831ab	0.9419cd	0.52500bcd	39.0976b	0.29420144c
31	19.7856a	3.2575a	0.2669e	1.9581a	0.32438ed	41.3087a	0.58606376b
32	17.8056b	2.9019ab	0.3706de	1.3069bc	0.28500ed	40.9453a	0.72634189a
33	14.6206ed	2.3013bc	0.4263cde	1.2138bc	0.24750e	39.5193b	0.37617585c
SEM							
P-value	**0.0086**	0.4650	0.1191	0.1865	**0.0015**	0.6831	0.0772

Supplementary results of Chapter 4

Table 2. Proportional hazards analysis on resistance to thermal stress, with focus on the two factors, recovery regime (Long recovery, mix recovery and short recovery) and time in every sampling time. Significant values in red**.** Control (C), Long recover (LR), short recovery (SR) and mixed recovery (MR).

Time 1	Glucose	Trehalose	Protein	Glycerol	Triglyceride	Glycogen
P value	0.6678	0.6148	0.9891	0.3769	0.2118	0.3113
C	1.0391a	0.9647a	9.2274a	1.05511a	0.9785a	0.70362a
LR	1.1302a	0.8053a	9.3746a	1.00248a	0.8497a	0.61004a
MR	1.2071a	1.0577a	9.0883a	0.95611a	0.7992a	0.70484a
SR	1.1214a	0.9660a	9.2154a	1.12758a	0.9219a	0.75597a

Time 2	Glucose	Trehalose	Protein	Glycerol	Triglyceride	Glycogen
P value	0.0002	0.0235	0.3874	<0.0001	<0.0001	<0.0001
C	1.1871c	0.9546a	9.7972a	1.09964b	1.02685a	0.69474b
LR	1.4290bc	0.8274ab	10.3884a	1.00612b	0.73537b	0.62908b
MR	1.7098ab	0.8097ab	10.7681a	1.20475b	0.54778b	0.62949b
SR	1.8851a	0.5061b	10.7023a	1.50866a	0.50352b	0.92256a

Time 3	Glucose	Trehalose	Protein	Glycerol	Triglyceride	Glycogen
P value	<0.0001	0.0728	<0.0001	0.2473	<0.0001	<0.0001
C	1.0794c	0.8484a	8.6082c	1.1559a	1.0110a	0.87148bc
LR	1.5678b	0.7686ab	10.0459b	1.0062a	0.7547b	0.73409c
MR	1.8094b	0.7856ab	10.9454b	1.0080a	0.5256c	0.90864bc
SR	2.2873a	0.4859b	13.7547a	1.2095a	0.4482c	1.22360a

Time 4	Glucose	Trehalose	Protein	Glycerol	Triglyceride	Glycogen
P value	<0.0001	0.0008	<0.0001	<0.0001	<0.0001	<0.0001
C	1.1370c	1.0084a	10.890bc	0.9763c	0.97a	0.81894b
LR	2.0396b	0.71abc	12.584b	1.4016b	0.71b	0.99773ab
MR	2.1214b	0.63bc	12.874b	1.7008ab	0.52c	1.00187ab
SR	2.9076a	0.4c	15.452a	1.8073a	0.42c	1.23648a