THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

Research Commons

http://researchcommons.waikato.ac.nz/

Research Commons at the University of Waikato

Copyright Statement:

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

The thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of the thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from the thesis.

The effect of warming temperatures on the physiology and behaviour of pollinating insects.

A thesis
submitted in partial fulfilment
of the requirements for the degree
Master of Ecology and Biodiversity in Science
at
The University of Waikato
by
René Devenish

2022

Abstract

Pollination is an important ecosystem service for both agricultural and natural systems. However, climate change is driving changes in the delivery of pollination services. One of the main influencing factors of climate change is rising temperatures. Most research on this issue has focused on phenological shifts (resulting in temporal mismatches between pollinators and plants), while the influence of warming on insect pollinator metabolism and pollination behaviour has not received equal attention. My research aims to fill both gaps by first testing if pollinator metabolism scales with temperature in pollinator body size according to the Metabolic Theory of Ecology (MTE). The MTE aims to explain a range of biological patterns and processes based on the underlying energetic constraints of organisms arising from metabolism. These constraints are primarily dependent on organismal body size and temperature, making the MTE highly relevant for predicting the impacts of warming on pollinating insects. Secondly, I tested if differences in air temperature can change pollinator foraging behaviour and if it can influence how pollinators alter their foraging behaviour in response to predation risk. I experimentally studied the patterns of foraging behaviour and metabolic scaling in four different pollinator species, *Apis mellifera* (honeybee), *Bombus terrestris* (bumblebee), *Eristalis tenax* (drone fly), and *Lucilia sericata* (blow fly) across a range of temperatures that represent a rise in average and maximum temperatures across New Zealand. My findings suggested that the MTE predictions only hold for certain species and only for the temperature-metabolism relationship for all species combined. Thus, the MTE may not be adequate for predicting the full impacts of warming on insect pollinators without further development, but the underlying theory itself still has uses in providing us with information on species-specific temperature sensitivities. Furthermore, I discovered that temperature and predation risk both affect insect pollinator behaviour, though not interactively, suggesting that warming will alter insect pollinator foraging behaviour, but not in concert with predation risk. In summary, this thesis provides meaningful insights into individual and species-level responses of pollinator behaviour and physiology to climate warming and the potential consequences for future pollination services.

This thesis is dedicated to Max and Mischief, the dogs that prove they truly are a man's best friend. May you both rest in peace.

Acknowledgements

First, I must thank my excellent supervisors, Dr Andrew Barnes and Dr Chrissie Painting. Andrew and Chrissie, not only have you been understanding and supportive during my struggles with mental health, but you have also been a constant source of inspiration in my life. You taught me so much about navigating the scary world of scientific research, and I do not believe I would’ve made it this far without the two of you guiding me through my master's. Andrew, I would like to thank you for always laughing at my awful jokes and for allowing me to become your sweet little girl's favourite person. And Chrissie, thank you for showing me that it is possible to succeed as a woman in STEM.

Secondly, I would like to extend my eternal gratitude to the University of Waikato for granting me the opportunity to study here and for the UoW Masters Scholarship, without which I would not have been able to afford to finish my master's.

Despite having two supervisors, I could pester, I was lucky enough to have Prof Mike Clearwater’s office close to the laboratory. Mike, not only were you kind enough to trust me with your Li-COR, but despite not being one of my supervisors, you were always willing to advise or help me whenever I experienced “technical difficulties” in the lab. For that, I will be forever thankful. I would also like to thank the University of Waikato (UoW) lab technicians, Danielle, Bruce, and Toni, for their help in ordering and organising all the equipment and other little things I needed.

To everyone who was part of the Paintings and Ecodiv lab groups, thank you for making my university experience so enjoyable. I enjoyed getting to know everyone and will greatly miss being part of our lab meetings and social gatherings.

Furthermore, I must thank the long list of people from organisations outside of the UoW who helped me in many ways. Dr Ummat Somjee for his help navigating the complex world of animal metabolism. Dr Anne Gasket for taking time out of her schedule to let me pick her brain about pollinator behaviour. Sam from Plant and Food research (PnF) Lincoln for supplying me with

drone flies and Biobees and Biosuppliers for supplying me with bumblebee hives and blow flies and for their excellent customer service

I especially want to thank the long list of people from Pnf Ruakura for their support and advice. This includes Dr Ashley Mortensen and Dr David Pattermore for granting me access to the labs at Ruakura, as well as for the help and advice they gave me along the way. They were a tremendous help when it came to planning my second chapter. Sarah, Grant, Max, Riva, and Lara for being ready and willing to answer all the many technical and theoretical questions and for their help in the lab. Bruce, for helping me figure out my lighting dilemma. Robin, Sarah and Ashley for their help guiding me through all the admin involved in gaining access to PnF Ruakura. And lastly, Lisa, for taking the time to meet with me to answer my questions on bumblebee behavioural experiments.

To the excellent artist, Auntie Hettie Rowley, thank you for your gift of beautiful sketches of my study species. I am beyond grateful for your contribution to my thesis. It really helped bring everything together in the end.

Next, I would like to thank all my friends in South Africa and Aotearoa who have supported me through this Masters. To Lara, Michaela, Grace, Tania, Milena, Simon, Gabby, Michelle, Margo, Jess, Emily, Welri, Dani, Cath, TJ, Amber, Megan, Sasha, Michael, Kahlia, Sean, Kyle, Jaden and Fez I couldn’t have done this without your love, friendship and endless encouragement.

Lastly, I must thank my family, including Ma, Pa and my favourite and only sister, Caitlin. You have always been able to see my potential even when I refused to believe in myself. Thank you for this and for always being my source of comfort and strength!

Table of Content

List of Figures

Chapter 2

List of Tables

Chapter 1

General introduction

1.1 Climate change and ecosystem services

1.1.1 Ecosystem Impacts of Climate change

Climate change is projected to cause increased air temperatures, lower precipitation, and more erratic and/or severe weather events (Massel et al., 2021; Tebaldi et al., 2011). Furthermore, these trends are not expected to occur in isolation. For example, higher mean temperatures are expected to result in more extreme, recurrent, and longer heat waves. Even geographical regions that are not currently as susceptible will experience increased heat wave severity in the future. (The Intergovernmental Panel on Climate Change [IPCC], 2022; Meehl & Tebaldi, 2004). Higher temperatures can also reduce soil moisture, increase surface runoff, and result in higher evapotranspiration (Dai, 2011; Massel et al., 2021), which can ultimately create drought stress, even in areas that are unlikely to have lower precipitation rates (Massel et al., 2021; Tebaldi et al., 2011). As established in the late 1900s, anthropological emissions of greenhouse gases are the main driving force behind climate change (Devi & Mishra, 2020; IPCC, 2022). Thus, without sustained, large-scale efforts to reduce greenhouse gas emissions and active management, our climate is likely to experience long-term changes resulting in ecosystem impacts that are permanent, severe, and widespread (Amano et al., 2010; IPCC, 2022). With all aspects of the global climate expected to change in some manner, along with climate change known to pose a threat to ecosystems, we must understand exactly how these changes can impact ecosystems in order to develop ecological mitigation strategies for the impacts of climate change (Devi & Mishra, 2020; Elias et al., 2017; IPCC, 2022).

In recent decades, there have been widespread research efforts to investigate how climate change impacts biodiversity, revealing several important conclusions. Firstly, we know impacts vary among species (Arribas et al., 2012), including those that are closely related. Secondly, we know climate change commonly causes extinction, as well as changes in species abundances and geographical distributions (Amano et al., 2010; Arribas et al., 2012; Elias et al., 2017; Parmesan, 2006; Traill et al., 2010), due to a reduction or loss of suitable habitats (Elias et al., 2017; Giannini et al., 2012; Traill et al., 2010). Distributional changes can result from either range shifts or reduced range size as species try to track geographical shifts in their preferred climatic niches (Elias et al., 2017; Kuhlmann et al., 2012). Other common impacts include physiological changes (Arribas et al., 2012; Parmesan, 2006) and changes in the timing of biological events (Amano et al., 2010; Arribas et al., 2012; Elias et al., 2017; Parmesan, 2006). The combination of these impacts will ultimately alter the ability of ecosystems around the globe to provide the many services humans rely upon.

1.1.2 Ecosystem services and the impact of climate change

Among the ecological impacts of climate change, the ability of the environment to continue supporting human needs through so-called ‘ecosystem services’ is expected to decline (Osman & Shebl, 2020; Scholes, 2016; Traill et al., 2010). While the exact definition is still debated, ecosystem services can be defined as “the set of processes through which natural ecosystems contribute to human well-being” or “the benefits which people derive from ecosystems” (Kerr et al., 2015; Kumar, 2012; Osman & Shebl, 2020; Scholes, 2016). These services are typically divided into four major categories, provisioning, supporting, regulating, and cultural services (Osman & Shebl, 2020; Reid et al., 2005; Scholes, 2016). As ecosystem services are essentially a way for humans to categorize the notoriously complex natural world, services may fall into multiple categories (Osman & Shebl, 2020). Pollination, the mutualistic plant-pollinator relationship in which animals transfer pollen in exchange for food (Devi & Mishra, 2020), is an excellent example of such an ecosystem service, which can be classified as either a regulating or supporting service (Scholes, 2016). This is because pollination can be considered to help “regulate” the reproduction of flowering plant species, thereby ensuring ecosystem stability or to indirectly “support” the delivery of other services such as carbon storage (Kremen et al., 2007; Osman & Shebl, 2020; Scholes, 2016).

Multiple sources have stated that an altered climatic environment plays a prominent role in biodiversity loss (Amano et al., 2010; Arribas et al., 2012; Elias et al., 2017; Kerr et al., 2015) and that the vulnerability of ecosystem services is expected to substantially increase with climate change (Osman & Shebl, 2020; Scholes, 2016; Traill et al., 2010). Furthermore, ecosystem services are expected to have greater vulnerability closer to the poles, in drier regions and where species or functional diversity is low (Scholes, 2016; Traill et al., 2010). Contrary to general expectations, and because of the non-linear relationship between temperature and metabolic rates, it has also been suggested that tropical organisms may be even more strongly impacted by global warming (Dillion et al., 2010). Thus, the risks posed to ecosystem services are not likely to be simple and will possibly depend on the specific organisms responsible for providing these services.

1.2 Insect pollination

1.2.1 Why is insect pollination important?

One of the most well-known ecosystem services at risk to a changing climate is pollination, and in particular, animal pollination. Pollinators are vital in maintaining global biodiversity (Elias et al., 2017; Giannini et al., 2012; Osman & Shebl, 2020) and the functioning of terrestrial ecosystems (Dalsgaard, 2020; Osman & Shebl, 2020). Their importance lies in the role they play in plant-pollinator interactions. Most flowering plants rely on animals for sexual reproduction through cross-pollination (Dalsgaard, 2020; Devi & Mishra, 2020; Osman & Shebl, 2020). It has been estimated that 78% of temperate and 94% of tropical plant species are pollinated by animals (Elias et al., 2017; Ollerton et al., 2011). Additionally, the loss of animal pollinators has been observed to alter network structure and could cause interaction networks to degrade overtime (Burkle et al., 2013). Furthermore, plant-pollinator interactions are one way that evolutionary pressures act on plants and their animal pollinators (Klein et al., 2007), with natural selection driving the coevolution of interacting species. However, evolutionary changes are not unique to individual interactions (Thompson et al., 2021). Changes in one species can trigger changes throughout a food web through direct and indirect feedbacks (Thompson et al., 2021). Thus, insect pollinators play a crucial role in maintaining biodiversity and ecosystems stability, meaning that pollinator loss would ultimately be detrimental for entire ecosystems (Dalsgaard, 2020; Osman & Shebl, 2020; Thompson et al., 2021; Vanbergen & Initiative, 2013).

The importance of animal pollination can also be seen in our everyday lives, with one-third of agriculture and about 70% of crops being dependent on pollinators to ensure high-quality crop production (Dalsgaard, 2020; Elias et al., 2017; Giannini et al., 2012; Klein et al., 2007; Kremen et al., 2007; Osman & Shebl, 2020). Pollinator diversity is closely related to sustainable food production, with diverse communities of pollinators contributing to the resilience of pollination services with high economic value (Vanbergen & Initiative, 2013). One source has estimated the annual economic value of pollination services to be over €153 billion (Gallai et al., 2009), while others have estimated it to be between 235–577 billion US dollars (Dalsgaard, 2020; Devi & Mishra, 2020; Klein et al., 2007; Gallai et al., 2009; Osman & Shebl, 2020; Vanbergen & Initiative, 2013). Regardless of the exact economic value, pollination plays a crucial role in maintaining ecosystem functioning and stability in both natural and agricultural ecosystems (Reddy et al., 2012).

Despite many staple crops being wind/self-pollinated, animal-pollinated crops provide most of our essential micronutrients (Chaplin-Kramer et al., 2014; Klein et al., 2007; Vanbergen & Initiative, 2013). For example, it has been shown that the human populations of multiple countries (including Thailand, Romania, and Australia) depend on animal-pollinated crops for 50% of their vitamin A intake (Chaplin-Kramer et al., 2014). The majority of animal pollinators important to agricultural systems are insects, especially bees, which pollinate the flowering plants responsible for about 35% of global food production (Devi & Mishra, 2020; Elias et al., 2017; Klein et al., 2007). Additionally, both native and introduced bee species have been shown to significantly increase fruit and seed production in multiple crop plants (De Marco & Coelho, 2004; Elias et al., 2017). However, the insect orders Diptera (flies), Lepidoptera (butterflies and moths) and Coleoptera (beetles and weevils) also make up a large portion of insect pollinators (Osman & Shebl, 2020). If global food demand continues to increase, the loss of insect pollinators will result in crop shortages (Gallai et al., 2009; Giannini et al., 2012; Vanbergen & Initiative, 2013). Thus, concern about the global decline in many pollinator species and its consequences for food production has made it essential to understand the effects of climate change on pollination (Dalsgaard, 2020; Giannini et al., 2012; Hoover et al., 2012).

1.3 Impact of climate change on insect pollination

Climate change poses the most geographically pervasive and rapidly increasing threat, with potential impacts on insect communities, even including those unaffected by other anthropogenic changes (Wilson & Fox, 2021). Insects, which are ectothermic, are especially vulnerable to rising temperatures (Devi & Mishra, 2020; González et al., 2009; Sharma & Singla, 2022) and also of particular economic importance due to their role in pollinating plants. Ultimately, climate change is anticipated to impact insect pollinator ecology through various mechanisms, including changes in abundance, diversity, distribution, phenology, physiology, behaviour, and species interactions (Sharma & Singla, 2022).

1.3.1 Commonly studied impacts of climate change on insects

Animal pollination is clearly vital to natural and agricultural systems, which is why the effect of climate change on this ecosystem service has been studied so widely for the past two decades. Results of these studies have provided convincing evidence that services provided by pollinators are currently at risk from climate change (Biesmeijer et al., 2006; Elias et al., 2017; Scholes, 2016). Due to their ectothermic physiology, insects are particularly sensitive to changes in rainfall and temperature, as their activity levels are more directly related to the environment than to many vertebrates (Devi & Mishra, 2020; González et al., 2009). Furthermore, insects are commonly used as model organisms when studying ectotherms and their relationships with temperature because of their diversity and easy husbandry (Chown & Storey, 2006). As such, there is already considerable knowledge about the general impacts of global warming on insects, including those crucial for pollination.

One of the main issues that research has focused on to date is species extinctions, which has emphasised great concern for global declines in the diversity and abundance of pollinating insects (Biesmeijer et al., 2006; Devi & Mishra, 2020; Elias et al., 2017, Giannini et al., 2012). Extinction estimates in the literature indicate that rates of insect decline may be equal to or greater than that of plant and vertebrate taxa (Wilson & Fox, 2021). Moreover, a recent review of local insect surveys has found that terrestrial insect abundance and biomass have been declining by 1.11% per year and 10.56% per decade between 1925 to 2018 (van Klink et al., 2020). The drivers of insect declines and extinctions have also received much attention (Wilson & Fox, 2021). One reason, which has been established across many insect taxa, is geographic shifts (Chen et al., 2011; Giannini et al., 2012). Geographic shifts in species distributions occur as the climatic conditions of low altitude and latitude warm to the extent that temperatures exceed the thermal tolerances of the species occupying those habitats. When temperatures exceed thermal limits, certain species that prefer cooler habitats move poleward and/or uphill, where these habitats are becoming more suitable because of climate change (Wilson & Fox, 2021). The warming of the poles and high-altitude areas also provide areas into which species with high tolerance to climatic variability can expand their ranges (Löffler et al., 2019; Thomsen et al., 2016; Wilson & Fox, 2021). Consequently, the more vulnerable species may experience reduced range size, competitive exclusion, or introgression (i.e., hybridizations through repeated backcrossing). These three effects can lead to declines in insect abundances or even to extinction (Giannini et al., 2012; Wilson & Fox, 2021).

Geographic shifts resulting from climate change may also lead to mismatches in the habitats that interacting species occupy (Giannini et al., 2012; Schweiger et al., 2008). The consequences of mismatches caused by geographic shifts could be severe, causing the disruption or destabilization of ecological interactions, such as those between plants and animal pollinators (Dalsgaard, 2020; Devoto et al., 2007; Memmott et al., 2007; Tylianakis et al., 2008). Moreover, if one interacting species is more vulnerable to environmental changes, the possibility of disruption or destabilization increases. (Devoto et al., 2009). Despite little being known about the mechanisms behind the disruption of pollination networks (Classen et al., 2020), there is little doubt in the literature that rising temperatures are responsible for disrupting the energy-flows and trophic interactions that stabilise network architecture (Devi & Mishra, 2020; Visser & Both, 2005). Furthermore, rising temperatures have been found to drive increases and decreases in energy fluxes and trophic interactions (Fussmann et al., 2014; Sentis et al., 2017). Thus, warming-induced mismatches in the area occupied by plants and their insect pollinators could destabilise pollination networks' interactions, thereby altering energy flux and network architecture.

Apart from spatial mismatches between interaction pairs, temporal mismatches can also affect the network architecture of pollination networks. In pollinator-plant interactions, temporal mismatches are usually a result of phenological mismatches, which are caused by changes in developmental timing in either plant and/or pollinator species (Amano et al., 2010; Dalsgaard, 2020; Hegland et al., 2009). Phenological mismatches occur because plant and insect development is usually controlled by different environmental cues (Hutchings et al., 2018; Scholes, 2016). Thus, temperature changes can differentially alter the timing of flowering in plants and emergence or flight periods in pollinating insects, which disrupts the synchrony between plant-pollinator interaction pairs (Borghi et al., 2019; Gilman et al., 2010; Scholes, 2016). In many cases, plants are flowering earlier than usual as temperatures increase, causing flowers to bloom when pollinators are absent. Pollinating insects may also emerge earlier in the season because of warming temperatures, which could mean they may miss the blooming of their preferred plant species (Devi & Mishra, 2020; Gilman et al., 2010). Consequently, the disruption of plant-pollinator mutualisms can reduce plant reproduction, outcrossing and growth rates (Borghi et al., 2019; Burkle et al., 2013; Dalsgaard, 2020; Devi & Mishra, 2020; Memmott et al., 2007; Rafferty & Ives, 2011). Phenological mismatches can also reduce the nectar and pollen available to pollinators (Memmott et al., 2007), causing malnourishment and reduced growth rates in insects (Devi & Mishra, 2020). In extreme cases, phenological mismatches can lead to local plant and/or pollinator extinction or the loss of these crucial interactions. (Burkle et al., 2013; Dalsgaard, 2020; Devi & Mishra, 2020; Memmott et al., 2007;). As phenological mismatches and the threat they pose to pollination services are a commonly predicted result of climate change (Devi & Mishra, 2020; Hegland et al., 2009; Hoover et al., 2012; Memmott et al., 2007), continued warming is expected to increase the frequency of plant-pollinator mutualism disruptions (Hutchings et al., 2018; Scholes, 2016).

1.3.2 Less studied impacts of climate change on pollinators

1.3.2.1 Functional traits, physiology, and rising temperatures

Aside from changes to functional traits, climate change research has also shown little focus on the influence of climate warming on pollinator physiology (Scaven & Rafferty, 2013). Despite the lesser focus on this area, it is highly important to understand how rising temperatures can indirectly affect plant-pollinator networks/communities via other physiological changes (Gilman et al., 2010).

In plants, rising temperatures significantly affect flower size, number, and shape (Hoover et al., 2012; Miller-Struttmann et al., 2015; Scaven & Rafferty, 2013). Changes in any floral functional traits (i.e., characteristics that influence an organism's performance or fitness; Nock et al., 2016) influence how insect pollinators interact with plants. For example, bumblebees prefer large flowers and so may switch their preferences to flowers that have increased in size (Goulson, 2009; Hoover et al., 2012; Scaven & Rafferty, 2013). Thus, any changes in floral dimensions would impact a pollinator's ability to acquire floral resources (Miller-Struttmann et al., 2015; Scaven & Rafferty, 2013). In specialized plant-pollinator mutualisms, the functional traits of the interaction partners evolve to perfectly match one another (e.g., long-tongued pollinators and flowers with long flower depths), so any changes in their functional traits could render their mutualism invalid (Scaven & Rafferty, 2013). For generalist pollinators, floral resources would still be available, but floral structural changes could increase the energy cost of obtaining these resources (Harder, 1983; Scaven & Rafferty, 2013). Ultimately, if rising temperatures change floral functional traits, pollinator species' performance could be under threat. However, Miller-Struttmann et al. (2015) found, over a 40-year period, that changing climate caused reductions in the tongue length of two bumble bee species but no changes to the co-occurring flowers. While it may seem like there was no effect on the plants, this could indicate that the evolutionary pressure has yet to reach a threshold where it is strong enough to drive the co-evolution of floral traits. Thus, regardless of whether plants or pollinators experience morphological changes (Miller-Struttmann et al., 2015), the asymmetrical effects of temperature on the pollinator versus the plant gives rise to potential disruptions of plant-pollinator interactions.

1.3.2.2 Metabolism and rising temperatures

In addition to effects on functional traits, temperature can also affect individual insect pollinators' biological rates, such as activity, life span, growth, and reproduction rates. With temperatures rising, the activity patterns of species are expected to be constrained by their thermal limits and ability to thermoregulate (Scaven & Rafferty, 2013). Changes in pollinator insect activity are usually implemented to reduce stress and meet new metabolic demands. For example, activity changes could involve avoiding heat stress by travelling shorter distances to forage or increasing foraging to meet greater demands for energy (Huey & Kingsolver, 2019; Scaven & Rafferty, 2013). Rising temperatures are also known to affect growth rates, which also change the body sizes of the various insect life stages and can thus change how species interact with their environment or other organisms (Gilman et al., 2010).

Among insects, developmental rates are faster at higher temperatures, which is expected to be why smaller adults are seen at higher temperatures (Kingsolver & Huey, 2008; Scaven & Rafferty, 2013). Consequently, body size can affect pollination by impacting the efficacy of pollen transfer and foraging distance (Greenleaf et al., 2007; Sahli & Conner, 2007; Scaven & Rafferty, 2013). Pollen transfer efficacy refers to how many pollen grains are deposited on flowers during a single visit by a pollinator. Thus, the more pollen transferred per visit, the more efficient the pollinators are. For both bumblebees and drone flies, larger individuals deposit significantly more pollen per visit, thereby increasing their overall pollination efficiency (Willmer & Finlayson, 2014). In general, large-bodied pollinators also travel further than small-bodied pollinators when foraging (Everaars et al., 2018). Foraging distances measure how far pollen is transported between flowers, and a longer foraging distance may imply that fewer flowers are being pollinated in a fixed period. However, large-bodied individuals must visit more flowers than smaller-bodied individuals to make up for their greater food requirements (Everaars et al., 2018). Thus, visiting a greater number of flowers and travelling longer distances makes large-bodied pollinators more efficient than their smaller counterparts. (Everaars et al., 2018; Larsen et al., 2005). Additionally, their ability to travel longer distances means larger-bodied pollinators can exploit resources further away during times of scarcity or escape local overpopulation (Everaars et al., 2018). Therefore, as the size of pollinators is predicted to decrease with global warming, the efficacy of pollination and pollinator adaptability could potentially diminish.

Warmer temperatures associated with climate change could also affect the life span of pollinating insects. (Bosch et al., 2000; Scaven & Rafferty, 2013; Sgolastra et al., 2011). Two studies focusing on the solitary bee *Osmia lignaria* highlighted how high temperatures experienced during "pre-wintering" led to high metabolic rates, increased consumption of energy reserves, and losses in body weight. These warming impacts all resulted in reduced over-wintering survival rates and springtime longevity (Bosch et al., 2000, Sgolastra et al., 2011). Moreover, the negative impacts were mainly limited to the bees that emerged earliest after pre-wintering (Bosch et al., 2000). These so-called "early bees", or those with short pre-wintering periods, are also expected to be selected for under future warming scenarios (Sgolastra et al., 2011). When combined with shifts in flowering times, the dominant presence of early bees could cause plants to experience fewer pollinator visits. Additionally, simulations of pollinator losses have shown that a drop in pollinator visits can cause pollen limitation, leading to reductions in fruit or seed set. (Thomson, 2019). Thus, reductions in insect pollinator life span resulting from global warming could pose a serious threat to pollination services.

In pollinating insects and other ectotherms, temperature effects on biological rates, such as life span, have long been linked to the relationship between metabolism and temperature (Gillooly et al., 2001; Gilman et al., 2010; Prather et al., 2018). For instance, species that show metabolic rates with higher thermal sensitivities are expected to experience greater energetic costs at higher temperatures. Higher costs would translate to greater constraints on an animal's ability to acquire resources for processes such as reproduction or growth, thereby causing a reduction in these biological rates (Dillon et al., 2010; Huey & Kingsolver, 2019; Shah et al., 2020). Apart from thermal sensitivity, changes in metabolic rate may also control other warming impacts on insects, such as changes in functional traits, phenological mismatches or geographical shifts, simply because of how an insect's metabolism underlies energy requirements and resource acquisition. Tomlinson et al. (2017) provided a recent example of how metabolism indicates energy expenditure and its implications for higher-level ecology. The study focussed on the links between metabolism, nectar consumption and landscape degradation. Using radio isotope analysis, they revealed that metabolic rates and food intake were linked, with nectar consumption being significantly different across landscape types (Tomlinson et al., 2017). The link between individual metabolism and large-scale ecological processes becomes clearer from studies such as this one. However, research investigating these links and the implications for pollination under global warming scenarios is limited and presents an important focus for future studies.

While understanding the influence of temperature on energetic demands is essential in determining how global warming will impact different species, the broader ecological implications for most insects remain unknown (Dillon et al., 2010; Huey & Kingsolver, 2019; Shah et al., 2020). The Metabolic Theory of Ecology (MTE; Brown et al., 2004) has been suggested as a unifying theoretical framework within which we can predict how individual-level responses scale up to the ecosystem (and even biogeographic) level. The MTE is based on an exponential relationship between temperature and metabolism, as described by the Arrhenius equation (Abram et al., 2017; Gillooly et al., 2001), and a sub-linear power law relationship between metabolic rate and body mass that has also been shown for insect pollinators (Kaefer et al., 2012). Ultimately the MTE explains the role of metabolism in controlling rates of biological processes across all levels of biological organisation, from molecules to ecosystems (Abram et al., 2017; Allen & Gillooly, 2007; Brown et al., 2004; Chown & Storey, 2006; Gillooly et al., 2001; West et al., 1997, 1999). Some studies have consequently used the MTE to predict ecological processes by scaling up metabolic rates. One study used rates of primary production and community respiration along with the MTE to predict whether marine communities would act as CO_2 sources or sinks under future global climate change scenarios (López-Urrutia et al., 2006). Another study used MTE models alongside others to quantitatively predict whether herbivory rates and temperature could calculate population abundances (O'Connor et al., 2011). Despite studies, such as those above, showing the potential of the MTE, it remains unknown whether the MTE can provide accurate predictions for how insect pollinators, from the individual to community level, and the ecosystem services they provide will be impacted by climate change (Brown et al., 2004; Chown & Storey, 2006).

1.3.2.3 Pollination behaviour and rising temperatures

Climate change and pollination literature commonly assert that rising temperatures can modify pollinator behaviour, either directly or indirectly (Dalsgaard, 2020; Elias et al., 2017; Sharma & Singla, 2022). In particular, pollinator foraging behaviour is assumed to be sensitive to changes in temperature (Gérard et al., 2020). Despite the assertion that temperature can alter behaviour, there is little research explaining exactly how and which behaviours may be affected. One reason for the lack of data on pollinator behaviour and climate change is that behavioural changes mediated by rising temperatures are difficult to detect and explain (Musolin & Saulich, 2012). However, research investigating climate change effects across insect taxa has provided valuable insights into the influence of rising temperatures on pollinating insect behaviours, including movement, reproduction, and foraging (Abram et al., 2017; Fucini et al., 2014; Jerbi-Elayed et al., 2015; Sharma & Singla, 2022).

While there is little conclusive understanding of how temperature directly alters insect behaviours, it is typically assumed that warming increases metabolic rates, which triggers shifts in foraging behaviour, allowing individuals to meet their higher metabolic demands (Abram et al., 2017; Phillips et al., 2010). For example, a study on honeybee crop-loading decisions showed that bees carried heavier loads when exposed to increasing ambient temperatures. The authors also proposed that the most likely reason for the loading decisions made by the bees was due to changes in metabolic rates forcing the bees to maximise energetic efficiency while foraging (Afik & Shafir, 2007). This study by Afik and Shafir (2007) did not actually measure bee metabolism, showing that the lack of metabolic measurement in studies that assume the importance of metabolism to pollinator forging decisions and efficiency is actually quite common.

Besides altering foraging behaviours, temperature also limits an insect pollinator's dispersal ability by altering its locomotive behaviours, as seen in another insect, the parasitoid wasp *Trichogramma brassicae* (Jerbi-Elayed et al., 2015; Suverkropp et al., 2001). When exposed to different temperature treatments, the wasps showed altered walking activity, which reduced the area *T. brassicae* could search when looking for potential hosts (Suverkropp et al., 2001). For insect pollinators, changes in locomotion behaviours are important for foraging, dispersal and locating mates (Jerbi-Elayed et al., 2015; Phillips et al., 2010). Thus, any changes to pollinator movement resulting from rising temperatures could threaten resource acquisition, individual fitness, and, ultimately, the resilience of plant-pollinator networks (Phillips et al., 2010).

Changes to insect fitness are not only an indirect impact of altered behaviours but could be caused by direct changes in reproductive behaviour (Abram et al., 2017; Fucini et al., 2014; Jerbi-Elayed et al., 2015). Animals often alter their reproductive behaviour to overcome physiological or environmental limits to maximize lifetime fitness (Jerbi-Elayed et al., 2022; Moiroux et al., 2015). However, it is also possible that reduced habitat quality resulting from environmental changes (e.g., rising temperatures) could force a species to alter its behaviour in a way that negatively impacts its fitness. For example, when exposed to a neonicotinoid pesticide, the ground-nesting bee *Eucera pruinose* reduced pollen harvesting behaviours, was less likely to start building nests and produced significantly fewer offspring (Willis & Raine, 2021). Studies such as this indicate how a pollinator's environment can significantly affect its behaviour and reproductive success. Despite the lack of direct evidence, the potential for climate change to have similar effects on insect pollinator behaviour and reproduction is strong.

Behavioural changes are not limited to adult stages. Temperature can also indirectly affect adult behaviour via its influence during developmental stages (Jerbi-Elayed et al., 2022; Wu et al., 2011). For example, Jerbi-Elayed et al. (2022) revealed that adults of the aphid parasitoid (*Aphidius colemani*), which had developed at higher temperatures, showed less optimal foraging behaviours than those reared at lower temperatures. Moreover, there is evidence that the temperatures to which honeybee pupae are exposed can affect their adult behaviour because temperatures can alter proteins that make up their nervous system (Groh et al., 2004). Therefore, the variability of insect development in response to temperature must be considered to improve predictions of climate change impacts on insect behaviour (Jerbi-Elayed et al., 2022).

1.3.3 Consequences of warming for Plant-pollinator Networks

While most of the global warming impacts mentioned above have focused mainly on individuals or single interaction pairs, it is important to understand how these impacts can scale up to plant-pollinator networks. For example, one study found that climate change-induced phenological shifts in plant and pollinator species had the potential to decrease and even completely remove floral resources from pollinator networks, causing extinction cascades (Memmott et al.,2007). Various other studies have also shown species-level warming impacts can alter plant-pollinator interactions, disrupting or destabilising them (Burkle & Alarcón, 2011; Devoto et al., 2007; Hegland et al., 2009; Memmott et al., 2007; Scaven & Rafferty, 2013; Tylianakis et al., 2008).

While there is still very little known about the mechanisms behind the disruption of pollination networks (Classen et al., 2020; Hegland et al., 2009), we know that species interactions are likely to be less resilient to climate change than other biodiversity measures (e.g., species richness). Interactions are affected by factors that may not influence other biodiversity measures, such as phenology, behaviour, and physiology, which explains their low resilience (Burkle & Alarcón, 2011; Tylianakis et al., 2008). Moreover, there is considerable evidence in the literature that rising temperatures are responsible for causing changes in energy flux and trophic interactions that stabilise network architecture (Devi & Mishra, 2020; Durant et al., 2007; Fussmann et al., 2014; Sentis et al., 2017; Stenseth & Mysterud, 2002; Visser & Both, 2005). If warming decreases the energy flux between consumers and producers relative to changes in metabolic rates, we may see stabilization of animal populations, though this is expected to lead to extinctions of consumer species at higher trophic levels, which could also be the case for pollinators (Fussmann et al., 2014).

1.3.4 Variable impacts of climate change across insect pollinator species

Not only are insect pollinators at risk from climate change, but there can be remarkable interspecific trait differences that affect how they respond to change. In particular, the level of specialisation can often explain differences in species' vulnerability to climate change. Plants and animals with specialized interactions are often more likely to be at risk from environmental changes such as climate change (Shah et al., 2020). Phenological mismatch, for example, affects specialist pollinators more severely than generalists despite also reducing generalist diet breadth (Memmott et al., 2007; Warren et al., 2001). For example, some bumblebee species had their foraging season curtailed by climate change, reducing the availability of floral resources for queens establishing colonies (Memmott et al., 2010). Yet, in Sweden, the well-known generalist *Bombus terrestris* was found to increase abundance with increasing temperature (Herbertsson et al., 2021), highlighting that the way warming impacts pollinators depends on pollinator specialisation.

Specialisation is not limited to plant-pollinator species interactions but can also describe the thermal tolerances of certain species. In some cases, species may be thermal specialists that are significantly more sensitive to temperature changes then thermal generalists (Shah et al., 2020). Differences in species' thermal sensitives will translate to differences in climate change impacts across species. While thermal specialists may be negatively impacted by rising temperatures, other generalist insect pollinators may remain virtually unaffected or even benefit from new climate regimes (Rafferty & Ives, 2011; Stelzer et al., 2010; Vanbergen & Initiative, 2013). While it has yet to be studied, understanding the thermal specialisation of insect pollinators shows the potential to facilitate better understanding and predictions of species-specific responses to climate change responses.

The difference between insect pollinator responses to new climate regimes is not only limited to intraspecific trait variation but can also be affected by intraspecific trait variation (Moran et al., 2016). For example, various traits, such as age, sex, and size, all vary among individuals of a specific species and affect how individuals respond to rising temperatures (Moran et al., 2016; Musolin & Saulich, 2012). Variation in body size is especially important as it affects an insect's ability to thermoregulate, with larger insects being better at physiological thermoregulation but at a higher risk of overheating (Scaven & Rafferty, 2013). Size may also determine activity periods, dispersal tendency, growth, and metabolic rates (Moran et al., 2016; Scaven & Rafferty, 2013). The relationship between body mass and metabolic rates has long been debated in the literature (Brown et al., 2004; Glazier, 2015; Kozłowski & Konarzewski, 2005; Lemaître et al., 2014; Savage et al., 2004; West et al., 2002 etc.). While there is considerable support for a universal law that defines how body size scales with metabolism (Brown et al., 2004; Savage et al., 2004; West et al., 2002), other research has suggested this idea to be flawed or too simplistic (Glazier, 2015; Kozłowski & Konarzewski, 2005; Lemaître et al., 2014). Nevertheless, the widely observed relationship between body size and metabolic rate provides important information that may help us predict how some biological processes, including those involved in pollination, may respond to altered climate regimes (Brown et al., 2004; Glazier, 2015). Intraspecific differences in warming impacts highlight that, despite all species being affected by the same environmental drivers, each pollinator species and individuals within populations will experience unique circumstances and thus respond in subtly different ways (Musolin & Saulich, 2012).

1.3.4.1 The difference in climate change impacts between dipteran and hymenopteran species

Two key groups of insect pollinators, dipterans (fly) and hymenopterans (bee), may differ significantly in their responses to climate change. These two major insect pollinator orders have often been found to show opposite responses to climate and, thus, tend to partially replace each other where they coexist (González et al., 2009; Howlett et al., 2013).

As eusocial bees are constantly foraging for resources to sustain themselves and their colonies, their lives are considerably more energetically demanding than other non-eusocial insects, such as flies. (Corbet et al., 1993; González et al., 2009; Howlett et al., 2013). Some evidence suggests bees are more sensitive to changes in certain climatic conditions, with bee foraging being reduced in the presence of rain and low temperatures (González et al., 2009; Le Conte & Navajas, 2008). Moreover, it only takes small temperature shifts for changes in bee behaviour to be observed (Elias et al., 2017; González et al., 2009). However, other studies suggest bees (particularly *Apis* species) are excellent at buffering against extreme heat waves by increasing water collection. Bees also regulate other behaviours, such as fanning their wings, to cool down their colonies (Cook & Breed, 2013; Le Conte & Navajas, 2008). The ability of these eusocial species to rely on other colony members to thermoregulate means bees can divide thermoregulation labour between workers, allowing them to maintain a constant temperature within the hive (Cook & Breed, 2013). However, there is considerable variation in the thermoregulation abilities across bee species, and those with less active colony thermoregulation tend to have nest temperatures closer to ambient temperature. Thus, under climate change, nest temperatures can more easily exceed the critical thermal limit of the species (da Silva et al., 2017). Species such as the European honeybee (*Apis mellifera*), which are very good at colony-level thermoregulation, show a remarkable ability to adapt to extreme heat. Furthermore, *Apis mellifera* is expected to do well in regions where temperatures are expected to increase substantially with climate change if sufficient water sources are present (Bordier et al., 2017) but may suffer in regions that will experience significant increases in rainfall (Le Conte & Navajas, 2008). Despite the suspected resilience of *Apis* species, other bees may be far more vulnerable to climate change.

Many bumblebee species are considered to be negatively impacted by altered climatic regimes (Herbertsson et al., 2021; Soroye et al., 2020). However, some species, such as *B. terrestris* (who are super-generalists), do well under present-day conditions and are expected to be less sensitive to climate change (Clavel et al., 2011; Herbertsson et al., 2021; Soroye et al., 2020). Nevertheless, at extreme temperatures, even *B. terrestris* is not expected to do as well as *Apis* species. For example, it has been shown that *B. terrestris* foraging rates at 26 °C are reduced in comparison to those observed at 21 °C (Decamps et al., 2021), whereas this is not the case for *A. mellifera*. Furthermore, *Bombus terrestris's* reproduction investment increases at higher temperatures, and because of faster colony development, they also need to invest more in wax and silk material to protect offspring and cool the colony (Zaragoza-Trello et al., 2021). On the other hand, *Apis mellifera* colonies need to invest more energy into warming their broods below temperatures of 33°C (Abou-Shaara et al., 2017). As temperatures rise, workers also get smaller, reducing foraging distance and efficacy (Everaars et al., 2018), which is expected to impact bumblebee colony fitness more negatively (Guiraud et al., 2021). Ultimately, many bees, including bumblebees, are expected to be at risk of climate warming, despite the expected variable responses of *B. terrestris* and *Apis* species.

Under future climatic conditions, flies are anticipated to become more important for pollination at sites with low temperatures and higher levels of rainfall (Devoto et al., 2005; González et al., 2009; Howlett et al., 2013). The increasing dominance of dipteran pollinators at cold and wet sites could be because flies have lower individual energetic demands than bees (González et al., 2009). Their lower energetic requirements are likely because flies tend to maintain lower thorax temperatures, sun-bask on heliotropic flowers to gain heat to sustain flight muscles and travel shorter distances between shelter and floral resources (González et al., 2009; Howlett et al., 2013). Furthermore, Diptera is one order of insects that often demonstrate rapid cold hardening, i.e. fast improvement in tolerance to low temperatures (Nyamukondiwa & Terblanche, 2010). Many dipteran taxa not only forage in cold and wet environments but can also be found across a broad range of climates. For example, the fly species *Eristalis tenax* and *Lucilia sericata* are both active across broader temperature ranges than the honeybee *A. mellifera* (Howlett & Gee, 2019; Shimomae et al., 2022). Broad environmental tolerances and greater foraging flexibility are why flies are expected to replace bees at cold and wet sites (Howlett et al., 2013).

If fly species are to avoid extinction due to climate change, they will do so either via dispersal to more suitable habitats or by adapting to new climatic conditions (Banda et al., 2021). Dipteran species are, in fact, more capable of avoiding unsuitable climates through dispersal than most hymenopterans because flies do not create nests or provide brood care as many bee species do. Thus, their movement is not as restricted (Howlett & Gee, 2019). For example, the drone fly (*E. tenax*) is able to migrate across vast distances, with individuals even flying over open oceans, crossing distances of at least 75 km (Howlett & Gee, 2019; Krčmar et al., 2010). Climate change may also cause some fly taxa to experience shorter developmental periods, with reduced pupae mortality rates and longer foraging seasons (Heath, 2021). For example, increases in mean air temperature are expected to cause the blow fly (*L. sericata*) to have more generations and longer developmental periods each year (Maksym et al., 2021). Increases in generation number and developmental periods may not necessarily improve fly survival, as higher metabolic demands during non-feeding life stages could offset any benefits gained from global warming. (Muntzer et al., 2015). Moreover, exposure to extreme temperatures (over 40ºC) can lead to the death or deformation of pupal stages, as seen in the oriental fruit fly, *Bactrocera dorsalis* (Xie et al., 2008). Presumably, fly species, such as *L. sericata* and *E. tenax,* will remain relatively resilient to climate change thanks to their broad environmental tolerance as long as they are not regularly exposed to extreme temperatures. These major differences in the way that flies and bees respond to changing temperatures make them interesting groups to compare when investigating the responses of pollinating insects to climate change. In doing so, these comparisons may shed light on the varying responses of pollinating insects and help to develop broader predictions of climate change's impact across multiple taxa.

1.4 Thesis outline

In this thesis, I will investigate the changes global warming could impose on insect pollinator physiology and behaviour by focusing on four generalist pollinator species, *Eristalis tenax, Lucilia sericata, Apis mellifera, and Bombus terrestris*. These four species are found in many countries across the globe (Howlett et al., 2013; Rader et al., 2020) and have strong evidence to support their importance as pollinators (Herrmann et al., 2019; Howlett & Gee, 2019; Klein et al., 2007; Rader et al., 2020; Velthuis & Van Doorn, 2006). Moreover, the broader environmental tolerance that makes these four species so successful globally (Carmo et al., 2022; Howlett & Gee, 2019; Shimomae et al., 2022) suggests that they will be more tolerant to global warming then native fly and bee species. Typically, climate change studies cover vulnerable species, but by focusing on more tolerant pollinating insects, I could possibly provide a more conservative estimate of the impacts of climate change. Furthermore, if I do identify any warming effects, it would emphasise how sensitive species are likely to be more severely affected in the future. Thus, the four species I chose will provide exciting subjects for investigating physiological and behavioural changes that may occur in insect pollinators.

Here, I address two major research aims that are fundamental to our understanding of how insect pollinators will respond to a warming climate. Firstly, using temperature-controlled water baths and an insect respiration chamber attached to a Li-COR 6400XT, I estimate changes in metabolic rates for each of the four species across a broad range of temperatures. I then use the metabolic rates to test the utility of the Metabolic Theory of Ecology for predicting climate change impacts on pollinating insects. Furthermore, I use the relationship between metabolism and temperature to elucidate the thermal sensitivities of each species, which can help understand how well each species will respond to climate warming. Secondly, I use highly controlled laboratory-based behavioural assays to test the effects of temperature and predation on insect pollinator foraging behaviours. I analyse behavioural recordings to measure each species' behavioural response to simulated predation risk and different temperature treatments. This allows me to investigate if and how temperature and predation interact to alter foraging behaviour in insect pollinators. Thus, my research develops an understanding of how insect pollinators may respond to multiple environmental change drivers in the future.

Chapter 2

Rising temperatures and insect pollinator metabolism.

2.1 Abstract

For decades, the broad application of the Metabolic Theory of Ecology (MTE) to predict ecological patterns and processes has been a major source of contention among ecologists. Nevertheless, the MTE could hold great promise for its ability to explain and predict the responses of functionally important organisms, such as pollinating insects, to a warming climate. Here, I tested whether the main predictions of the MTE hold for four pollinating insects, namely, *Eristalis tenax*, *Lucilia sericata*, *Apis mellifera*, and *Bombus terrestris*. In addition to measuring the body mass of each individual, a closed respirometry system was used to measure insect CO_2 production rates across a range of temperatures (15- 35°C). Data from these laboratory experiments allowed me to test the hypotheses that: 1) metabolism will scale with body mass following a ¾ power-law relationship, 2) metabolism positively scales exponentially with temperature according to Arrhenius' Law, 3) the slopes (i.e., activation energy) of the temperature-metabolism relationship falls within the range of -0.60 and -0.70 eV, and 4) hypotheses 1-3 hold for individual species and across multiple species. My findings suggest that the Metabolic Theory of Ecology, as it stands, is only partially applicable across different species of insect pollinators, such that MTE-derived predictions of temperature responses can be made, even though body size does not appear to scale with metabolism as expected. Nevertheless, the scaling relationships presented in this study provide species-specific metabolic scaling coefficients that are crucial for developing models of how pollinator species may be affected by climate change.

2.2 Introduction

Environmental temperature plays a central role in the ecology of ectothermic animals, including pollinating insects. Since their body temperatures rely so heavily on ambient temperatures, it affects everything from their physiology to the ecology of the systems they are a part of (Buckley et al., 2012; Irlich et al., 2009). Temperature is possibly the most critical factor affecting insect metabolism, with metabolic rates known to increase exponentially along with temperature in these organisms (Angilletta et al., 2004; Bjørge et al., 2018; Brown et al., 2004; Dillon et al., 2010; Gillooly et al., 2001; Prather et al., 2018). The effect of temperature on metabolism is crucial for individual fitness (Pettersen et al., 2016; Réveillon et al., 2019), as metabolic rates determine other biological rates such as growth and development (Bjørge et al., 2018; Brown et al., 2004; Dillon et al., 2010; Gillooly et al., 2001; Irlich et al., 2009; Kozłowski & Konarzewski, 2004; Prather et al., 2018; Shah et al., 2020;). Consequently, these biological rates are known to be a key predictor of ectotherm population dynamics (Angilletta et al., 2004; Bjørge et al., 2018) and can influence their geographic distribution and local abundance (Shah et al., 2020; Terblanche & Chown, 2007; Vannote & Sweeney, 1980).

With rising temperatures being an immediate consequence of climate change (Arribas et al., 2012; Tewksbury et al., 2008) and continuing to increase in record time, understanding the biological responses of pollinating insects to warming temperatures is vital to predicting current and future warming impacts (Arribas et al., 2012; Shah et al., 2020). Our ability to predict these impacts is essential as animal pollination is crucial to the function of natural and agricultural systems, but the ecosystem services provided by pollinators are at risk from climate change (Biesmeijer et al., 2006; Elias et al., 2017; Goulson, 2009; Potts et al., 2010; Scholes, 2016; Steffan-Dewenter et al., 2005). One area that has received considerable attention, but less so concerning pollinating insects, is how metabolism varies with temperature and, thus, how this affects species' vulnerability to climate change (Colwell et al., 2008; Parmesan et al., 2003; Shah et al., 2020). For example, only two studies have separately investigated how variation in ambient temperature can affect the non-flight metabolic rate of bumblebees (*Bombus terrestris*; Silvola, 1984) and honeybees (*Apis mellifera carnica*; Stabentheiner et al., 2003). In contrast, there are no studies to my knowledge that examine the temperature-metabolism relationship in drone flies or blow flies, both of which are important insect pollinators (Herrmann et al., 2019; Howlett & Gee, 2019). Moreover, studies that have focused on temperatures' effect on insect metabolism have primarily investigated responses to extreme temperatures by defining lethal and critical thermal limits (da Silva et al., 2017; Deutsch et al., 2008; Frazier et al., 2006; Kenna et al., 2021). To a lesser extent, the Metabolic Theory of Ecology (MTE) has also been used as a standard against which to compare the thermal responses of organismal metabolic rates (Irlich et al., 2009; Regaudie-de-Gioux & Duarte, 2012). However, the MTE literature has mainly been centred on other taxa apart from insects. The few studies that do focus on insects and the MTE have mostly tested the validity of this theory by measuring metabolic rates (Chown et al., 2007; Gudowska et al., 2017) or by testing if the thermal responses of other ecological patterns and biological rates match MTE predictions (Irlich et al., 2009; Keil et al., 2008). However, such tests are completely lacking for pollinating insects. Hence, there is a need for more research centring around temperature's influence on insect pollinator metabolism, with a particular focus on the MTE and its usefulness in predicting species' vulnerability to warming.

The MTE, in the simplest sense, is used to quantitatively define how metabolism scales with body size and temperature. However, its significance as a biological theory (Price et al., 2012) lies in its ability to explain how metabolism, because of its importance in resource assimilation and allocation, governs processes at all levels of biological organisation (Brown et al., 2004; Gillooly et al., 2001; West et al., 1997, 1999). In other words, it links species performance to large-scale patterns in biodiversity, such as the impact of climate change on ecosystem processes (Brown et al., 2004; Irlich et al., 2009). For example, by applying MTE concepts to the relationship between temperature and both handling time and attack rate of consumers, Rall et al. (2012) showed that population and food-web stability increased with temperature while attack efficiency decreased, lowering feeding rates and consequently reducing interaction strengths. Given the ability of the MTE to predict the impacts of warming on food web ecology, this suggests there is further scope to apply these concepts to understanding interactions in plant-pollinator systems.

For the MTE to fulfil its potential as a general mechanistic theory, the foundations on which this theory was built must be sound. The primary theoretical basis of MTE can be summarised in one equation (Arrhenius, 1889; Boltzmann, 1872; Gillooly et al., 2001; Kleiber, 1932),

$$ln\left(IM^{-\frac{3}{4}}\right) = -E\left(\frac{1}{kT}\right) + ln\,(i_0) \qquad (1)$$

that explains the mass-corrected metabolic rate, where I is the whole-organism metabolic rate and M is body mass, with a ¾ allometric exponent (Kleiber, 1932). This part of the equation (based on Kleiber's Law) predicts that metabolism scales with body size to ¾ power (i.e. an exponent of 0.75) (Kleiber, 1932). The right side of the equation is based on Arrhenius's Law ($e^{-E/kT}$), which predicts that metabolic rate scales exponentially with temperature, where E is the activation energy, k is Boltzmann's constant, and T is the absolute temperature in K (Arrhenius, 1889; Boltzmann, 1872). Therefore, the rearranged expression presented in equation 1 predicts that the natural logarithm of the mass-corrected metabolism should increase linearly as temperature decreases, i.e. as the inverse absolute temperature (1/kT) increases. Using 1/kT may seem counterintuitive, but it is merely a simplified way of mathematically expressing how metabolism increases exponentially with temperature. The slope of the equation, E, corresponds to the activation energy required by the biochemical reactions that make up metabolism which, for aerobic respiration, is predicted to fall within the range from 0.60 eV – 0.70 eV (Gillooly et al., 2001). Since E demonstrates how rapidly temperature causes metabolism to change, the MTE predicts that the thermal sensitivity of metabolic rate should be remarkably similar across different taxa (Gillooly et al., 2001; Irlich et al., 2009). Lastly, the intercept, i_0) gives the natural logarithm of the normalisation constant, which is used to explain residual variation (Brown et al., 2004; Price et al., 2012; Réveillon et al., 2019; Tarahovsky et al., 2020).

The assumption that activation energies and the ¾ mass scaling law are highly consistent across all living organisms allows the MTE to link individual physiology to higher levels of organisation and is why the MTE is considered fundamental to explaining ecological patterns (Brown et al., 2004; Irlich et al., 2009; Jerde et al., 2019; Réveillon et al., 2019). Therefore, the relationship between metabolism and temperature predicted by the MTE could allow us to predict how warming temperatures affect pollination success (McCallum et al., 2013; Shah et al., 2020; Terblanche & Anderson, 2010) across any species of pollinator. However, the theorised consistency of these relationships across all scales is still considered highly controversial, despite decades of studies testing its fundamental theories and validity (Irlich et al., 2009; Jerde et al., 2019; Price et al., 2012; Réveillon et al., 2019). Additionally, there has been little focus on pollinating insects and MTE. Thus, if one were to attempt to predict the ecological and evolutionary consequences of climate change for pollinating insects using the MTE, the validity of the theory's fundamental predictions must first be confirmed (Chown & Storey, 2006; Clarke, 2006; Irlich et al., 2009; Price et al., 2012).

This study aims to determine whether the MTE can be applied across two levels of organisation (i.e., the community and population level) and if the MTE can accurately predict the thermal responses of different insect pollinator species under warming temperatures. Using an experimental approach, I tested the ability of the fundamental equation of the MTE to predict the relationship between metabolism, temperature, and body mass for four key pollinating insects; European honeybee (*Apis mellifera),* buff-tailed bumblebee (*Bombus terrestris)*, drone fly (*Eristalis tenax)* and blow fly (*Lucilia sericata).* Specifically, I test four hypotheses central to the MTE: 1) temperature-corrected whole-organism metabolic rates should scale with body mass by an exponent of approximately 0.75 (i.e. the ¾ power allometric exponent), 2) mass-corrected metabolic rates (*ln*) should scale linearly and negatively with inverse absolute temperature, 3) the slopes from the relationships between temperature and metabolism should fall within the predicted range for the activation energy of $E \approx 0.60$–0.70 eV, and 4) the three hypotheses above should hold across the community level (i.e., across a group of species) and the population level. To address these hypotheses, I measured the respiration rates of 126 individual pollinator insects by running laboratory assays, in a closed respirometry system, across a series of different temperatures ranging from 15 to 35ºC. The respirometry experiments allowed me to fully characterise the four study species' metabolic response to increasing temperature. I then modelled these responses using the central equation of the MTE to determine whether the predictions of the MTE hold at both the population and community level for an experimental assemblage of insect pollinators. In doing so, this study determines if the MTE can provide a basis from which predictions can be made on how global warming may influence insect pollinators from individuals to communities.

2.3 Materials and Methods

2.3.1 Supply and care of study species

Eristalis tenax (Diptera: Syrphidae) and *Lucilia sericata* (Diptera: Calliphoridae) pupae close to hatching age were provided by Plant and Food Research (Lincoln) and Biosuppliers Live Insects, respectively. The pupae were kept in a large plastic container (60L) with sawdust as hatching media. The pupae and adult flies were kept in the Invertebrate Behavioural Ecology lab at the University of Waikato at 27°C. Once a minimum of five adults hatched out, respirometry experiments were started. Once the adult flies hatched, they were provided with food and water *ad libitum* using dental cotton rolls soaked in sucrose water (1:2 sucrose to water ratio) and placed in a petri dish. The cotton rolls were replaced every two days to ensure the flies had sufficient food and to prevent mould growth.

Adult *Apis mellifera* (Hymenoptera: Apidae) were collected from hives at Plant and Food Research (Ruakura) and kept in a glass mason jar (500ml) with a mesh lid. Individuals were collected by plugging the entrance to the hive with a sponge, vacuuming the returning foragers with an insect vacuum and then transferring them to the mason jar. Individuals were collected from a single hive to prevent them from attacking and killing each other in the jars. Using this method, I ensured all the honeybees collected were foragers of a similar age. After transportation to the Terrestrial Biology lab at Waikato University, they were kept at room temperature. The honeybees were supplied with water and food *ad libitum* via water-soaked dental cotton rolls and sugar cubes. All honeybees were kept overnight before trials were conducted the next day.

A standard hive of *Bombus terrestris* (Hymenoptera: Apidae) was obtained from Biobees Ltd. The hive was 34.5 x 24 x 21 cm in size and came with approximately 40 adults, including a queen. The hive was kept in the Invertebrate Behavioural Ecology lab at Waikato University at 27°C. Pellets of frozen pollen were defrosted and dropped through the trap door daily to ensure the bees had ample food. Additionally, the hive box was supplied with a sugar bladder in the base, enough to last the hive's lifetime.

For both fly species, sex was determined by looking at the size and placement of their eyes. The males of both species have larger eyes placed closer together than the females (Metcalf, 1913; Williams & Villet, 2014). Drone fly adults were large enough that the sexes could be differentiated with the naked eye. However, blow fly adults were too small, so they were frozen after each respirometry experiment and then viewed under an Olympus SZ61 dissecting microscope.

2.3.2 Respirometry assays

Prior to each respirometry assay, honeybees were placed in a fridge (2-4°C) for 15 minutes to reduce activity. An individual was selected at random, removed using soft plastic forceps and placed into a small urine sample container. The mass of the individual was weighed using a (Mettler Toledo NewClassic MF ML204 balance) to the nearest 0.001g. The bee was then transferred to the insect respiration chamber (IRC)

Bumblebees were collected using a urine sample jar by opening the manual entrance of the hive and waiting for a bee to climb out into the jar. The bees were then weighed, as explained above and transferred to the IRC. Both species of flies were calm and posed no threat of stinging, so they were not cooled in a fridge before handing. Like the bumblebees, the flies were collected in a urine sample container, and their weight was determined as above. Again, the flies were transferred to the IRC after weighing.

There is currently no standard experimental protocol for measuring insect respiration rates (Mänd & Karise, 2015). Therefore, I adapted methods from multiple sources to suit my study (Glazier et al., 2016; Lalouette et al., 2011; Muljar et al., 2012; Piccolomini et al., 2018; Salvucci & Crafts-Brandner, 2000). Respiration measures were recorded using a Li-COR 6400XT with a 6400-89 insect respiration chamber attachment. The instrument measures respiration rates as the change in VCO_2 (carbon dioxide production) over time. Incoming air was scrubbed of all CO_2 using soda lime. After scrubbing, a CO_2 mixer was used to inject a constant concentration of 400 µml CO_2 (as recommended by Li-COR). Dendrite was used as a desiccant to control humidity in the IRC. The desiccant control was set halfway between scrub and bypass, so the insects did not experience desiccation or overwhelmingly low humidity.

The desiccant and soda lime was replaced at the beginning of each day. Before each assay was run, a set of pre-assay checks were conducted in a standard order: the CO_2 mixer was calibrated, air and block temperature was confirmed to be the same, the pressure was established to be close to 100kPa, the leaf fan was checked, the flow control was tested to be working at both high and low levels, the H_2O and CO_2 sample and reference concentrations were set to zero, after the CO_2 mixer had been allowed to run for 5-10min it was set to the correct concentration, the Li-COR 6400XT was checked for leaks, and lastly, the reference and sample concentrations were matched

To control airflow, a set flow rate of gases coming into the IRC was determined and maintained for the duration of the metabolic assays, to control airflow. The flow rate was based on the trade-off between data quality (temporal resolution) and the potential for mixing problems. Mixing problems occur when a mixture of gases within a respiration chamber is not homogenous, which is problematic because the underlying mathematical assumptions involved in respirometry require the equal mixing of gases within the respiration chamber (Lighton, 2017). Low flow rates can increase the temporal resolution (reduces noise around the data) of the respiration data but can lead to mixing problems within the IRC. The opposite is true for high flow rates (Lighton & Halsey, 2011). I found the best flow rate from pilot experiments to be 110 µml/min, which was established by observing an individual's respiration rate for each species in real-time using the graphing function on the Li-COR 6400XT while changing the flow rate every 5 minutes. I tested the flow rate between 170-50 µml/min based on rates used in other studies (Muljar et al., 2012; Salvucci & Crafts-Brandner, 2000). The same flow rate was applied for all four insect species to ensure comparable results.

An iButton® was placed in the IRC to record air temperature every 60 seconds across each 1hr respiration trial, from which average temperature per trial was calculated. The iButton also provided a perch for each insect to rest (Somjee et al., 2021). When observing the bumblebees during pilot trials, I often found them dead or convulsing on their backs at the end of an experiment, which I assumed to be a result of stress. Therefore, bumblebee stress was alleviated by packing the IRC with inert cotton wool, which acted as a secondary perch. To keep the treatment consistent, I added inert cotton to the IRC for all the insect species except drone flies because the respirometry trials for drone flies were conducted before the bumblebee trials, and the flies showed no signs of stress without a second perch.

The IRC was placed into a water bath to control temperature. The insect was allowed to acclimate to the chamber and experimental temperature for 15 minutes before the V CO_2 was logged every 5s for 1hr. The acclimation period also allowed the air inside the IRC to reach the temperature. During pilot experiments, I started logging the data when the respiration rate was low enough to indicate the insect was in a stable state of rest (or inactivity). Insect activity was visually observed to ensure this was accurate. The insect was assumed to be resting when not flying or crawling around the IRC and stability was defined as a respiration rate change of 20 units or less (which was determined from previous observations). Visually observing the insects may have disturbed them, as large spikes in respiration were observed at the same time. Furthermore, I found that the insects never rested for a sufficient time to get an adequate measure of the resting respiration rate using the method above. However, making the room dark during the trial and not causing stress by observing them every few minutes allowed the insects to reach a low and stable respiration rate. The addition of inert cotton as a secondary perch also helped reduce activity. Instead, the activity was determined by observing the pattern of the recorded respiration data.

2.3.3 Temperature control/variation

Insects were exposed to 11 temperature treatments of between 15-35°C, with an approximate 2°C difference between each temperature treatment (table 2.1). The VCO_2 was recorded for a minimum of three individuals per temperature, thus, giving a total sample size of 33 insects per species. This temperature range was chosen to cover the average daytime temperatures and extremes New Zealand is likely to experience under a high carbon scenario leading to almost 4°C warming (Kenny, 2011; Ministry for the Environment, 2016) and based on the mean annual temperature data for New Zealand (from https://cliflo.niwa.co.nz/).

To determine the effect of temperature on pollinator respirometry, I controlled temperature within the IRC by placing it in a (Julabo F10 Immersion circulation) water bath. Before using the water bath, I attempted to set the temperature treatments using a cooler box fitted with a Tropicool

XC1204 power supply, Tropicool XC3000 thermoelectric refrigeration unit and a GCS-300 Micro-computer-based Temperature Indicating Controller. A thermocouple was placed within the cooler box alongside the IRC to record air temperature in the cooler box. Additionally, an ibutton was placed within the IRC to record the air temperature to which the insects would be exposed. The Li-COR 6400XT's gas supply lines entered the cooler box through a hole cut into the lid with cotton wool packed around the tubes to maintain the box's insulation capacity. Ultimately the aim of using the cooler box was to keep the temperature constant. However, testing showed the air temperature within the cooler box varied more than 2°C during the respirometry trials making it unsuitable for my purposes. I, therefore, used a (Julabo F10 Immersion circulation) water bath as an alternative, in which I completely submerged the IRC. Two egg fishing sinkers (55g) were affixed to ensure the IRC did not float. The water bath also proved useful for identifying leaks in the IRC.

Table 2.1: An overview of body size and temperature range for each insect species (n gives the sample size).

	Body mass (mg)		**Temperature (°C)**		
Species	*minimum*	*maximum*	*minimum*	*maximum*	*n*
Eristalis tenax	71.00	135.00	14.73	35.08	33
Lucilia sericata	8.49	34.17	14.61	34.55	33
Apis mellifera	69.00	184.00	15.08	35.02	33
Bombus terrestris	159.00	403.00	15.10	35.08	33

2.3.4 Data processing and analyses

Resting VCO_2 was determined by averaging 10 minutes of the 1hr recorded data that showed the lowest VCO_2. A scatterplot was used to identify areas of low VCO_2 in the dataset for every individual (figure 1.1). Mean resting VCO_2 from each trial was collated in a single dataset with the live body mass of each individual and the experimental temperature. The resting VCO_2 was converted to metabolic rate in Watts using the equation:

$$I = \frac{R \times M \times 21.1}{0.68 \times 10^6 \times 60} \tag{2}$$

where *I* represents whole-organism metabolic rate in Watts, *R* is the respiration rate in µg.g^{-1}.min^{-1}, and *M* is body mass in grams. By multiplying the respiration rate with body mass, I was able to generate whole-organism metabolic rates, which differed from mass-specific metabolic rates in that they define the energy consumption of an entire organism. In contrast, mass-specific metabolic rates define the rates at which energy is consumed at the cellular level (Savage et al., 2007). A conversion factor of 0.680x10-6 is used to convert from micrograms to millilitres. A conversion factor of 60 was used to convert from minutes to seconds, and a conversion factor of 21.100 was used to convert metabolic rate in ml.s^{-1} to joules per second (equivalent to watts).

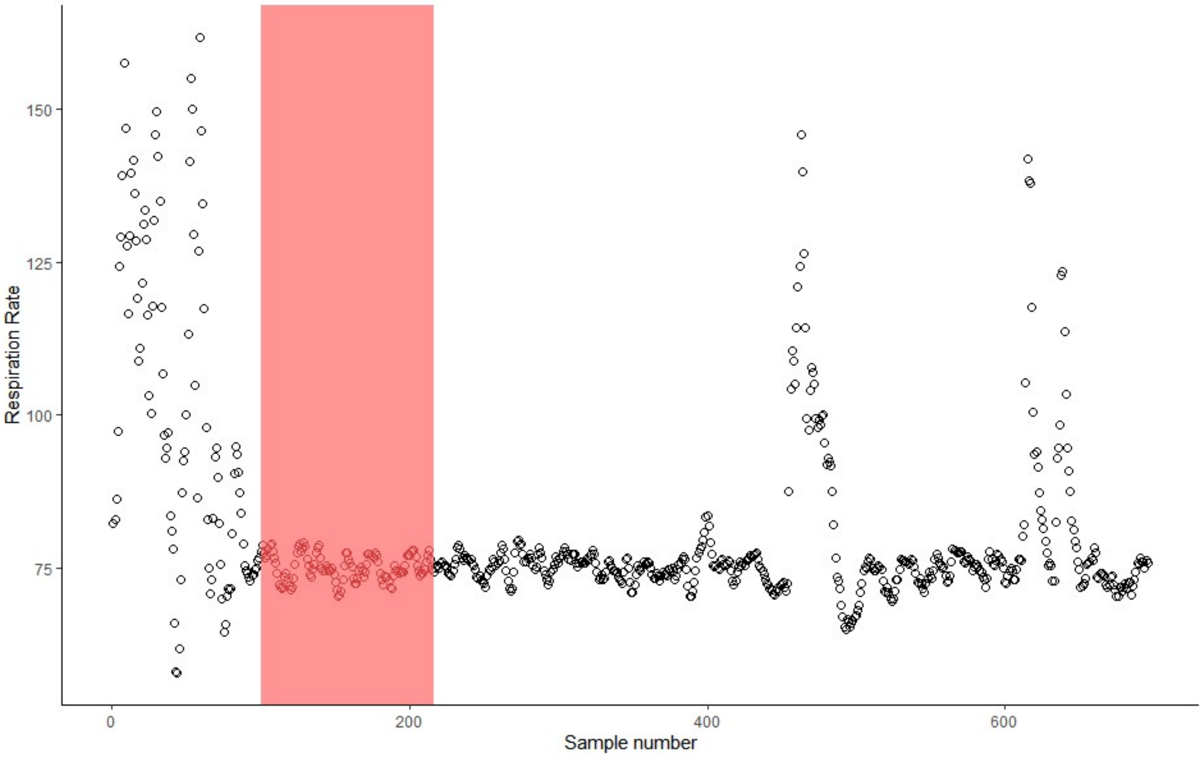

Figure 2.1: Example of scatterplot for the hour of respiration data collected from a sample drone fly (*Eristalis tenax*) at 35°C. The red box represents the 10 minutes of data averaged to estimate the resting respiration rate.

To assess the mass and temperature dependence of resting metabolic rate in pollinating insects, I used the equation described by Brown et al. (2004):

$$ln\ (IM^b)\ = -E\left(\frac{1}{kT}\right) + ln\ (i_o) \qquad (3)$$

where *b* is the allometric exponent that explains how metabolism scales with body size. The second part of the equation defines the relationship of metabolism with temperature and is described by the Van't Hoff-Arrhenius relationship where E is the activation energy, k is Boltzmann's constant, and T is the absolute temperature in Kelvin (Brown et al., 2004). Lastly, i_o is a normalisation constant independent of body size and temperature.

Prior to statistical analysis, the dataset was scanned for outliers, and consequently, 12 sets of individual insects' data were removed. Outliers were defined as a trial showing a respiration rate >300 $\mu g.g^{-1}.min^{-1}$, which was deemed as active rather than resting metabolism. Because metabolic rates of both sexes for the two fly species were recorded, we conducted an ANOVA between whole-organism metabolic rate and sex to determine if sex influences metabolism. We did not find a statistically significant difference in metabolic rate between males and females for both drone flies (F = 0.010, df = 1, P = 0.921) and blow flies (F = 0.647, df = 1, P = 0.428). Therefore, I pooled data across both sexes in all further analyses.

To determine the activation energy (E) and normalisation constant (i_o) for each species, Ordinary Least Squares (OLS) regression models were fitted to the relationship between mass-corrected whole-organism metabolic rate, $ln\ (IM^b)$ and temperature, $\frac{1}{kT}$. The allometric exponent had to be defined as 0.75, the body mass scaling exponent predicted by the metabolic theory of ecology (MTE) (Brown et al., 2004), to allow E and i_o to be determined. The fitted slope from this relationship gave E, and the intercept described i_o. From the results of the temperature-metabolism model, the activation energies were inserted into an OLS model with temperature-corrected metabolic rate, $ln\ (I\ e^{E/kT})$, as the response variable and body mass, $ln\ (M)$, as the explanatory variable. From this OLS, the actual allometric exponents for each species are equivalent to the slope, and the normalisation constants are equivalent to the intercepts. A 95% confidence interval was calculated from each OLS to estimate variance around model estimates. All analyses were conducted in R version 4.1.3 (R Core Team, 2022).

2.4 Results

2.4.1 The influence of temperature and insect pollinator metabolic rates

Using my respiration measurements from 120 individual pollinator insects across temperatures ranging from 15 – 35 °C, I found an overall slope between temperature and metabolism of -0.60 to -0.70 electron volts (eV). The slope falls within the activation energy range predicted by the metabolic theory ecology. The relationship between temperature and mass-corrected metabolism was statistically significant (table 2.2, figure 2.2), though it had an *adjusted* R^2 of 0.040, indicating that only a low proportion variance in pollinator metabolism could be explained by temperature.

Table 2.2: Results from the five Linear Model regressions testing the relationship between mass-corrected metabolic rate and temperature for all four species together and separately. For each regression, the results for the normalisation constants (i_o) and activation energies (E) are reported. Includes estimates, standard errors, t-values, P-values (significant values in bold font), 95% confidence intervals (CI), and adjusted R^2 values.

					CI (95%)		
	Estimate	*Standard error*	*t-value*	*P-value*	*lower*	*upper*	*Adjusted* R^2
Grouped							
i_o	8.502	9.402	0.904	0.368			
E	-0.600	0.242	-2.485	**0.014**	-1.079	-0.122	0.040
Apis mellifera							
i_o	10.290	7.404	1.390	0.176			
E	-0.610	0.190	-3.243	**0.003**	-1.007	-0.227	0.247
Bombus terrestris							
i_o	16.566	6.006	2.758	**0.010**			
E	-0.753	0.154	-4.877	**<0.001**	-1.068	-0.438	0.424
Eristalis tenax							
i_o	1.683	2.354	0.715	0.480			
E	-0.420	0.061	-6.941	**<0.001**	-0.544	-0.296	0.603
Lucilia sericata							
i_o	4.293	5.609	5.609	0.450			

E	-0.580	0.144	0.144	**<0.001**	-0.874	-0.285	0.329

Linear model (eqn 1): $ln\ (IM^{0.75})\ = -E\left(\frac{1}{kT}\right) + ln\ (i_o)$ where b is substituted for 0.75 to ensure the metabolic rate is “mass-corrected”.

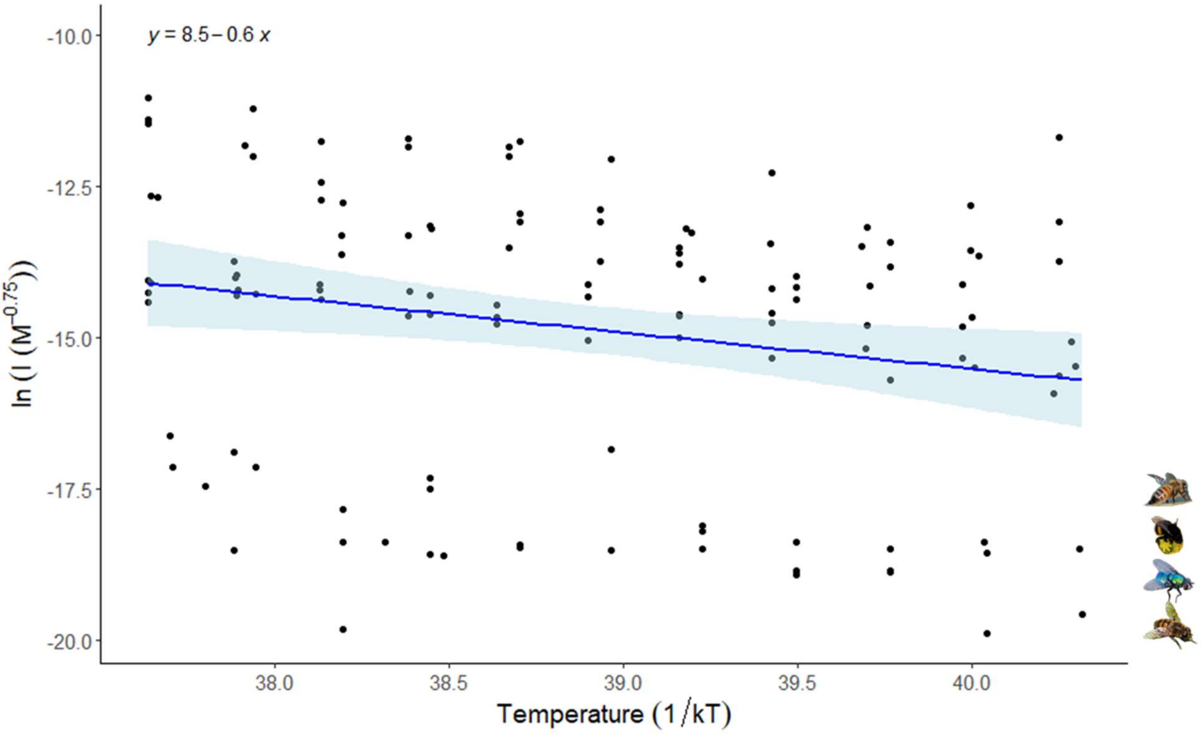

Figure 2.2: Relationship between the natural log of mass-corrected metabolic rate and Arrhenius temperature for all four study species combined (‘community-level’ analysis). The Blue shaded area denotes the 95% confidence interval. The slope, intercept, and R^2 values are presented in Table 2.2

In contrast to the community-level temperature response, I found strongly varying activation energies among species when population-level temperature responses were modelled, all of which showed a significant relationship between mass-corrected metabolic rate and temperature (table 2.2, figure 2.3). All individual regressions showed adjusted R^2 values greater than the grouped species regression, with drone flies ($R^2 = 0.603$) showing the best fit and honeybees ($R^2 = 0.247$) showing the poorest fit (table 2.2). The three species, honeybees, bumblebees, and blow flies, had negative slopes that fell within MTE’s predicted activation energy range of -0.60 to -0.70eV, whereas the activation energy for drone flies did not (eV of -0.42). It is important to note that the 95% confidence interval (CI) needs to be calculated when determining whether the slope fits the predicted results. These provide us with a confidence of 95% that the slope falls within the upper and lower values calculated. Thus, we can see that the negative 0.420 slope for drone flies with confidence intervals of -0.544 and -0.296 is just out of the predicted activation energy range (table 2.2). From these results, it is clear that the linear regression I used in this study explains the relationship between temperature and metabolism well (or that the data strongly support all results). Still, not all species match the predictions made by the MTE.

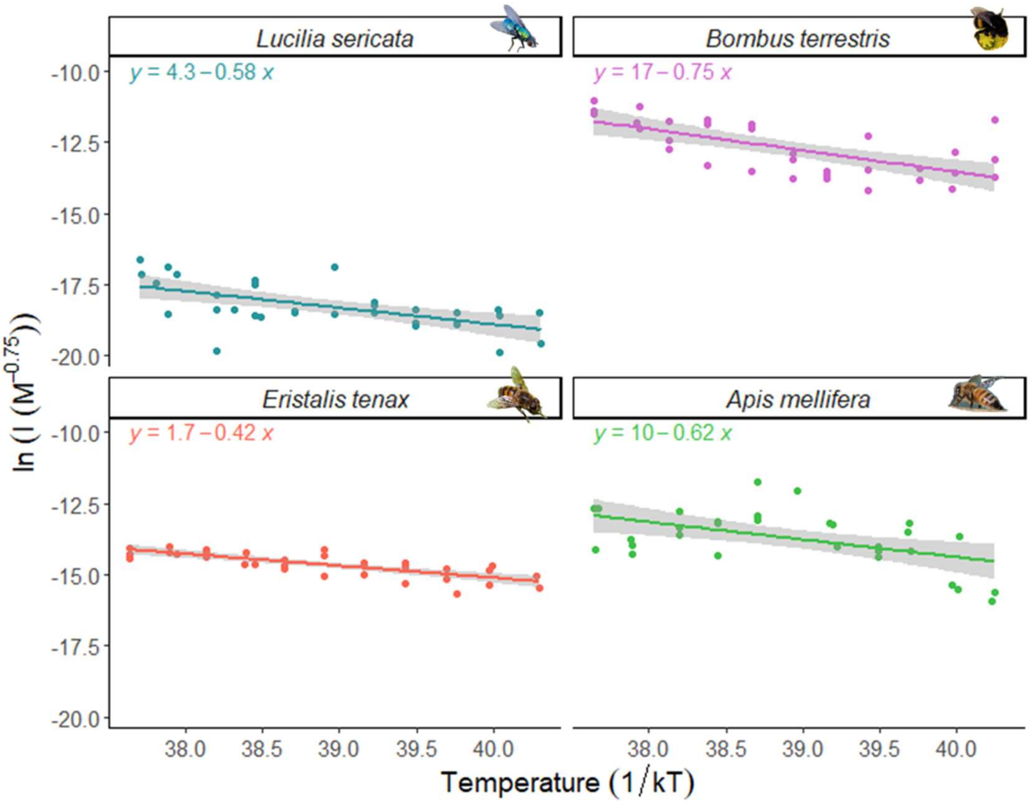

Figure 2.3: Relationship between the natural log of mass-corrected metabolic rate and Arrhenius temperature for all four study species separately. Shaded areas denote the 95% confidence intervals. The slopes, intercepts, and R^2 values are presented in Table 2.2

2.4.2 Insect pollinator body mass and its relationship with metabolism

The slope of 1.166 for the relationship between body mass and temperature-corrected metabolism at the community level did not match the predicted 0.75 slope expected by MTE. While the slope was positive, as would be expected under the MTE, it was considerably steeper than predicted, with the lower bound of the CI (1.063) being considerably higher than the predicted 0.75 (table 2.3, figure 2.4). This relationship was, however, statistically significant and had a high *adjusted* R^2 value indicating that metabolic rate is strongly dependent on body mass (table 2.3). Nevertheless, analyses of the relationship between body mass and temperature-corrected metabolic rate for these four pollinator species combined do not support the ¾ power law used to define body mass-metabolism scaling in the MTE.

Table 2.3: Results from the five Linear Model regressions testing the relationship between temperature-corrected whole-organism metabolic rate and body mass for all four species together and separately. The results for the normalisation constant (io) and allometric scaling exponent (b) are reported for each regression. Includes estimates, standard errors, t-values, P-values (significant values in bold font), 95% confidence intervals (CI), and adjusted R^2 values.

					CI (95%)		
	Estimate	*Standard error*	*t-value*	*P-value*	*lower*	*upper*	*Adjusted R^2*
Grouped							
i_o	13.380	0.143	93.250	**<0.001**			
b	1.166	0.052	22.340	**<0.001**	1.063	1.270	0.801
Apis mellifera							
i_o	12.310	1.312	9.382	**<0.001**			
b	0.198	0.612	0.324	0.748	-1.055	1.451	-0.032
Bombus terrestris							
i_o	19.201	0.515	37.261	**<0.001**			
b	1.045	0.361	2.895	**0.007**	0.308	1.781	0.192
Eristalis tenax							
i_o	3.266	0.566	5.772	**<0.001**			
b	-0.049	0.249	-0.198	0.844	-0.559	0.460	-0.032
Lucilia sericata							
i_o	9.602	0.938	10.230	**<0.001**			
b	0.467	0.210	2.150	**0.0403**	-0.022	0.912	0.111

Linear model (eqn 3): $ln\left(IM^b\right) = -E\left(\frac{1}{kT}\right) + ln\left(i_o\right)$ where E is substituted with the values shown in table 2.2 to ensure the metabolic rate is "temperature-corrected".

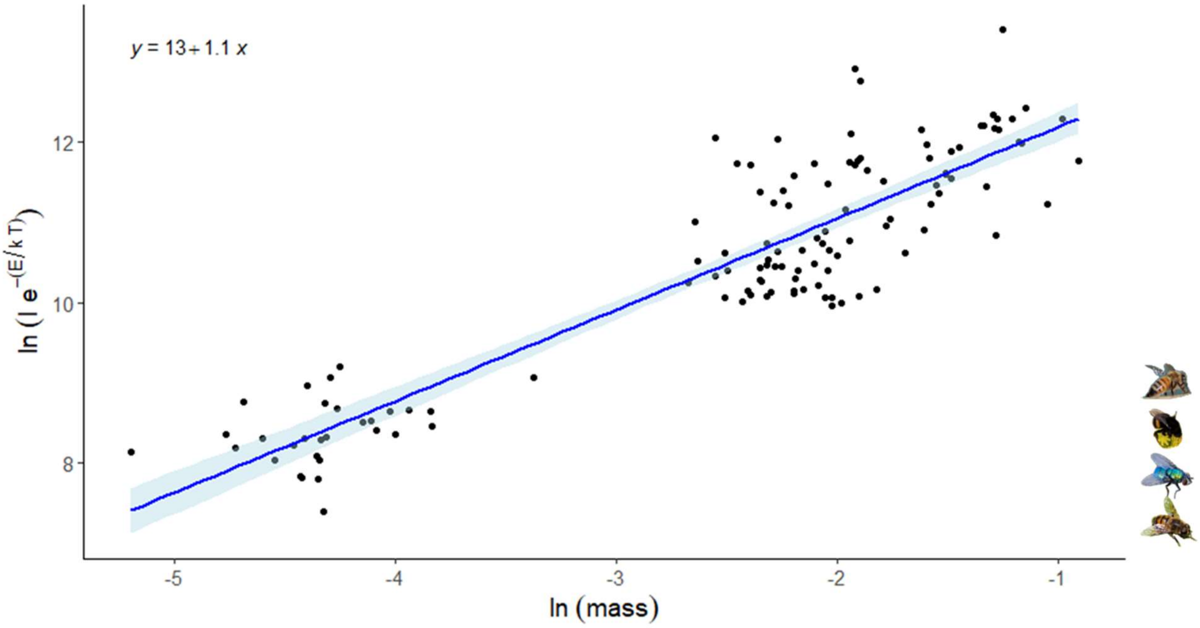

Figure 2.4: Relationship between the natural log of temperature-corrected metabolic rate and the natural log of body mass for all four study species combined. The shaded area denotes the 95% confidence interval. The values for slope, intercept, and R^2 value is presented in Table 2.3.

For the individual species regression, the results showed that three out of the four species (honeybees, bumblebees, and blow flies) demonstrated a positive relationship between the natural log of temperature-corrected metabolic rate and the natural log of body mass. Furthermore, these three species' relationships all produced slopes that fall close to the MTE predicted slope of 0.75 (table 2.3, figure 2.5). However, the drone flies' body mass-metabolism relationship differed from the predicted positive slope of 0.75, with a negative slope of 0.050and no incorporation of the predicted value falling within the 95% CI of -0.559-0.460 (table 2.3, figure 2.5). Moreover, only bumblebees (P = 0.007, *adjusted-*R^2 = 0.192) and blow flies (P = 0.403, *adjusted-*R^2 = 0.111) showed significant linear relationships between temperature-corrected metabolic rate and the natural log of body mass (table 2.3). Thus, because these data only support the relationship between body mass and metabolism for bumblebees and blowflies, the results suggest that the MTE cannot be applied at the species level for all pollinators.

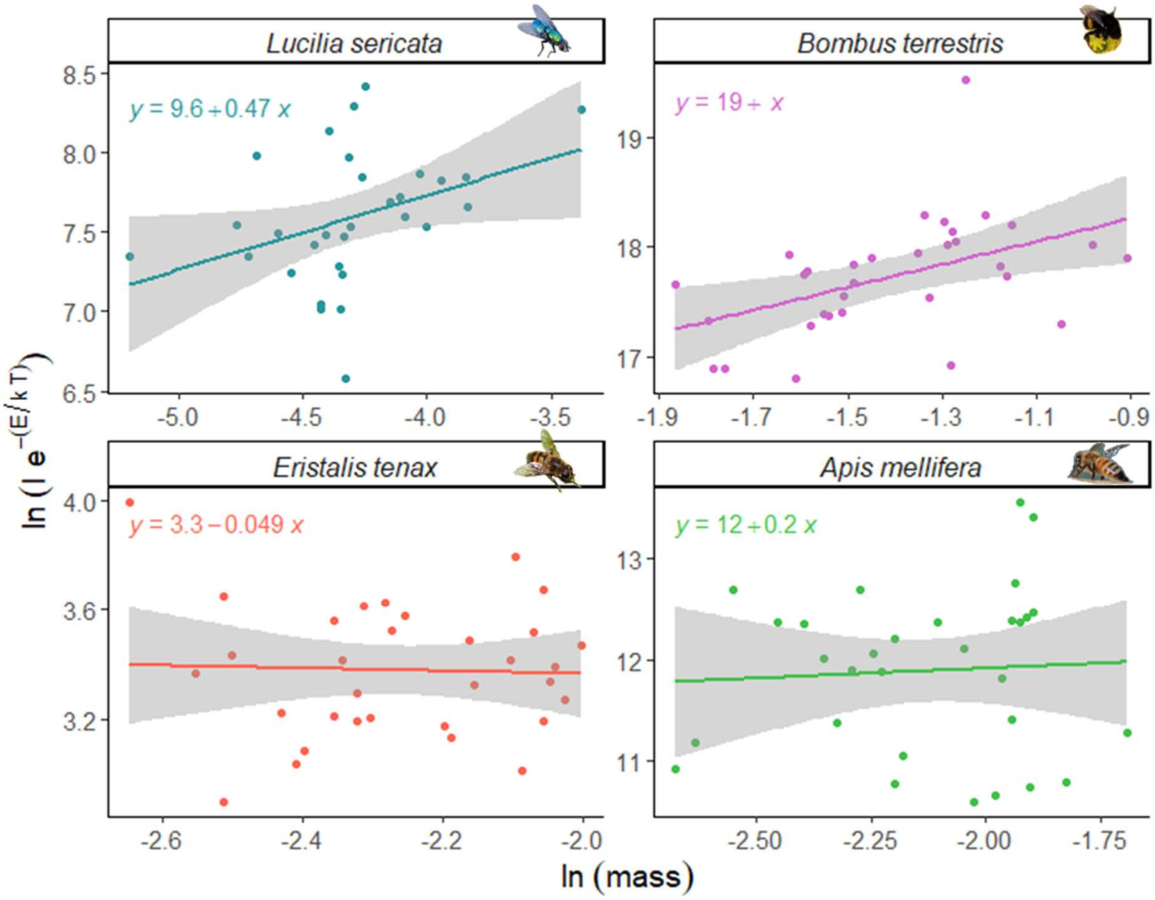

Figure 2.5: Relationships between the natural log of temperature-corrected metabolic rate and the natural log of body mass for all four study species separately. The shaded areas describe 95% confidence intervals. The values for slopes, intercepts, and R^2 values are presented in Table 2.3.

The results of this study are best represented in figure 2.6, where allometric scaling exponents, activation energies and their 95% CI are shown in comparison to the predicted slopes. These results demonstrate how the drone flies' allometric scaling exponent and activation energy do not fall within the values predicted by the MTE. It is also evident that the grouped species' metabolic rates do not scale with body mass according to an exponent of 0.75, as predicted by the MTE (figure 2.6 A).

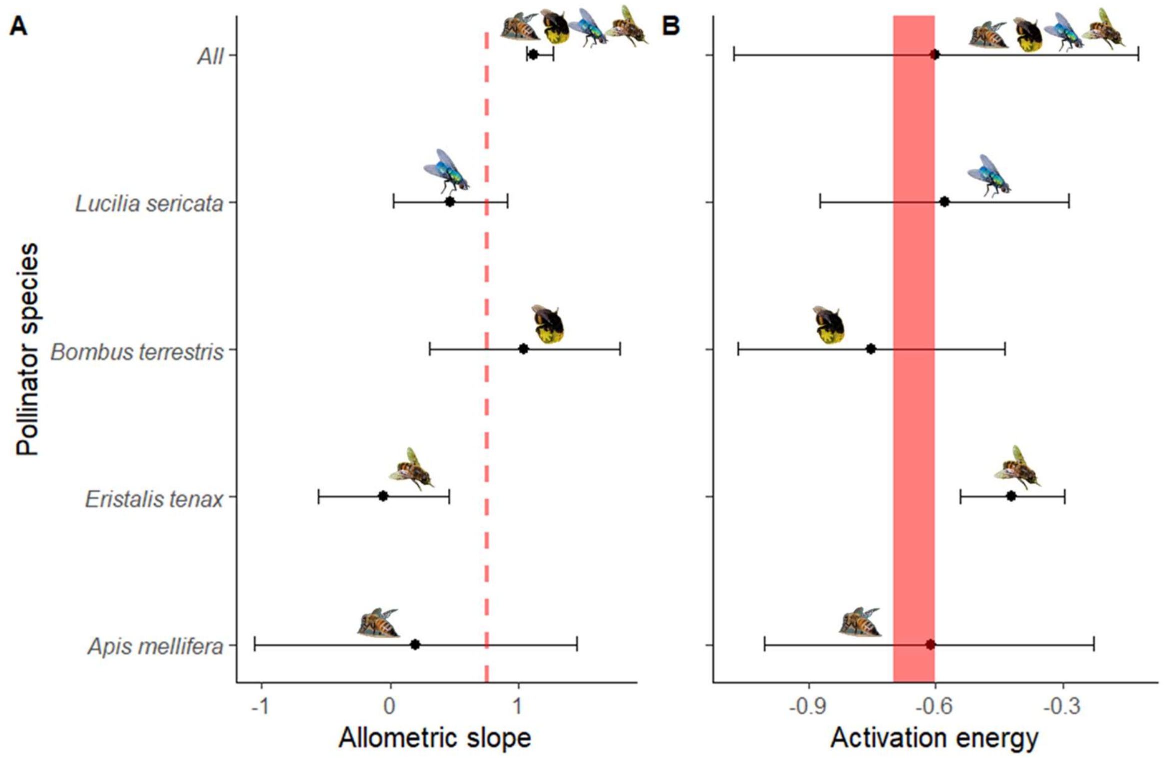

Figure 2.6: Predicted values from the relationships between **A)** body mass and metabolic rate and **B)** temperature and metabolic rate compared across individual species and all four species combined. Predicted values for the allometric slope (mass-metabolism scaling exponent) and activation energy are represented by black points, ± 95% confidence intervals represented by horizontal black bars. The vertical red dashed line (**A**) and shaded bar (**B**) denote the allometric slope and activation energy range predicted by the MTE.

2.5 Discussion

This study demonstrates that the community-level relationship between resting metabolic rate (RMR) and temperature for pollinating insects scales as predicted by the MTE. However, it was not always valid for the species level RMR and temperature regressions, with each species showing different slopes and only three out of the four species showing activation energies that fell within the predicted range (0.60-0.70eV). Furthermore, for allometric scaling relationships between RMR and body mass, neither the community nor species-level regressions were entirely consistent with the MTE predictions. Both the community-level and drone fly (*Eristalis tenax*) specific regressions fell far from the predicted ¾ scaling relationship. Interestingly, the direction of the drone fly RMR-body mass regression was the only model that produced a negative relationship rather than the positive relationship predicted by the MTE.

From these results, the metabolic theory of ecology is seemingly only applicable to temperature-metabolism relationships at the community level for pollinating insect species. Furthermore, it appears that the MTE cannot be used to reliably predict relationships at the population level or for pollinator community metabolism-body size relationships. So why do these allometric scaling and activation energy differences exist among the community and population levels? The lack of consistency in results across the literature and the fact different ecological, physiological, and behavioural factors that can influence metabolism and scaling relationships were not included in the MTE (Jerde et al., 2019) has resulted in other researchers developing different theories in an attempt to explain the inconsistency in their results (Kaefer et al., 2012).

Regarding the inconsistency in the literature, the allometric scaling relationship between mass-specific metabolism has received the most attention. Previous studies found results that refute some of the predictions put forth by the MTE (Bokma, 2004; Chown et al., 2007; Dodds et al., 2001; Hui & Jackson, 2007; Kozłowski & Konarzewski, 2005; Lighton et al., 2001; Niven & Scharlemann, 2005; Roberts et al., 2004; Savage et al., 2004; Weibel et al., 2004; White & Seymour, 2003), while other studied presented observations of scaling exponents equal or close to 0.75 (Belgrano et al., 2002; Farrell-Gray & Gotelli, 2005; Hui & Jackson, 2007; Lemaître et al., 2014; Niven & Scharlemann, 2005; Somjee et al., 2021; West & Brown, 2005; West et al., 1999, 2002). One important reason for these differences could lie in the differences in the body size ranges across species (Chown & Gaston, 2010; Somjee et al., 2021). Or in other words, if the variation among species' individual metabolic rate (response variable) is higher than their variation in body mass (explanatory variable), the slopes of the scaling relationship will not be reliably estimated (Somjee et al., 2021). Thus, differences in the insects' size ranges are a possible reason for the variation in species-specific scaling exponent estimates found in my results. Moreover, it can explain the slight negative relationship between mass and RMR, as seen for blow flies (*Lucilia sericata*) in my study. Across species, however, the body masses ranged between 5 and 400mg (an 80-fold difference in size from smallest to largest individual), which should be large enough to detect any relationship between mass and metabolism. Niven & Scharlemann (2005) support the suitability of our size range by suggesting that for insects, body mass ranges of around 10 to 100mg are better for obtaining accurate estimates of scaling relationships. However, it is possible that any unexplained variation can be attributed to differences in phylogeny, which is a fruitful avenue for future research (Capellini et al., 2010; Niven & Scharlemann, 2005; Symonds & Elgar, 2002).

Another commonly suggested reason for RMR not scaling with mass according to the MTE prediction could be due to inaccuracies in the underlying mechanistic theory (Jerde et al., 2019). The mechanistic theory underlying the MTE is known as the "nutrient supply network model", which postulates that a scaling relationship of 0.75 is predicted because metabolic rate is constrained by the oxygen supply being limited by hierarchical branching networks as an organism's size increases (Clarke et al., 2010; Brown et al., 2004; Harrison et al., 2014; Jerde et al., 2019; West et al., 1997). Studies have both presented data suggesting that in insects, the allometric scaling of RMR is not caused by limited oxygen supply (Harrison et al., 2014, 2018) and that other mechanistic hypotheses, such as the alternative cell size hypothesis described by Kozłowski et al., (2003), may better explain the RMR-mass scaling relationships (Chown et al., 2007). However, there is yet to be a consensus in the literature on the accuracy of the nutrient supply network model or any of the other alternative hypotheses (Dodds et al., 2001; Harrison et al., 2014, 2018; Niven & Scharlemann, 2005; Price et al., 2012; Weibel 2004; West et al., 2002; West & Brown, 2005). Hence, I cannot be certain this is the reason why my results deviate from the 0.75 scaling exponent.

Regarding the temperature dependence of metabolism, the inaccuracies in the mechanistic hypothesis used to support MTE predictions are the most likely explanation for my results showing more variation than expected. The universal thermal dependence (UTD) hypothesis is the theory on which MTE thermal relationship is based and assumes that activation energy shows slight variation across scales, inferring that an organism's metabolic rate is equally sensitive to temperature (Labocha et al., 2004; Réveillon et al., 2019). However, studies have questioned the logic of the UTD, with some researchers suggesting that tropical and temperate species show considerably different MR-temperature relationships (Chown & Gaston, 1999; Irlich et al., 2009; Yamahira et al., 2007), while others suggest that without natural variation there would be nothing on which natural selection can act and so we would not see any adaptations in sensitivity to temperature (Labocha et al., 2004; Réveillon et al., 2019). Consequently, Clarke (2004, 2006) has suggested an alternative hypothesis combining these ideas, stating that natural selection and local adaptation produces differences in organisms' thermal sensitivities (Murren et al., 2014; Réveillon et al., 2019). A recent study by Réveillon et al. (2019) tested the variability of thermal sensitivities among individuals of *Gammarus fossarum* ants and found that their results supported Clarke's (2004, 2006) alternative hypothesis rather than the UTD hypothesis. Thus, Clarke's (2004, 2006) alternative hypothesis provides a logical explanation for the variation seen in my results.

It must be noted that another reason for the variation seen across species could be that flying insects are heterothermic (Gooley & Gooley, 2020; Stabentheiner et al., 2003; Oyen et al., 2016). Heterothermic means they show ectothermy when resting or walking but endothermy when flying (Stabentheiner et al., 2003). Moreover, the relationship between metabolic rate and temperature has been shown to change directionality depending on the thermal regulatory strategy (Gooley & Gooley, 2020; Woods et al., 2005). However, the size of the respiration chamber I used in this study prevented the insects from flying, so their heterothermy would not have impacted the results. It is also possible that using a closed respirometry system could have made detecting movement difficult (Chown et al., 2007; Terblanche et al., 2004), impacting the data in a way that elevated the slopes from the relationships above what would be considered realistic (Deere & Chown, 2006; Irlich et al., 2009; Terblanche et al., 2004). However, the closed system I used in this study had a clear respiration chamber, which made movement detection easy; thus, this is unlikely to have been a problem.

As is clear from the uncertainty in mechanistic explanations for MTE predictions, further research is required before there can be any certainty that the theories presented would accurately explain why my results deviated from the MTE predictions. Furthermore, other key assumptions not mentioned here remain untested and thus pose an important avenue for future research (Harrison et al., 2014, 2018; Price et al., 2012; Terblanche et al., 2007). However, I would argue that while a strict use of MTE should be done with caution, considering all the potential flaws, the fundamental equation on which it is based is still highly valuable in understanding climate responses. Firstly, here, I show that MTE may still be relevant to community-level pollinating insect responses. However, studies that control for phylogeny are needed before we can be sure of this (Capellini et al., 2010; Niven & Scharlemann, 2005; Symonds & Elgar, 2002). Secondly, studies based on the MTE's fundamental equation could still prove helpful in providing species-specific metabolic scaling coefficients that could improve understanding of how pollination services are likely to change in the future. For example, scaling relationships have been used to forecast the effects of global change on fisheries (Cheung et al., 2008; Jerde et al., 2019). The evidence from these studies suggests that species-specific scaling exponents are preferable to community-level scaling exponents as they are less likely to under or overestimate a species' sensitivity to environmental changes (Jerde et al., 2019). Finally, I show that species' thermal sensitivities estimated by their activation energy could be useful in providing species-specific sensitivities to global warming (Gillooly et al., 2001; Réveillon et al., 2019). For example, one could hypothesise from my results that the bumblebee would be the most sensitive to climate change as it showed the highest activation energy. This measure of sensitivity could potentially be included in models to aid in the prediction of the vulnerability of individual species to climate change. However, before doing so, studies of real-world responses of these species to climate change would be important to ensure that the activation energies produced by the MTE equation provide accurate estimates of a specie's sensitivity to its thermal environment.

In conclusion, the strict application of the metabolic theory of ecology cannot accurately predict all the scaling relationships we see in pollinating insects. Moreover, more research is needed before we can fully understand the reasons why deviations from the strict MTE prediction exist in these and other organisms so that we can improve upon the current theory. However, the fundamental equation on which the MTE is based could still prove helpful outside the strict predictions made by the MTE. The equation’s usefulness could lie in producing species-specific scaling coefficients that could be used to enhance our understanding of a species’ vulnerability to environmental change.

Chapter 3

Does temperature alter the risk-avoidance behaviour of pollinating insects?

3.1 Abstract

The energetic trade-offs between predation risk and foraging have been well studied across many animal taxa, including insect pollinators, showing that predation is a key factor that influences foraging behaviour. However, temperature also likely plays an important role, especially in ectothermic animals, due to its influence on metabolic rates. With increasing global average temperatures, it is imperative to understand how warming interacts with other factors, such as predation risk, to alter the foraging behaviour of pollinators and their ability to provide vital pollination services. In this study, I ran a series of behavioural assays under laboratory conditions to test whether insect pollinators increase foraging at higher temperatures to compensate for increased energetic demands. Further, I tested whether pollinating insects show a smaller reduction in foraging at higher versus lower temperatures under simulated predation risk (i.e. if they are more risk-prone at high temperatures and more risk-averse at low temperatures). My results showed that pollinator metabolism and foraging behaviour increased with temperature in the absence of predation risk. I also found that all species foraged less at high and low temperatures when a predator risk was included but were more likely to continue foraging at warmer temperatures (more risk-prone). My study demonstrates how warming temperatures could cause pollinators to increase foraging rates in order to maintain their energetic demands, but predation risk may limit their ability to meet these increased demands under warming. This highlights a vital fitness trade-off for insect pollinators that could have significant consequences for pollination services in the future.

3.2 Introduction

Animals must constantly make decisions that balance energetic gains and losses while also considering potential fitness risks (Higginson & Huston, 2015). However, decision-making during foraging could be influenced by environmental changes, such as warming, which could have important consequences for ecosystem functions linked to foraging, such as plant pollination. For example, pollinators are expected to choose nectar-rich flowers over flowers that produce smaller volumes of nectar, but complex foraging environments could force them to sacrifice energetic gains from foraging in an effort to avoid predation (Bednekoff, 2007; Jones & Dornhaus, 2011). However, a warming climate will increase the metabolic losses of animals (Vucic-Pestic et al., 2011), which could intensify pressure on pollinators to take greater risks while foraging in order to maintain a positive energy balance.

The importance of avoiding predators lies in the severe fitness cost that a successful predation event imposes on a prey animal (BedneKoff, 2007). However, predators can also affect their prey in a non-lethal way by inducing fear in their prey, causing the prey animal to alter their behaviour to avoid being attacked or eaten (Tan et al., 2013). Pollinating insects will usually change their behaviour by altering their habitat or flower preference and foraging rates in response to the presence of predators (Gavini et al., 2020; Jones & Dornhaus, 2011; Llandres et al., 2012; Romero et al., 2011; Tan et al., 2013). For example, Jones and Dornhaus (2011) demonstrated that honeybees exposed to simulated crab spider attacks reduced foraging activity if attacked on low-reward flowers or changed their preference to low-reward flowers when attacked on a high-reward flower (Jones & Dornhaus, 2011). Honeybees have also been shown to alter predator avoidance behaviour according to the level of danger posed by different predators (Tan et al., 2013). Additionally, a study on bumblebees showed reductions in foraging worker visitation rate and time when exposed to artificial spiders (Gavini et al., 2020). These so-called “non-consumptive effects” (Romero et al., 2011; Tan et al., 2013) are non-lethal but involve prey animals altering their behaviour to reduce predation risk and typically pose an energetic cost. As insect pollinators more commonly experience non-consumptive effects than direct predation effects through consumption (Romero et al., 2011; Tan et al., 2013), this is likely a major factor driving pollinator behaviour and the associated pollination services they provide.

The role that pollinators play in plant-pollinator interactions makes them essential for plant fitness and the maintenance of plant biodiversity (Bascompte et al., 2003; Ings et al., 2009; Llandres et al., 2012). Thus, any non-consumptive effect that predation has on a pollinator's behaviour can filter through to alter interaction networks which, in turn, can have serious consequences for ecosystem functioning (Gonclaves-Souza et al., 2008; Llandres et al., 2012; Suttle, 2003). For example, Ings and Chittka (2009) found that crab spiders increased flower avoidance by bumblebees, which could consequently reduce the reproductive success of flowers. Due to the importance of non-consumptive effects for pollination, research into behavioural trade-offs between foraging and predation risk in pollinating species has received a great deal of attention (Romero et al., 2011). However, there has been little investigation into how environmental change drivers, such as climate warming, influence the complex interaction between pollinator foraging and predation avoidance behaviour (Ma & Ma, 2012).

As I show in Chapter 2, insect pollinator metabolic rates increase exponentially with temperature. As a result, higher temperatures can create sustained periods of high metabolic rates, increasing animal energy expenditure (Bordier et al., 2018; Gillooly et al., 2001; González-Tokman et al., 2020; Prather et al., 2018). Thus, with limited energy budgets, individuals are likely faced with an important trade-off (Gooley & Gooley, 2020; Maryanski et al., 2002) when choosing between allocating energy to avoid predators versus foraging to sustain the higher energetic demands at warmer temperatures. The little research that has focused on this topic has only been conducted on non-pollinating insect species. For example, Moiroux et al. (2015) found that female aphid parasites (*Aphidius ervi*), which had developed under high temperatures, adopted more risk-prone behaviours to compensate for lower reproductive success expected at higher temperatures. However, to my knowledge, the potential trade-off between pollinator behavioural responses to high temperature and predation risk remains unexplored. Understanding how temperature and predation-risk interact may explain how warming will influence pollinator foraging behaviour and performance, which could help to understand and predict how pollination services will be affected under future climate change scenarios.

Here, using laboratory behavioural assays, I compared the foraging behaviour of four insect pollinators, *Apis mellifera* (honeybee), *Bombus terrestris* (bumblebee), *Eristalis tenax* (drone fly) and *Lucilla sericata* (blow fly), when placed in a novel environment, before and after exposure to a simulated predation threat, and at two different temperature treatments. Specifically, I tested whether pollinators altered their latency and likelihood of foraging to make accommodation for the energetic trade-off that exists between an individual's need to avoid predators and forage under different temperatures. I predicted that the four insect pollinator species studied would show a greater tendency to forage at high temperatures in order to balance their energetic expenditure with increased energy intake. I further predicted that the simulation of a predation disturbance would reduce the pollinators' foraging tendency but that this reduction would be less pronounced at high temperatures because the increased demands to obtain energy would counteract predation risk. Lastly, and because of my previous findings in Chapter 2, I predicted that the behavioural responses would differ among species. In Chapter 2, each species differed in the strength of metabolic responses to temperature. Specifically, the strength of the species' responses increased from drone flies, with the lowest sensitivity to temperature, through to blow flies, honeybees, and bumblebees, which have the highest temperature sensitivity. Thus, I expected behavioural responses would differ across taxa, with drone flies showing the weakest behavioural responses and bumblebees the strongest. This research could potentially emphasise the importance of studying multiple drivers of behavioural change to understand how climate change will impact pollination services in the future.

3.3 Materials and Methods

3.3.1 Supply and care of study species

All four insect pollinator species *Apis mellifera* (honeybee), *Bombus terrestris* (bumblebee), *Eristalis tenax* (drone fly) and *Lucilla sericata* (blow fly) studied in the metabolic assays (Chapter 2) were used in the following experiments to determine the effects of predation disturbance and warming on pollinator behaviour. The same suppliers provided all insects at the same life stage, as mentioned previously. Furthermore, drone flies, blow flies, and bumblebees were kept under conditions almost identical to those described in Chapter 2. The only difference was that they were fed 66% sugar solution and honey *ad libitum*, so they became accustomed to the sugar solution used in the behavioural assays.

Honeybees were the only insects that I collected and kept under different conditions than described in Chapter 2. Instead of collecting fresh adults from a hive each day, I collected a few hundred adult females on 13th February / 2022. I transferred them into a "miniature" hive, which I kept in the laboratory at ambient room temperature. The miniature hive consisted of a 15 cm tall cylindrical container in which I placed a plastic strip infused with queen mandibular pheromone (QMP). All the equipment I used to keep honeybee workers in the lab was supplied by Plant and Food Research. The QMP strips allowed the honeybees to function normally in the absence of a queen (Slessor et al., 2005). The miniature hive was then placed in a 30 x 30 x 30 cm mesh cage (Bioquip). A sugar feeder was made using a 50 ml centrifuge tube with a small hole drilled into the bottom and filled with 66% sugar solution. The sugar feeder was placed at the top entrance of the mesh cage outside the miniature hive to ensure that the bees had continuous access to food.

3.3.2 Temperature treatments and experimental set up

Each species was exposed to two temperature treatments: 15º C and 35º C. These temperatures were chosen to mimic the lowest and highest temperature treatment of the metabolic assays carried out in Chapter 2. For each temperature treatment, the behavioural responses of 30 individuals per species were tested, thereby giving a total sample size of 240 trials. I used a 500 ml labserv cylindrical screw cap container as the experimental arena (figure 3.2), which was placed in a water bath during assays to control the temperature. A hole was drilled into the lid so that an aquarium aeration pump could be attached to the experimental arena, thereby allowing air flow. The tubing that connected the aquarium pump was submerged in the temperature-controlled water so that the air entering the arena was the same temperature as the water. This helped to ensure the air inside the arena reached temperature quickly and remained at the desired experimental temperature throughout each assay. A HOBO MX2202 Pendant data logger was also placed at the bottom of the container to record air temperature, and a second hole was drilled into the lid to place a thermocouple connected to a digital thermometer. This allowed the air temperature inside the container to be monitored instantaneously while the data logger was used to collect more precise temperature data that could be used after the experiment. To ensure enough visual contrast between the insects and the container, I placed a piece of opaque white plastic at the bottom of the experimental arena. This allowed me to easily detect the insect against the background and record its behaviour. Lastly, a flexible metal wire was attached to the water bath and slid over the container to keep it submerged.

Figure 3.1: Screenshot captured from one of the behavioural assays, clearly showing the experimental arena setup described above. On the lid of the container, you can see a drone fly (*Eristalis tenax*) resting.

Two small changes were made to the arena for blow flies and bumblebees. For blow flies, a 70ml Labserv screw cap container with the fly inside was placed over the petri dish. This was because in trial assays, blow fly individuals consistently ignored the food source in the larger 500 ml arena, while more foraging was observed when the arena size was reduced (figure 3.2B)). For bumblebees, cotton wool was placed at the bottom of the container to reduce the occurrence of any initial stress-induced defence responses (i.e. lifting their legs into the air or lying on their backs with abdomens curled upwards) (Varnon et al., 2021; figure 3.2B)).

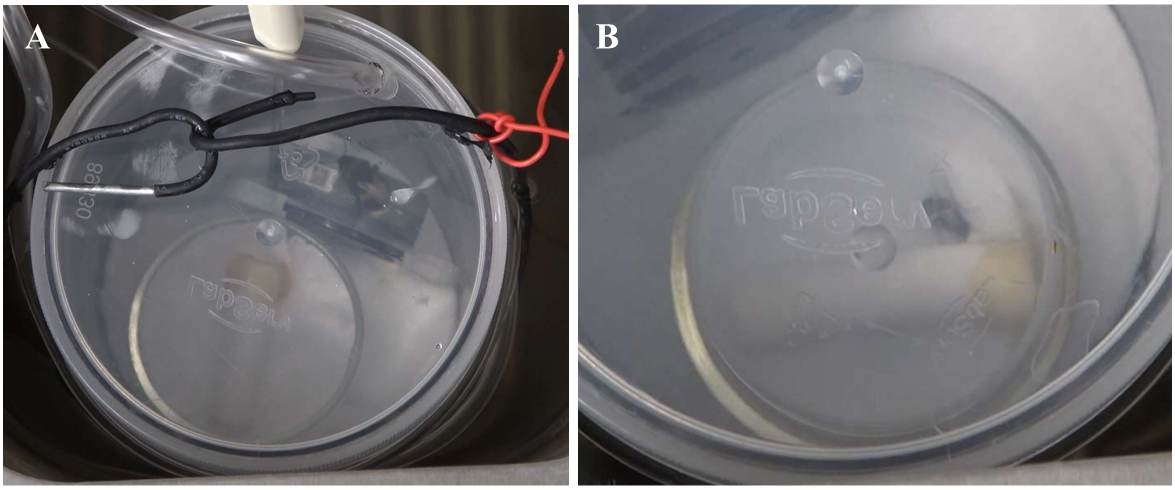

Figure 3.2: Behavioural assay stills showing (**A**) the cotton wool added to the bottom of the container for the bumblebee (*Bombus terrestris*) trails and (**B**) the reduced arena size used for blow fly (*Lucilia sericata*). In (**A**) a bumblebee can be seen grooming on the hobo, while in (**B**) a blow fly is feeding on honey.

3.3.3 Behavioural assays

An hour before the day's assays started, any food was removed from the enclosures. The data logger was set up and placed alongside a small glass petri dish at the bottom of the container. The experimental arena was then submerged in the water bath and allowed to reach temperature before any behavioural assays were started. At the start of each assay, a cotton roll soaked at one end with 66% sugar solution and honey on the opposite end was placed in the petri dish at the bottom of the container. The petri dish prevented the cotton roll from rolling around the bottom of the arena and spreading the food around. Lastly, a Sony FDRAX53 video camera was placed on a tripod alongside the water bath so that the camera could be situated directly above the experimental arena.

It is important to note that neither of the two bee species were trained to forage in the arena as it was meant to replicate a novel environment.

Once the experimental arena reached the required temperature, the behavioural assays were conducted as follows; the video recorder was started, the insect was placed into the arena, and its behaviours were recorded at 25 frames per second. During each assay, I observed the insect and recorded when an insect started feeding on either the sugar solution or the honey. An insect's behaviour was defined as 'feeding' when its proboscis made clear contact with the food source for a minimum of 2 seconds. Once the insect had been observed feeding, I simulated a predator threat by tapping the container against the side of the water bath twice. Tapping provided a visual and auditory signal that simulated a bird attack (Jones & Bulbert, 2020; Umbers & Mappes, 2015). This technique consistently disturbed each of the four study species, which was defined as when the insect moved away from the food source or displayed a defensive response (e.g., abdomen curls in bees). I continued to observe the subject until it had resumed feeding or for a maximum of 10 minutes, after which the insect was removed, regardless of whether it had fed or not. This time limit was decided upon as pilot observations showed that all four species were unlikely to forage if they had not done so within 10 minutes of entering the arena. I also recorded whether the subject foraged more than once after a disturbance. After each assay, I humanely euthanized each subject by freezing to ensure the same individual was not used again and to prevent any potential information from being passed between individuals within their rearing containers.

3.3.4 Video recording analysis

A universal ethogram was constructed for all four species and included all the behaviours that had been observed during the assays (Table 3.1). I used Boris v.7.13 (Friard & Gamba, 2016) to create a time-budget from the video recordings based on the ethogram. Each individual's behaviour was tracked immediately after it was placed within the water bath because the intention of the study was to test a forager's response to a novel environment, and allowing the insect to acclimatise to the arena would negate this. This approach has been applied in other studies of thermoregulatory behaviour in honeybees that use acclimation for behavioural rather than thermoregulatory reasons (Cook & Breed, 2013; Cook et al., 2016). Furthermore, studies testing short-term temperature responses of adult fruit flies (*Drosophila melanogaster*) did not mention any use of behavioural or physiological acclimation (Bahrndorff et al., 2016; MacLean et al., 2017). However, if the insect foraged before the temperature in the arena stabilised, this behaviour was ignored.

Table 3.1: Ethogram of all behaviours shown by insects during behavioural assays. *Refers to behaviours only displayed by bumblebees (*Bombus terrestris*).

Behaviour	Definition
Eat	Using proboscis to ingest food over a minimum period of 2 seconds.
Disturbed	External stressor causes the animal to move away from food.
Rest	Remains mostly stationary and does not take part in grooming.
Crawl	Moves from one area to another on its legs
Fly	Moves from one area to another with the use of its wings.
Groom	Animal engages in cleaning themselves by wiping their legs through their mouthparts and then over the rest of their body.
Investigate	Proboscis touches an object, but nothing is ingested.
Stressed*	Thrashes around on back with mandibles open and abdomen curled upwards or had one or more legs raised straight into the air.
Defecate	Waste elimination

3.3.5 Data manipulation and analysis.

The observations were exported from Boris as CSV files and compiled into a single data frame. Using R version 4.1.3 (R Core Team, 2022), the occurrences and durations of the different behaviours displayed during each assay were compiled. From these data, I calculated latency to forage (seconds) before and after a disturbance and foraging likelihood (as binary data) before and after a disturbance to be calculated and included in the final data frame.

To first model the variability in the likelihood of foraging across temperature treatments and species identity, I constructed a logistic Generalised Linear Model (GLM) with a binomial distribution that included foraging likelihood as the binary response (describing if the insect foraged or not) and temperature, species identity, and their interaction as predictors. A second

GLM was constructed using a Poisson distribution to test if latency to forage (time in seconds before foraging began) varied with temperature and species identity or their interaction. For foraging latency, the individuals that did not forage at all were included as missing values.

Secondly, I tested for the effects of temperature and species identity on the likelihood of foraging and latency to forage before and after a simulated predator attack. To do so, I constructed Generalised Linear Mixed Models (GLMM) that included foraging likelihood and latency to forage as the response variables, temperature, species, and predator disturbance as fixed effects, and experimental individuals as a random effect to account for the repeated sampling of individuals before and after the simulated predation disturbance. Models were run with foraging likelihood modelled on a binomial distribution and latency to forage modelled using a Tweedie Compound Poisson distribution to account for zero inflation in the data (Zhang, 2013).

After constructing each model with the predictor's temperature, species identity, predation, and their interactions, I performed model simplification to identify the best set of predictors for each response variable based on the Akaike Information Criterion (AIC). To do so, I constructed multiple models with all possible combinations of predictors for each predictor, including a null intercept-only model, to allow comparisons among explanatory variable combinations using AIC. If two or more models yielded AIC scores with < 2 units difference (i.e., $\boldsymbol{\Delta}$AIC < 2), I chose the most parsimonious model with the fewest parameters (table 3.2). For the final model, I used ANOVAs to compare models with and without each predictor to assess their significance. All analyses were conducted using R software version 4.1.3 (R Core Team, 2022).

3.4 Results

3.4.1 Warming effects on pollinator foraging behaviour

When testing for the effects of temperature on foraging likelihood, the best model included temperature, species identity, and their interaction (table 3.2). I found a significant difference in the likelihood of foraging between the high and low temperatures ($X^2 = 7.162$, df = 1, $P = 0.007$; table 3.3), whereby pollinators were more likely to forage at the warmer versus cooler temperatures, as can be seen in the consistently higher mean predicted probabilities at the high-temperature treatment compared to the low temperature (figure 3.3A). The likelihood of foraging also varied significantly among species ($X^2= 17.223$, df = 3, $P = <0.001$, figure 3.3A, table 3.3). Furthermore, I found the interaction between species identity and temperature to be significant ($X^2= 13.054$, df = 3, $P = 0.005$), suggesting that the effect of temperature on foraging likelihood varies significantly among pollinator species. In particular, the response of foraging likelihood to temperature in bumblebees (*Bombus terrestris*) showed the largest drop in predicted probability from high to low temperatures, while drone flies (*Eristalis tenax*) showed the smallest drop (figure 3.3A, table 3.3). Interestingly, the predicted probability at high temperatures for drone flies (0.642) was far lower than for the other three species, which all demonstrated probabilities between 0.933 and 0.967 (figure 3.3A, table 3.3).

Table 3.2: Model comparisons with the model formulas, AIC scores, and delta AIC. All four models constructed are listed, with model variations ranked by **ΔAIC** (bold values correspond to the models selected based on AIC scores).

Nr.	Formula	AIC	ΔAIC
Binomial GLM			
1	Foraged (yes/no) ~ species * temperature	**231.060**	**0**
2	Foraged (yes/no) ~ species + temperature	238.114	7.054
3	Foraged (yes/no) ~ temperature	246.768	15.708
4	Foraged (yes/no) ~ 1	269.008	37.948
GLM			
1	Log(latency to forage) ~ species + temperature	685.322	0
2	Log(latency to forage) ~ species * temperature	686.227	0.905
3	Log(latency to forage) ~ temperature	686.689	1.367

4	Log(latency to forage) ~ 1	**686.018**	**0.696**
Binomial GLMM			
1	Foraged (yes/no ~ species + temperature + predation + species* temperature + (1|individual),	**486.510**	**0**
2	Foraged (yes/no) ~ species + temperature + predation + species * temperature + temperature * predation + (1|individual)	488.505	1.995
3	Foraged (yes/no) ~ species + temperature + predation + temperature* predation + (1|individual),	505.545	19.035
4	Foraged (yes/no) ~ species*temperature*predation + (1|individual)	498.785	12.275
5	Foraged (yes/no) ~ species + temperature + predation + (1|individual),	503.556	17.046
6	Foraged (yes/no) ~ temperature + predation +(1|individual)	526.068	39.558
7	Foraged (yes/no) ~ temperature + (1|individual)	530.595	44.085
8	Foraged (yes/no) ~ predation + (1|individual)	579.418	92.908
9	Foraged (yes/no) ~ 1 + (1|individual)	583.205	96.695
Tweedy GLM			
1	Latency to forage ~ predation + (1|individual)	**3491.368**	**0**
2	Latency to forage ~ temperature+ predation + (1|individual)	3491.636	0.268
3	Latency to forage ~ species + temperature + predation + temperature* predation + (1|individual)	3498.179	6.811
4	Latency to forage ~ species + temperature + predation + species * temperature + temperature * predation + (1|individual)	3497.186	5.818
5	Latency to forage ~ species + temperature + predation + (1|individual)	3496.605	5.237
6	Latency to forage ~ species + temperature + predation + species *temperature + (1|individual)	3495.636	4.268
7	Latency to forage ~ temperature + (1|individual)	3517.834	26.466
8	Latency to forage ~ 1 + (1|individual)	3517.004	25.636

Table 3.3: Results from the logistic regression predicting the likelihood of four different pollinator species foraging at low and high temperatures (before a disturbance was introduced). Includes odds ratio (with 95% confidence interval (CI)), predicted probability of foraging (with 95% CI), z-value, and P-value (significant values in bold font) from the logistic regression model: likelihood of foraging ~ species * temperature.

	Foraging likelihood					
Predictors	*Odds Ratio*	*CI (95%)*	*Probability*	*CI (95%)*	*z-value*	*P-value*
Intercept	29.000	6.210-516.790	0.967	0.798-0.995	3.311	**0.001**
Bombus terrestris	1.000	0.040-26.100	0.967	0.798-0.995	0.000	1.000
Eristalis tenax	0.060	0.000-0.360	0.642	0.453-0.796	-2.548	**0.011**
Lucilia sericata	0.480	0.020-5.310	0.933	0.769-0.983	-0.581	0.561
Temperature (low)	0.090	0.000-0.570	0.733	0.550-0.860	-2.146	**0.032**
*Bombus terrestris** Temperature (low)	0.220	0.010-6.690	0.379	0.224-0.564	-0.974	0.330
*Eristalis tenax** Temperature (low)	7.810	0.930-173.190	0.571	0.387-0.738	1.675	0.94
*Lucilia sericata** Temperature (low)	2.070	0.150-53.920	0.733	0.550-0.861	0.527	0.527

The model selected based on AIC for explaining variation in latency to forage included none of the predictors (i.e., the intercept-only model; table 3.2). Thus, there was no detected effect of species or temperature on latency to forage before a disturbance (table 3.4), which is evident in Figure 3.3B, where the difference between latency to forage at low and high temperatures is negligible. While there appeared to be some difference among species in their latency to forage (Figure 3.3B), there was no significant effect of species detected in the GLM, suggesting species identity is relatively unimportant.

Table 3.4: Results from the GLM comparing latency to forage (ln) for the four pollinator species at low and high temperatures (before a disturbance was introduced). Includes estimates, 95% confidence intervals (CI), t-values, P-values (significant values in bold font), from logistic regression model: latency to forage ~ temperature.

	Log (latency to forage)			
Predictors	*Estimates*	*CI (95%)*	*t-value*	*P-Value*
Intercept	4.005	3.680 – 4.330	23.999	**<0.001**

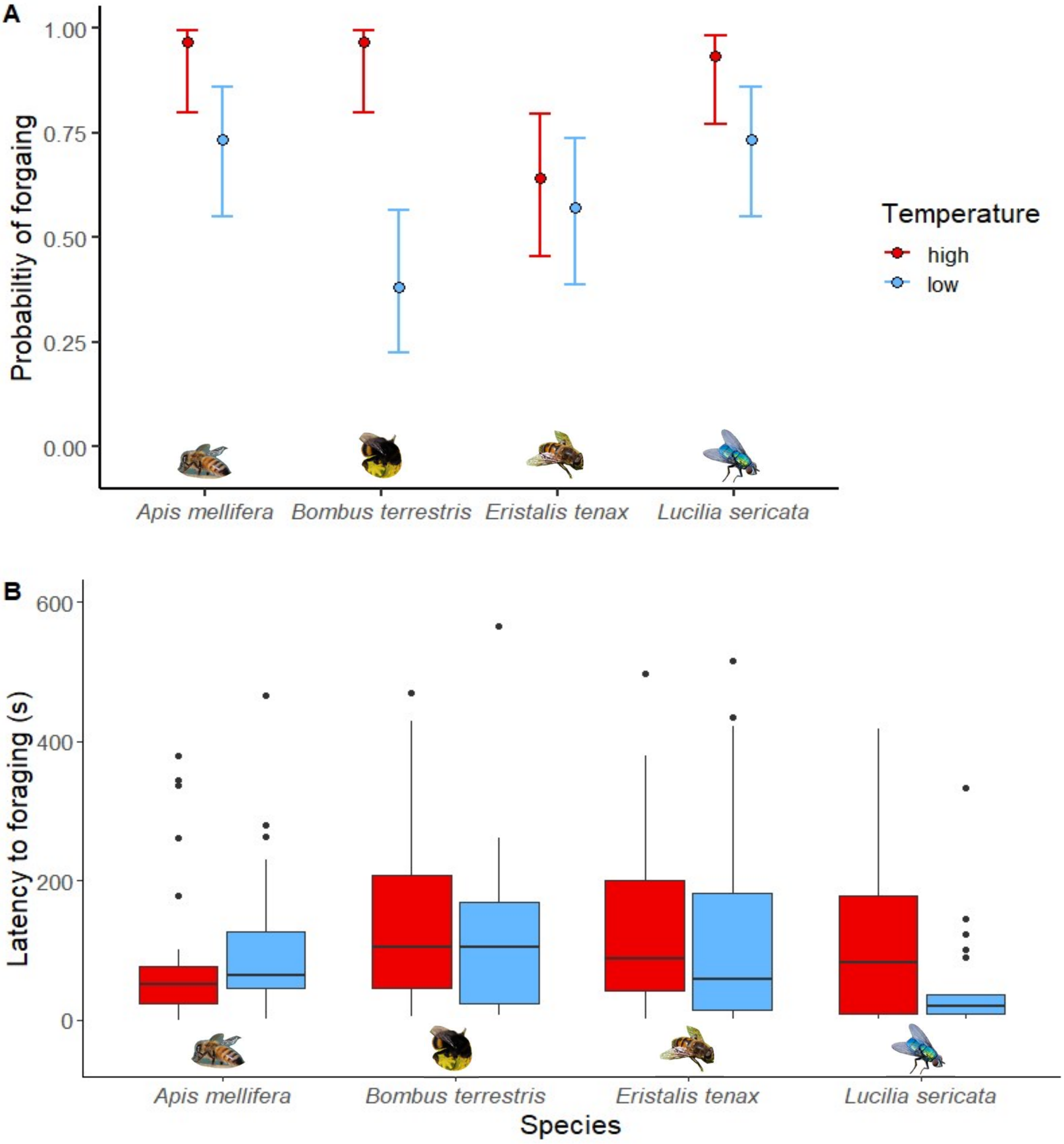

Figure 3.3: Comparisons of (**A**) the likelihoods of the four pollinator species foraging and (**B**) the time taken for each of the four species to start foraging (before a disturbance was introduced) when placed in the experimental arena. High and low-temperature treatments in (**A**) are represented by red and blue points (mean probability of foraging) and standard error bars, respectively. In (**B**), the bold horizontal black line represents the median, with the whiskers showing the maximum and minimum data values for latency to forage, black points showing outliers, and boxes showing the interquartile range. High and low-temperature treatments are represented by red and blue, respectively.

3.4.2 Effects of warming on predator avoidance behaviour of pollinators

When testing for the effects of predation-risk (i.e., the simulated predation disturbance) and temperature on foraging likelihood, I found that species identity, predation disturbance (before or after disturbance) and the interaction between species identity and temperature were included in the best model based on AIC selection (table 3.2; table 3.5). The interaction between temperature and predation was removed from the model as its exclusion improved the model's AIC score (table 3.2). However, I found a significant overall difference in the likelihood of foraging before and after the predation disturbance was introduced ($X^2 = 8.710$, df = 1, $P = 0.010$; table 3.5), whereby pollinators were less likely to forage after the predation disturbance. Similar to the analysis on pollinator foraging in the absence of the simulated disturbance, I found that pollinators were significantly more likely to forage at high temperatures ($X^2 = 8.710$, df = 1, $P = 0.003$; table 3.5), and there was a significant difference in the likelihood of foraging among species ($X^2 = 26.356$, df = 3, $P = <0.001$; table 3.5). Additionally, there was a significant interaction between species identity and temperature ($X^2 = 17.988$, df = 3, $P = <0.001$), which like the analysis on foraging likelihood in the absence of the predators, suggests that the effect of temperature on foraging likelihood depends on species identity.

Bumblebees showed the largest change, and the drone flies the smallest change in predicted foraging probability with temperature, both before and after a disturbance (figure 3.4(B), table 3.5). Nevertheless, the foraging likelihood was still higher for pollinators at higher temperatures, regardless of the disturbance treatment (figure 3.4), which was supported by the lack of a significant interaction between predation and temperature. Finally, the effect of predation was clear, with all species showing a drop in predicted foraging probability after a disturbance at both high and low temperatures (figure 3.4, table 3.5).

Table 3.5: Results from the generalised linear mixed model (GLMM) predicting the effect of temperature, disturbance, and species identity on foraging likelihood across four pollinating insect species. Includes odds ratio (with 95% confidence interval (CI)), predicted probability of foraging (with 95% CI), z-value, and P-value (significant values in bold font) from binomial GLMM: likelihood of foraging ~ species + temperature + predation + species * temperature + (1|individual).

	Foraging likelihood					
Predictors	*Odds Ratios*	*CI (95%)*	*probability*	*CI (95%)*	*z-value*	*P-Value*
Intercept	8.460	3.120 – 22.920	0.894	0.106 – 1.000	4.200	**<0.001**
Bombus terrestris	1.270	0.320 – 5.030	0.915	0.000 – 1.000	0.347	0.729
Eristalis tenax	0.130	0.040 – 0.380	0.514	0.387 – 0.648	-3.640	**<0.001**
Lucilia sericata	1.270	0.320 – 5.030	0.915	0.124 – 1.000	0.347	0.729
Temperature (low)	0.190	0.060 – 0.570	0.619	0.252 – 0.987	-2.951	**0.003**
Predation before	1.850	1.160 – 2.950	0.750	0.438 – 1.000	2.583	**0.010**
Bombus terrestris (temp low)	0.170	0.030 – 0.860	0.396	0.375 – 0.417	-2.144	**0.032**
Eristalis tenax (temp low)	3.610	0.960 – 13.530	0.578	0.330 – 0.825	1.902	0.057
Lucilia sericata (temp low)	0.620	0.130 – 3.010	0.705	0.383 – 1.000	-0.587	0.557

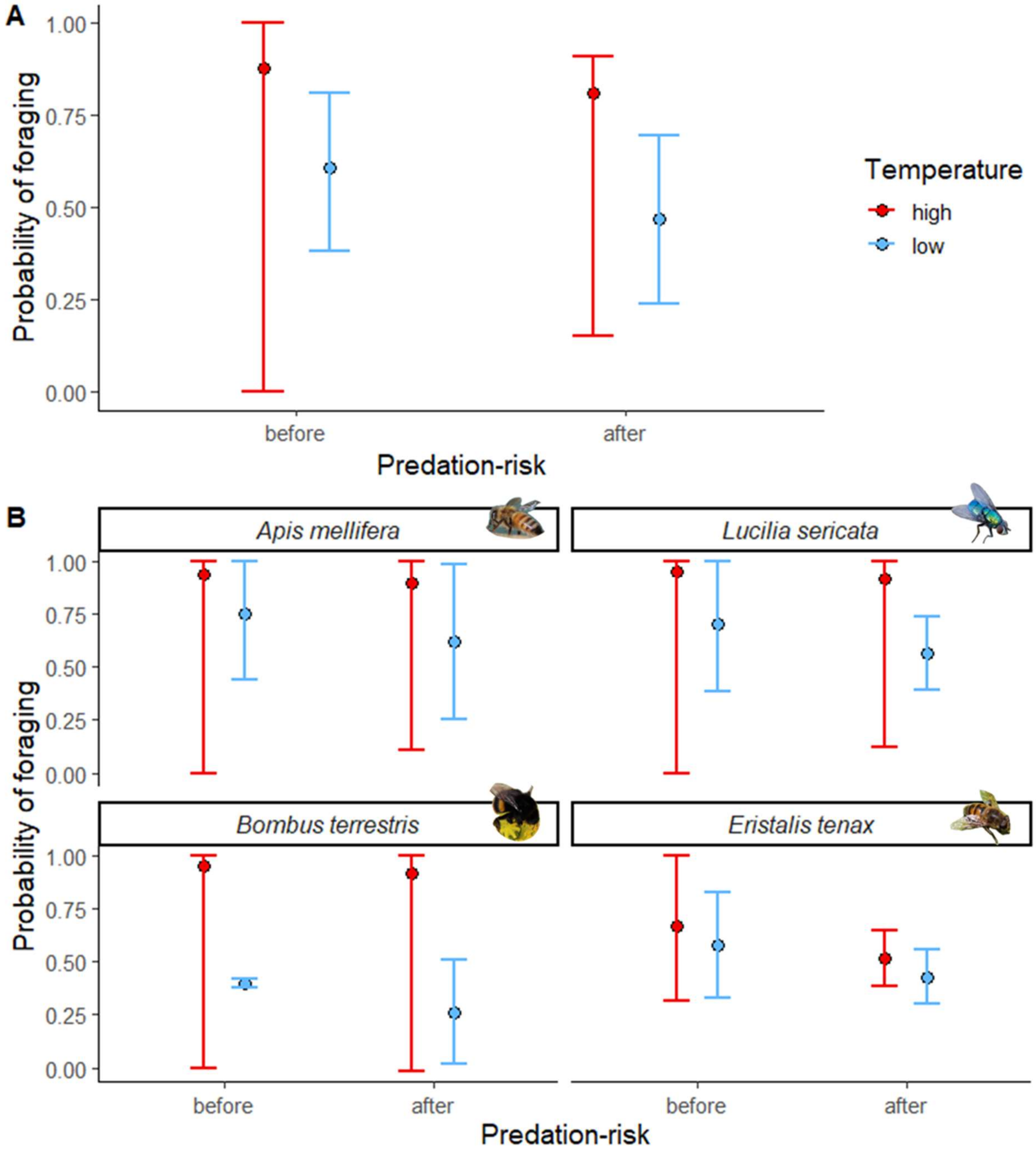

Figure 3.4: Comparisons of (**A**) the likelihood of foraging before and after predation across all species and, (**B**) the likelihood of foraging before and after predation for each species. In both (**A**) and (**B**), the high and low-temperature treatments are represented by the red and blue points (mean probability of foraging) and standard error bars, respectively.

When analysing the effect of temperature and predation on latency to forage, the best model included only predation as a predictor (table 3.2). Thus, species identity, temperature and any interactions between these explanatory variables had little power to explain variation in latency to forage (table 3.6). My results demonstrated that latency to forage differed significantly before and after a disturbance was introduced (X^2 = 27.959, df = 1, *P* = <0.001; table 3.4). In particular, there was a higher latency to forage before a disturbance was introduced then after (Fig. 3.5). The blow flies' latency to forage at the low-temperature treatment before predation seemed to be the one exception, as it remained seemingly unchanged after predation. However, the lack of any significant difference in latency to forage between temperature treatments or among species suggests that these effects were negligible.

Table 3.6: Results from the tweedy GLMM comparing latency to forage for the four pollinator species before and after a disturbance was introduced. Includes estimates, 95% confidence intervals (CI), z-value, P-value (significant values in bold font) from the tweedy GLMM: latency ~ predation + (1|individual).

		Latency to forage		
Predictors	*Estimates*	*CI (95%)*	*z-value*	*P-Value*
Intercept	28.470	21.270 – 38.110	22.514	**<0.001**
Predation before	2.350	1.710 – 3.220	5.288	**<0.001**

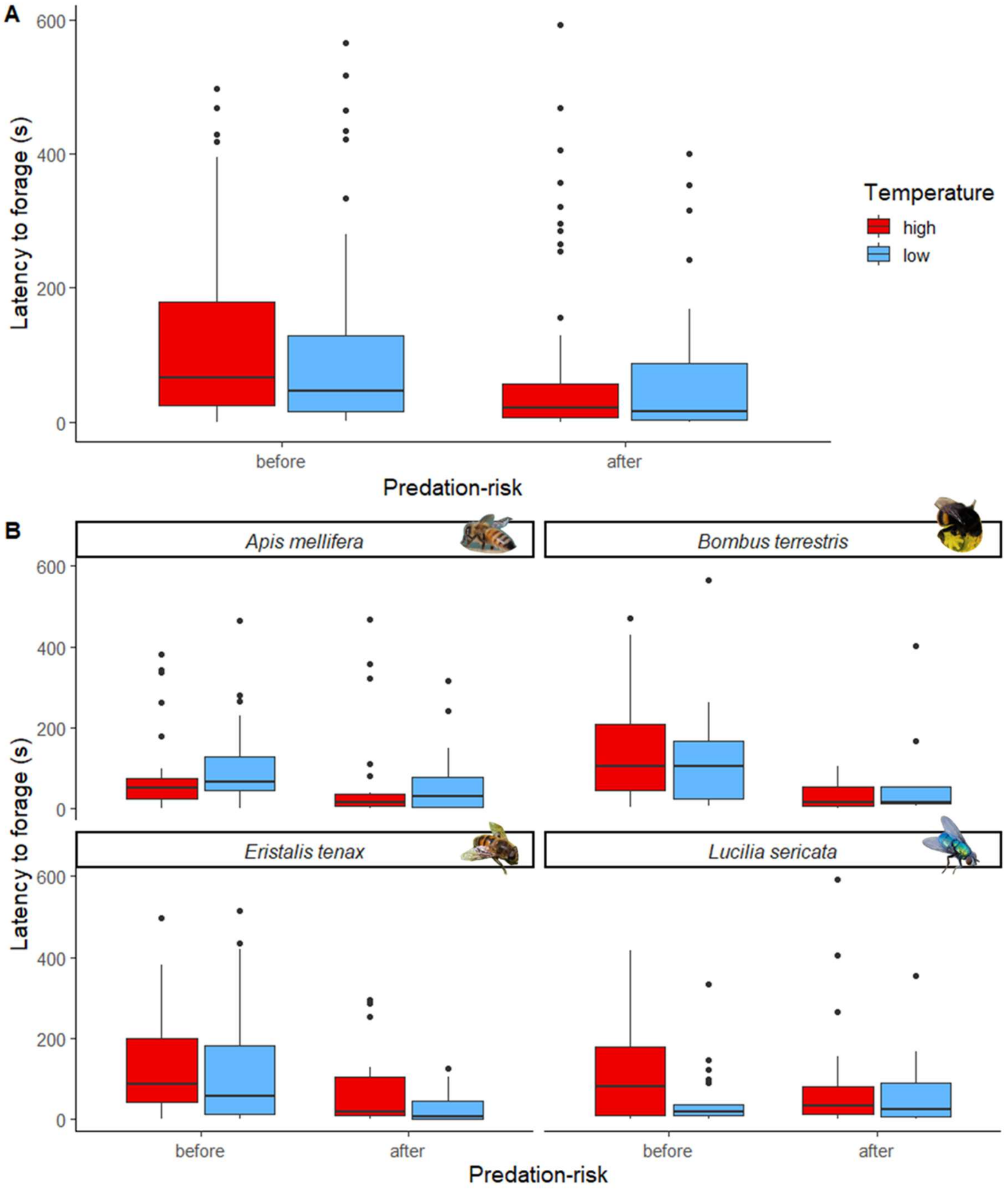

Figure 3.5: Comparisons of latency to forage (s) before and after a disturbance at high and low temperatures across (**A**) all four pollinator species and (**B**) for each species separately. In both figures, the thick black line represents the median latency to forage, with the whiskers showing the maximum and minimum data values for latency to forage. The black points show outliers, and the high and low-temperature treatments are represented by the red and blue boxes consecutively.

3.5 Discussion

Results from this study show that the likelihood of pollinating insects to forage is significantly dependent on temperature, predation risk and species identity, while their foraging latency is only influenced by predation risk. These results suggest that pollinators alter their foraging behaviour in response to predation and temperature in order to minimise fitness costs of predation and also compensate for increased energetic costs at warmer temperatures. However, the lack of any interaction between the temperature and predation risk suggests that warming temperatures may override the predator avoidance behaviour induced by non-consumptive predation effects.

I found that all pollinating insects were more likely to forage at high temperatures than at low temperatures. However, this effect of temperature depended on the species in question. It has been established that as environmental temperatures rise, insect pollinator resting metabolic rates increase (Chapter 2; González-Tokman et al., 2020; Irlich et al., 2009). Moreover, pollinators' energetic requirements are likely related to their field metabolic rates (McCallum et al., 2013; Nagy, 1987), so to avoid energy depletion at higher temperatures, pollinators may need to increase their intake rates (McCallum et al., 2013; Stabentheiner & Kovac, 2016). Given the strong relationship between temperature and metabolic rate in insects, it is therefore highly likely that the increased foraging likelihoods at the higher temperature in my experiments were driven by the pollinators attempting to meet rising energetic demands under warmer conditions. If this is the case, the positive effect of temperature on pollinator foraging could result in increased pollination rates in a warmer climate, having a potentially positive implication for plant fitness (Scaven & Rafferty, 2013). However, if there are not enough floral resources available in the landscape to meet the increased energetic demands of pollinators, their reproductive fitness or survival could be jeopardised by global warming (Scaven & Rafferty, 2013).

While I found that honeybees (*Apis mellifera*) and blow flies (*Lucilia sericata*) had similar responses in foraging likelihoods across temperature treatments, bumblebees (*Bombus terrestris*) *w*ere the most temperature sensitive, and drone flies (*Eristalis tenax*) showed significantly lower foraging likelihoods at high temperatures than the other three species. The species-level differences in foraging likelihood at different temperatures could arise for various reasons. Firstly, there could be differences in thermal tolerances among the species that caused the observed species-specific differences in foraging behaviour. Warming may allow species that are more tolerant to higher temperatures to show superior foraging performance (Cerdá et al., 1998). Furthermore, flies and bees are believed to have different peak foraging temperatures, which have been estimated as 20.8ºC for dipterans and 24.5ºC for hymenopterans (Kühsel & Blüthgen, 2015). The similarity found in my study between foraging likelihoods in blow flies and honeybees, rather than between species in the same insect order, suggests that order-specific thermal tolerances are unlikely to explain the variation among species. Moreover, bumblebees are commonly thought to forage at cooler temperatures when compared to honeybees because their larger size makes them better at maintaining body temperatures higher than ambient (Goulson et al., 2002). Honeybees have also been seen foraging at hot mid-day temperatures, while bumblebees avoided foraging at this time (Schaffer, 1997). In contrast, however, I found that bumblebees were less likely to forage at low temperatures but had similar foraging likelihoods to honeybees at high temperatures. Thus, predictions for species-level thermal preferences based on past findings from other studies would also not appear to match the results from my study. Taken together, my results indicate that a better understanding of species-specific responses of pollinator behaviour to warming is needed but will require targeted experiments to better tease apart more universal mechanisms that underlie these differences.

My results revealed that both temperature and predation risk influence the foraging behaviour of pollinators. Here, all pollinator species in my experiments altered their behaviour in response to predation risk, demonstrating that pollinating insects do respond to non-consumptive predation effects. In particular, foraging likelihoods significantly declined after being disturbed, regardless of the experimental temperature to which they were exposed. Indeed, many animals, including pollinating insects, have been shown to react to predator presence by changing their behaviour to minimise their predation risk while foraging (Goncalves-Souza et al., 2008; Ings & Chittka, 2009; Pierce, 1988). Pollinators, in particular, typically reduce visitation frequencies, residence times, and foraging efficiency when faced with a predation risk (Gavini et al., 2020; Ings & Chittka, 2009; Romero et al., 2011), which likely results in fitness costs to the pollinators and the plants. For example, Gonçalves-Souza et al. (2008) showed that the total visitation rate of a group of insect pollinators decreased when artificial predators were introduced into the system. Another study showed that bumblebees reduce visitation and change their flower preference when exposed to a predation event in order to avoid flowers of the same colour where they previously had experienced simulated predator attacks (Ings & Chittka, 2009). Both studies' results suggest that the behaviourally mediated indirect effects of cryptic predators could impact plant reproductive success more severely than conspicuous predators My results, in conjunction with previous findings, provide convincing evidence that predation risk alters can reduce pollinator foraging the likelihood of foraging, which could have important consequences for plant-pollinator interactions in future climatic conditions.

The simulated increase in predation risk also affected foraging latency, with all species showing shorter latencies after being exposed to a disturbance. In other words, pollinating insects took less time to return to foraging immediately after experiencing increased predation risk. This non-consumptive predation effect on foraging latency was contrary to my expectation that pollinators would forage more cautiously under higher perceived predation risk (Gavini et al., 2020; Goncalves-Souza et al., 2008; Ings & Chittka, 2009; Romero et al., 2011; Tan et al., 2013). Furthermore, this also seems to contradict the finding that the likelihood of pollinator foraging decreased following the predation disturbance. So why are pollinators less likely to forage, but those that do are quicker to initiate foraging after a predation disturbance? As the tests of behavioural responses to predation risk were repeated on individuals, the pollinators could have learnt where the food was, allowing them to return to forage quickly. In fact, a large number of studies focussing mostly on *Drosophila* fruit flies and honeybees have shown them to be capable of several forms of learning (Kawecki, 2009; Perry & Barron, 2013). This research has also been extended to other insect pollinator species, such as a study on *Eristalis* flies that found they could learn to find food based on model flower colour (Ilse, 1949). Furthermore, bumblebees have been shown to use colour to distinguish between flowers with and without rewards and were even able to retain what they had learned overnight (Dukas & Real, 1991). Despite the possibility that the insects in my study could have learnt the food reward location in the experimental arena, more careful behavioural analyses are needed to rule out that this is not just a simple behaviour response to food being interpreted as reward learning (Perry & Barron, 2013).

Another explanation for the reduced foraging latency following increased predation risk could be that insects foraged quickly to obtain food before the perceived predator returned. As mentioned earlier, pollinators reduce visitation frequencies and the time spent foraging when faced with a predation risk (Gavini et al., 2020; Romero et al., 2011; Tan et al., 2013). Furthermore, a study on honeybees by Tan et al. (2013) found that the presence of hornets caused bees to reduce visitation rates and residence times. Additionally, the bees took into account the level of danger different hornet species presented and reduced their foraging accordingly (Tan et al., 2013). Thus, reductions in foraging latency due to the presence of predation risk could be a measure that, similarly to residency times, indicates how foragers will spend less time foraging when predators are nearby.

Despite the obvious effect that predation risk had on pollinator foraging behaviour, there was no interaction between predation and temperature, suggesting that warming does not strengthen the non-consumptive effects of predation. Instead, my results suggest that pollinators are more likely to continue foraging at warmer temperatures, regardless of perceived predation risk. Interestingly, while my results are not in accordance with some other studies, research on interactions between temperature and non-consumptive predation effects has yielded variable conclusions. For example, a study by Barton and Schmitz (2009) revealed that high temperature strengthened single predator effects of spiders on grasshopper prey species, whereas a study of marine invertebrates found that warming reduced the non-consumptive effects that marine predators had on their prey (Miller et al., 2014). In contrast, Barton (2010) found that increased air temperatures did not change the impact of spider predator presence on grasshopper behaviour. Scaling up my results to real ecosystems, however, should be done with some caution, given that pollinators were isolated from natural pollination networks and may have been buffered from other indirect effects such as exploitative and apparent competition. Nevertheless, by controlling for the myriad of other abiotic and biotic factors that these pollinators will experience in real ecosystems, my study demonstrates that pollinators are likely to expose themselves to greater predation risks with rising temperatures.

To conclude, both temperature and simulated predation risk impact the foraging behaviour of pollinating insects. Honeybees, bumble bees, drone flies and blow flies all respond to warming temperatures by increasing their foraging likelihood, while predation risk reduces their foraging likelihoods but also shortens their latency to return to foraging. Interestingly, I found that warming seemingly increases pollinator foraging, even when there is an increase in the perceived risk of predation. These findings suggest that, rather than warming temperatures influencing the strength of non-consumptive predator effects, the energetic costs of warming may outweigh the perceived fitness costs of predation causing insect pollinators to take greater foraging risks in the future. These impacts of temperature and predation on insect pollinator behaviour can alter pollination rates, which could have serious consequences for the reproductive fitness and productivity of flowering plants. While the increased foraging behaviour of the four study species under warming could increase their flower visitation rates and indirectly improve plant pollination, this is likely to come at the expense of pollinator fitness as they take greater risks in a warmer world. Therefore, warming climatic conditions could have the potential to destabilise plant-pollinator and erode pollinator services by imposing greater energetic costs and predation risk on insect pollinators.

Chapter 4

General discussion

In this thesis, I have highlighted the importance of understanding the effects of warming on insect pollinator physiology and behaviour. My research has focused on the effects that global warming would have on the responses of metabolism and foraging behaviour in four common insect pollinators, honeybee (*Apis mellifera*), bumblebee (*Bombus terrestris*), drone fly (*Eristalis tenax*), and blow fly (*Lucilia sericata*), in Aotearoa New Zealand. My results highlighted that universal scaling laws could not be indiscriminately applied across insect pollinator species to predict widespread climate change impacts (Chapter 2). Additionally, this thesis was the first such study to examine the combined effect of warming and predation risk on foraging behaviour in animal pollinators (Chapter 3). While warming may alter foraging behaviour, the four pollinators studied here continued to show high foraging probabilities, regardless of increased predation risk. Thus, these four species are unlikely to experience changes in their non-consumptive predation effects because of climate change (Chapter 3). Below, I discuss why the unified metabolic theory is impractical, how metabolism and behavioural responses to temperature are linked, what insect pollinator responses may mean for the future of pollination, and potential areas for expanding upon pollination research.

4.1 How practical is the Metabolic Theory of Ecology?

The results from my work have added to the growing consensus that the current Metabolic Theory of Ecology (MTE) is not suitable for using metabolic rates of individual species to infer universal patterns of biological processes at higher levels of organisation. The inconsistency in the scaling relationships found across the four insect pollinator species implies that if the metabolic rates of individual species were to be used to make predictions for other species or for whole communities, the results might be imprecise (Chapter 2), but this is not necessarily true for all species metabolic rates. However, my results do show that the fundamental equation on which the MTE is based has the potential to provide information on the thermal (and thus global warming) sensitivity of individual species. For example, from the scaling relationships presented in Chapter 2, it is evident that bumblebee metabolic rates had the strongest response to temperature, implying that this species would be more sensitive to warming than the other four species. Moreover, the fact that the grouped species data (at the ‘community’ level) agreed with the scaling relationships upon which the MTE is based suggests that this theory has some potential for higher levels of biological organisation (Chapter 2). However, if one needed a dataset that included the metabolic rates of an entire group of species for the MTE to make accurate predictions, then it does not appear to be capable of fulfilling its aim of predicting how metabolic rate governs ecological processes from an individual level through to biosphere level (Brown et al., 2004).

4.2 Are temperature, metabolism and behaviour connected?

Across both Chapters 2 and 3 I found similar temperature-induced metabolic and behavioural responses for bumblebees and drone flies. The drone flies, for example, showed very small metabolic and behavioural changes with temperature, whereas the bumblebees showed more dramatic changes for both response variables. The similarity in responses across chapters suggests that temperature, through its effect on metabolic rates, could limit the foraging behaviour of insect pollinators. In contrast to the bumblebees and drone flies, honeybees and blow flies' metabolic and behavioural thermal sensitivities across chapters did not show similar sensitivities. In other words, honeybees' metabolic rates were the second most thermally sensitive, but their behaviour response was the second least sensitive to temperature. In comparison, blow flies had the second least sensitive metabolic response and the second most sensitive behavioural response to temperature. In addition to the difference in the honeybees' and blow flies' sensitivities, only foraging probability showed species-specific changes with temperature. The inconsistency in these results across chapters and in both behavioural measures responses implies that temperature influence on metabolic rates might not be acting alone to control insect pollinator behaviour.

These incongruities in the species-specific responses of metabolic rates and foraging behaviour to increasing temperature could be attributed to differences in activity levels. This is because, while metabolic rates may determine ectotherm activity to some extent, activity levels can also significantly affect measured metabolic rates (Glazier, 2020; Halsey et al., 2015; Harrison & Fewell, 2002; Woods et al., 2005). For example, a study by Woods *et al.* (2005) demonstrated that metabolic rates for flying honeybee individuals decreased as air temperature increased as opposed to resting metabolic rates, which increased with temperature. Importantly, I only measured resting metabolic rates, i.e. in the absence of activity (Chapter 2). Therefore, the variation in pollinator activity levels (i.e. walking vs flying) during the behavioural assays could have interacted with temperature to alter the metabolic rate and energetic demands in a way that differed from what I hypothesised. The inclusion of individual activity levels in future studies of metabolic and behavioural responses to global warming could help to elucidate the importance of pollinator activity levels for the variation between metabolic and behavioural responses.

Conversely, in Chapter 3's results, I found that temperature effects on foraging behaviour are stronger than predation effects. Moreover, I did not detect an interaction between temperature and predation risk, meaning that temperature did not determine the strength of the predator avoidance response. Thus, temperature seems to have a stronger influence on foraging behaviours than on antipredator behaviours. Foraging under high temperatures, as well as in the presence of predators, both come with energetic and potential fitness costs. I initially expected that when temperatures were high, increased metabolic rates would alter the energetic balance of insect pollinators and that this would force the insects to lower their predator avoidance behaviour to balance energetic gains and losses (Bednekoff, 2007; Jones & Dornhaus, 2011; Vucic-Pestic et al., 2011). What I found, instead of the non-consumptive predation effects' strength being altered by warming, was that when faced with the trade-off, insect pollinators' drive to forage at high temperatures was stronger than their drive to avoid predators. Thus, temperature may be increasing metabolic rates to the point that this overrides non-consumptive predation effects. From the ideas presented above, my results may suggest that insect pollinator foraging choices all come down to metabolism, regardless of predation risk, but that temperature alone does not determine pollinator metabolic rates.

4.3 Will climate change in Aotearoa, New Zealand, improve pollination services?

The temperature range over which I tested the physiological and behavioural responses of pollinators was chosen to represent a potential 3 – 4°C rise in mean and very high annual temperatures across New Zealand (Kenny, 2011, Ministry for the Environment, 2016; *Welcome to the Climate Database*, n.d.). Furthermore, I studied the potential physiological and behavioural impacts of warming for four important agricultural pollinator species. Thus, from the results of my work, I can make broad theoretical predictions about how pollination services may be affected by future warming.

Firstly, the pollination of agricultural crops may be improved because of climate warming. This theory is alluded to by three lines of evidence. The first is that at high temperatures, metabolic rates increase (Chapter 2). High metabolic rates will ultimately raise energetic demands requiring pollinators to boost their foraging activity, and increased foraging by pollinators will increase pollen transfer, thereby enhancing plant pollination (Scaven & Rafferty, 2013). Second, the consistency of this study's grouped species temperature-metabolism relationship with the exponential relationship predicted by the MTE suggests the thermal limits of insect pollinators used in my study are not reached in temperatures ranging from 15 – 35°C (Chapter 2). This was assumed because the Arrhenius relationship on which the MTE has been partially based moves away from being perfectly exponential at temperatures near an organism's thermal limits (Molnár, 2017). If their thermal limits were to have been breached, the loss of pollinators (da Silva et al., 2017) and, thus, reductions in pollination would be highly likely under the warming scenarios tested here. Thirdly, I showed that high temperatures are responsible for increases in foraging probability and reductions in the latency to forage (Chapter 3), which could increase flower visits, boost pollen transfer, and improve pollination services (Scaven & Rafferty, 2013). Together all three results support the notion that the provisioning of pollination services across New Zealand may improve with global warming.

The second theory that I investigated is that while pollination may improve overall with climate change, each species will respond differently to rising temperatures. For example, evidence from both chapters 2 and 3 suggests that bumblebee individuals will be more sensitive to temperature change, resulting in more dramatic changes in foraging behaviours with warming. The exact opposite is expected for drone fly individuals, in that they should show the smallest behavioural change. Honeybee and blow fly individuals will likely be impacted by warming similarly to each other, with both being more sensitive than drone flies but less so than bumblebees. It is also important to note that the physiological and behavioural responses shown in my thesis could also cause the loss of pollination services. For example, climate change could reduce the food resources available to pollinators because of extinctions, geographical shifts and/or changes in the developmental timing of the plants they rely on for food (Dalsgaard, 2020; Devi & Mishra, 2020; Giannini et al., 2012; Wilson & Fox, 2021). As a result, pollinating insects would no longer be able to support higher energetic demands due to warming temperatures, causing reductions in fitness or even mortality (Devi & Mishra, 2020). This evidence suggests that warming may have the potential to improve pollination services in New Zealand, but its impact will depend on the local context, such as the availability of resources. Furthermore, my results from Chapter 3 demonstrated that pollinators increase their foraging even at the expense of increased predation risk. These results suggest that if pollinators also experience higher rates of predation in the future, it could lead to reductions in pollinator populations and destabilisation of plant-pollinator networks. In other words, the nature of the pollinator-plant networks being affected and the additional warming impacts playing a role in these systems will ultimately determine the future outcome of pollination in New Zealand.

4.4 Limitations and future research directions

My research exclusively focused on insect pollinator metabolism and foraging behaviour responses to temperature, limiting my ability to make predictions about the effects of climate change on pollination under varying future scenarios. In future studies, I would thus aim to incorporate more variables. For example, including variation in the concentration or volume of floral resources in behavioural assays manipulating temperature (such as those I ran in Chapter 3) would allow me to investigate if the effects of temperature and/or predation risk on energetic demands and an insect's ability to meet these demands are dependent on resource quality. Llandres et al. (2012) conducted a study that tested pollinator responses to habitat patches that varied in nectar availability and predator presence. Thereby including temperature variation into a setup similar to the one used by Llandres et al. (2012). would also allow me to test if temperature influences the non-consumptive effects of predation, making pollinators more risk-prone when food is limited. Moreover, testing the temperature response of a broader range of pollinator behaviours, such as foraging distance, visit duration, visitation rate and number of visits (Descamps et al., 2021; Everaars et al., 2018), could allow me to identify behaviours most important for plant pollination services and those most vulnerable to temperature changes.

By studying pollinators that were isolated from their natural systems, I was able to isolate and identify the effects of temperature and predation on specific foraging behaviours. However, studying insect pollinators separately from the pollination networks, as I have done herein, reveals only certain impacts pollinators encounter with climate change but can hide impacts that affect their interaction partners. By adding more response and explanatory variables into future experiments, I could enhance future predictions about climate change impacts. For example, since I only offered pollinators excess sucrose solution during my behavioural assays, incorporating differences in nectar availability could allow me to test if resource limitation makes pollinators more risk-prone under high temperatures. This could be done in ways similar to Llandres et al. (2012) or even Gavini et al. (2020), the latter of which investigated the effect of worker size on bumblebee decisions to trade off between nectar reward and predation risk. Additionally, if flowering plants instead of nectar rewards or their own or in fake flowers were included in future research, I could explore how temperature and predation affect plant fitness through shifts in pollinator foraging behaviour, allowing me to make real-life predictions about the effects of global warming on pollination services. Finally, the behaviour of pollinator predators may also be impacted by climate change. Thus, incorporating live predators into studies investigating the effect of warming and predation risk on pollinators could reveal interactive impacts not seen in my results. For example, warming was shown to cause trophic cascades by altering predation risk behaviour in Barton and Schmitz's (2009) study on grasshoppers and their spider predators. While my study has shown important variation in the response of pollinator foraging to temperature and predation risk, adding additional complexity to future experiments would allow the capture of interactive effects taking us closer to understanding the effects of climate warming on pollination services.

Studying pollinators in isolation from pollination networks can also conceal impacts that affect the competitive interactions between species. A good example of a concealed impact is how climate change may alter niche complementarity. Niche complementarity describes a scenario when multiple species have functional niches that do not overlap, allowing them to completely exploit a suite of resources and, therefore, can lead to increased rates of ecosystem functioning at the community level (Loy & Brosi, 2022). Because the effect of rising temperature on resource usage by pollinators has not been studied in depth, future studies, including entire pollinator guilds and their interaction partners, could investigate the effects of climate change on niche complementarity and, thus, ecosystem functioning of pollinator assemblages. Lastly, my work only focused on introduced insect pollinator species. Expanding any pollination research in New Zealand to include native pollinator and native plant communities could allow us to understand how global warming will impact the future of native pollination networks compared to introduced pollinator networks.

4.5 Concluding remarks

My thesis examined the physiological and behavioural impacts of rising temperatures on insect pollinators. I have also demonstrated how, without revision, the Metabolic Theory of Ecology may be incapable of fulfilling its primary aim across all taxa. Despite this, the MTE appears useful for providing information on species' thermal sensitivity. My work has also highlighted the role that multiple environmental drivers (i.e. temperature and predation) have in influencing pollinator behaviour. The results herein show a potentially positive future for pollination services in New Zealand, despite the predicted rise in air temperature associated with climate change. However, there is still much to be investigated if we are to fully understand all the potential impacts climate change will have on insect pollinators, both introduced and native.

References

Abou-Shaara, H. F., Owayss, A. A., Ibrahim, Y. Y., & Basuny, N. K. (2017). A review of impacts of temperature and relative humidity on various activities of honeybees. *Insectes Sociaux*, *64*(4), 455-463. https://doi.org/10.1007/s00040-017-0573-8

Abram, P. K., Boivin, G., Moiroux, J., & Brodeur, J. (2017). Behavioural effects of temperature on ectothermic animals: Unifying thermal physiology and behavioural plasticity. *Biological Reviews*, *92*(4), 1859–1876. https://doi.org/10.1111/brv.12312

Afik, O., & Shafir, S. (2007). Effect of ambient temperature on crop loading in the honeybee, *Apis mellifera* (Hymenoptera: Apidae). *Entomologia generalis*, *29*(2-4), 135-148.

Aizen, M. A., Garibaldi, L. A., Cunningham, S. A., & Klein, A. M. (2008). Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. *Current biology*, *18*(20), 1572-1575.

Allen, A. P., & Gillooly, J. F. (2007). The Mechanistic Basis of the Metabolic Theory of Ecology. *Oikos*, *116*(6), 1073-1077.

Amano, T., Smithers, R. J., Sparks, T. H., & Sutherland, W. J. (2010). A 250-year index of first flowering dates and its response to temperature changes. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1693), 2451-2457. https://doi.org/10.1098/rspb.2010.0291

Angilletta Jr, M. J., Steury, T. D., & Sears, M. W. (2004). Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integrative and comparative biology, 44*(6), 498-509.

Arrhenius, S. (1889). Über die Reaktionsgeschwindigkeit bei der Inversion von Rohrzucker durch Säuren. *Zeitschrift für Physikalische Chemie, 4*(1), 226-248. https://doi.org/10.1515/zpch-1889-0416

Arribas, P., Abellán, P., Velasco, J., Bilton, D. T., Millán, A., & Sánchez-Fernández, D. (2012). Evaluating drivers of vulnerability to climate change: A guide for insect conservation strategies. *Global Change Biology*, *18*(7), 2135-2146. https://doi.org/10.1111/j.1365-2486.2012.02691.x

Bahrndorff, S., Gertsen, S., Pertoldi, C., & Kristensen, T. N. (2016). Investigating thermal acclimation effects before and after a cold shock in *Drosophila melanogaster* using behavioural assays. *Biological Journal of the Linnean Society*, *117*(2), 241-251. https://doi.org/10.1111/bij.12659

Banda, A., Madamba, D. C., Gumbo, T., & Chanyandura, A. (2021). Climate Change Extent and Dipteran Pollinators Diversity in Africa. In W. Filho, J. Luetz, & D. Ayal (Eds.) *Handbook of Climate Change Management* (1-20). Springer, Cham. https://doi.org/10.1007/978-3-030-22759-3_306-1

Barton, B. T. (2010). Climate warming and predation risk during herbivore ontogeny. *Ecology, 91*(10), 2811-2818. https://doi.org/10.1890/09-2278.1

Barton, B. T., & Schmitz, O. J. (2009). Experimental warming transforms multiple predator effects in a grassland food web. *Ecology Letters, 12(*12), 1317-1325. https://doi.org/10.1111/j.1461-0248.2009.01386.x

Bascompte, J., Jordano, P., Melián, C. J., & Olesen, J. M. (2003). The nested assembly of plant–animal mutualistic networks. *Proceedings of the National Academy of Sciences, 100*(16), 9383-9387. https://doi.org/10.1073/pnas.1633576100

Bednekoff, P. A. (2007). Foraging in the face of danger. In: D. W. Stephens, J. S. Brown, & R. C. Ydenberg (Eds.) *Foraging: behavior and ecology* (305329). The University of Chicago Press, Chicago.

Belgrano, A., Allen, A. P., Enquist, B. J., & Gillooly, J. F. (2002). Allometric scaling of maximum population density: A common rule for marine phytoplankton and terrestrial plants. *Ecology Letters*, *5*(5), 611-613. https://doi.org/10.1046/j.1461-0248.2002.00364.x

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science 313*, 351-354.

Bjørge, J. D., Overgaard, J., Malte, H., Gianotten, N., & Heckmann, L. H. (2018). Role of temperature on growth and metabolic rate in the tenebrionid beetles *Alphitobius diaperinus* and *Tenebrio molitor*. *Journal of Insect Physiology*, *107*, 89-96. https://doi.org/10.1016/j.jinsphys.2018.02.010

Bokma, F. (2004). Evidence against Universal Metabolic Allometry. *Functional Ecology*, *18*(2), 184-187.

Boltzmann, L. (1872) Weitere Studien über das Wärmegleichgewicht unter Gasmolekülen. *Sitzungsberichte Akademie der Wissenschaften, 66*, 275-370. (English translation: Boltzmann, L. (2003) Further Studies on the Thermal Equilibrium of Gas Molecules. *The Kinetic Theory of Gases. History of Modern Physical Sciences, 1*, 262-349.) https://doi.org/10.1142/9781848161337_0015

Bosch, J., Kemp, W. P., & Peterson, S. S. (2000). Management of *Osmia lignaria* (Hymenoptera: Megachilidae) Populations for Almond Pollination: Methods to Advance Bee Emergence. *Environmental Entomology*, *29*(5), 874–883. https://doi.org/10.1603/0046-225X-29.5.874

Bordier, C., Dechatre, H., Suchail, S., Peruzzi, M., Soubeyrand, S., Pioz, M., Pélissier, M., Crauser, D., Conte, Y. L., & Alaux, C. (2017). Colony adaptive response to simulated heat waves and consequences at the individual level in honeybees (*Apis mellifera*). *Scientific Reports*, *7*(1), 3760. https://doi.org/10.1038/s41598-017-03944-x

Bordier, C., Klein, S., Le Conte, Y., Barron, A. B., & Alaux, C. (2018). Stress decreases pollen foraging performance in honeybees. *The Journal of Experimental Biology*, *221*(4), jeb171470. https://doi.org/10.1242/jeb.171470

Borghi, M., Souza, L. P. de, Yoshida, T., & Fernie, A. R. (2019). Flowers and climate change: A metabolic perspective. *New Phytologist*, *224*(4), 1425-1441. https://doi.org/10.1111/nph.16031

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a Metabolic Theory of Ecology. *Ecology, 85*(7), 1771-1789. https://doi.org/10.1890/03-9000

Buckley, L. B., Hurlbert, A. H., & Jetz, W. (2012). Broad-scale ecological implications of ectothermy and endothermy in changing environments. *Global Ecology and Biogeography*, *21*(9), 873-885. https://doi.org/10.1111/j.1466-8238.2011.00737.x

Burkle, L. A., & Alarcón, R. (2011). The future of plant–pollinator diversity: Understanding interaction networks across time, space, and global change. *American Journal of Botany*, *98*(3), 528-538. https://doi.org/10.3732/ajb.1000391

Burkle, L. A., Marlin, J. C., & Knight, T. M. (2013). Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science*, *339*(6127), 1611-1615.

Capellini, I., Venditti, C., & Barton, R. A. (2010). Phylogeny and metabolic scaling in mammals. *Ecology, 91*(9), 2783-2793. https://doi.org/10.1890/09-0817.1

Carmo, R. F. R., Astúa, D., & Vasconcelos, S. D. (2022). Environmental conditions differently affect the wing size and shape of two blow fly species (Calliphoridae) of forensic importance in the Brazilian tropical ecosystems. *International Journal of Tropical Insect Science*, *42*(2), 1903–1911. https://doi.org/10.1007/s42690-021-00720-3

Cerdá, X., Retana, J., & Cros, S. (1998). Critical thermal limits in Mediterranean ant species: Trade-off between mortality risk and foraging performance. *Functional Ecology, 12*(1), 45-55. https://doi.org/10.1046/j.1365-2435.1998.00160.x

Chaplin-Kramer, R., Dombeck, E., Gerber, J., Knuth, K. A., Mueller, N. D., Mueller, M., Ziv, G., & Klein, A. M. (2014). Global malnutrition overlaps with pollinator-dependent micronutrient production. *Proceedings of the Royal Society B: Biological Sciences, 281*(1794), 20141799. https://doi.org/10.1098/rspb.2014.1799

Chen, I. C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024-1026.

Cheung, W. W., Close, C., Lam, V., Watson, R., & Pauly, D. (2008). Application of macroecological theory to predict effects of climate change on global fisheries potential. *Marine Ecology Progress Series*, *365*, 187-197.

Chown, S. L., & Gaston, K. J. (1999). Exploring links between physiology and ecology at macro-scales: The role of respiratory metabolism in insects. *Biological Reviews of the Cambridge Philosophical Society, 74*(1), 87–120. https://doi.org/10.1017/S000632319800526X

Chown, S. L., & Gaston, K. J. (2010). Body size variation in insects: A macroecological perspective. *Biological Reviews*, *85*(1), 139-169. https://doi.org/10.1111/j.1469-185X.2009.00097.x

Chown, S. L., Marais, E., Terblanche, J. S., Klok, C. J., Lighton, J. R. B., & Blackburn, T. M. (2007). Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Functional Ecology*, *21*(2), 282-290. https://doi.org/10.1111/j.1365-2435.2007.01245.x

Chown, S. L., & Storey, K. B. (2006). Linking Molecular Physiology to Ecological Realities. *Physiological and Biochemical Zoology*, *79*(2), 314-323. https://doi.org/10.1086/499989

Clarke, A. (2004). Is there a universal temperature dependence of metabolism? *Functional Ecology, 18*(2), 252-256.

Clarke, A. (2006). Temperature and the metabolic theory of ecology. *Functional Ecology, 20*(2), 405-412. https://doi.org/10.1111/j.1365-2435.2006.01109.x

Clarke, A., Rothery, P., & Isaac, N. J. B. (2010). Scaling of basal metabolic rate with body mass and temperature in mammals. *Journal of Animal Ecology, 79*(3), 610-619. https://doi.org/10.1111/j.1365-2656.2010.01672.x

Classen, A., Eardley, C. D., Hemp, A., Peters, M. K., Peters, R. S., Ssymank, A., & Steffan-Dewenter, I. (2020). Specialization of plant–pollinator interactions increases with temperature at Mt. Kilimanjaro. *Ecology and Evolution*, *10*(4), 2182-2195. https://doi.org/10.1002/ece3.6056

Clavel, J., Julliard, R., & Devictor, V. (2011). Worldwide decline of specialist species: Toward a global functional homogenization? *Frontiers in Ecology and the Environment, 9*, 222-228.

Colwell, R. K., Brehm, G., Cardelús, C. L., Gilman, A. C., & Longino, J. T. (2008). Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science, 322(5899), 258-261.

Cook, C. N., & Breed, M. D. (2013). Social context influences the initiation and threshold of thermoregulatory behaviour in honeybees. *Animal Behaviour*, *86*(2), 323-329. https://doi.org/10.1016/j.anbehav.2013.05.021

Cook, C. N., Kaspar, R. E., Flaxman, S. M., & Breed, M. D. (2016). Rapidly changing environment modulates the thermoregulatory fanning response in honeybee groups. *Animal Behaviour*, *115*, 237-243. https://doi.org/10.1016/j.anbehav.2016.03.014

Corbet, S. A., Fussell, M., Ake, R., Fraser, A., Gunson, C., Savage, A., & Smith, K. (1993). Temperature and the pollinating activity of social bees. *Ecological Entomology, 18*(1), 17-30. https://doi.org/10.1111/j.1365-2311.1993.tb01075.x

Dai, A. (2011). Drought under global warming: a review. *Wiley Interdisciplinary Reviews: Climate Change*, *2*(1), 45-65.

da Silva, M. A., Ferreira, N. da S., Teixeira-Souza, V. H. da S., Maia-Silva, C., de Oliveira, F. de A., & Hrncir, M. (2017). On the thermal limits for the use of stingless bees as pollinators in commercial greenhouses. *Journal of Apicultural Research*, *56*(1), 81-90. https://doi.org/10.1080/00218839.2016.1260380

Dalsgaard, B. (2020). Land-Use and Climate Impacts on Plant-Pollinator Interactions and Pollination Services. *Diversity*, *12*(5), 168. https://doi.org/10.3390/d12050168

Deere, J. A., & Chown, S. L. (2006). Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. *The American Naturalist, 168*(5), 630-644.

De Marco, P., & Coelho, F. M. (2004). Services performed by the ecosystem: forest remnants influence agricultural cultures' pollination and production. *Biodiversity & Conservation*, *13*(7), 1245-1255.

Descamps, C., Jambrek, A., Quinet, M., & Jacquemart, A. L. (2021). Warm Temperatures Reduce Flower Attractiveness and Bumblebee Foraging. *Insects*, *12*(6), 493. https://doi.org/10.3390/insects12060493

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences, 105*(18), 6668-6672.

Devi, S., & Mishra, R. P. (2020). A mathematical model to see the effects of increasing environmental temperature on plant–pollinator interactions. *Modelling Earth Systems and Environment*, *6*(3), 1315-1329. https://doi.org/10.1007/s40808-020-00763-4

Devoto, M., Medan, D., & Montaldo, N. H. (2005). Patterns of interaction between plants and pollinators along an environmental gradient. *Oikos*, *109*(3), 461-472. https://doi.org/10.1111/j.0030-1299.2005.13712.x

Devoto, M., Medan, D., Roig-Alsina, A., & Montaldo, N. H. (2009). Patterns of species turnover in plant-pollinator communities along a precipitation gradient in Patagonia (Argentina). *Austral Ecology, 34*(8), 848-857. https://doi.org/10.1111/j.1442-9993.2009.01987.x

Devoto, M., Zimmermann, M., & Medan, D. (2007). Robustness of plant-flower visitor webs to simulated climate change. *Ecología Austral*, *17*(1), 37-50.

Dillon, M. E., Wang, G., & Huey, R. B. (2010). Global metabolic impacts of recent climate warming. *Nature*, *467*(7316), 704-706.

Dodds, P. S., Rothman, D. H., & Weitz, J. S. (2001). Re-examination of the “3/4-law” of Metabolism. *Journal of Theoretical Biology*, *209*(1), 9-27. https://doi.org/10.1006/jtbi.2000.2238

Dukas, R., & Real, L. A. (1991). Learning foraging tasks by bees: A comparison between social and solitary species. *Animal Behaviour, 42*(2), 269-276. https://doi.org/10.1016/S0003-3472(05)80558-5

Durant, J. M., Hjermann, D. Ø., Ottersen, G., & Stenseth, N. C. (2007). Climate and the match or mismatch between predator requirements and resource availability. *Climate Research, 33*(3), 271-283. https://doi.org/10.3354/cr033271

Elias, M. A. S., Borges, F. J. A., Bergamini, L. L., Franceschinelli, E. V., & Sujii, E. R. (2017). Climate change threatens pollination services in tomato crops in Brazil. *Agriculture, Ecosystems & Environment*, *239*, 257-264. https://doi.org/10.1016/j.agee.2017.01.026

Everaars, J., Settele, J., & Dormann, C. F. (2018). Fragmentation of nest and foraging habitat affects time budgets of solitary bees, their fitness and pollination services, depending on traits: Results from an individual-based model. *PloS one, 13*(2), e0188269. https://doi.org/10.1371/journal.pone.0188269

Farrell-Gray, C. C., & Gotelli, N. J. (2005). Allometric Exponents Support a 3/4-Power Scaling Law. *Ecology, 86*(8), 2083-2087. https://doi.org/10.1890/04-1618

Frazier, M. R., Huey, R. B., & Berrigan, D. (2006). Thermodynamics Constrains the Evolution of Insect Population Growth Rates: “Warmer Is Better.” *The American Naturalist, 168*(4), 512-520. https://doi.org/10.1086/506977

Fucini, S., Uboni, A., & Lorenzi, M. C. (2014). Geographic Variation in Air Temperature Leads to Intraspecific Variability in the Behavior and Productivity of a Eusocial Insect. *Journal of Insect Behavior*, *27*(3), 403-410. https://doi.org/10.1007/s10905-013-9436-y

Friard, O., & Gamba, M. (2016). BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325-1330. https://doi.org/10.1111/2041-210X.12584

Fussmann, K. E., Schwarzmüller, F., Brose, U., Jousset, A., & Rall, B. C. (2014). Ecological stability in response to warming. *Nature Climate Change, 4*(3), 206-210. https://doi.org/10.1038/nclimate2134

Gallai, N., Salles, J. M., Settele, J., & Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological economics*, *68*(3), 810-821.

Gavini, S. S., Quintero, C., & Tadey, M. (2020). Intraspecific variation in body size of bumblebee workers influences anti-predator behaviour. *Journal of Animal Ecology*, *89*(2), 658–669. https://doi.org/10.1111/1365-2656.13135

Gérard, M., Vanderplanck, M., Wood, T., & Michez, D. (2020). Global warming and plant–pollinator mismatches. *Emerging Topics in Life Sciences*, *4*(1), 77–86. https://doi.org/10.1042/ETLS20190139

Giannini, T. C., Acosta, A. L., Garófalo, C. A., Saraiva, A. M., Alves-dos-Santos, I., & Imperatriz-Fonseca, V. L. (2012). Pollination services at risk: Bee habitats will decrease owing to climate change in Brazil. *Ecological Modelling*, *244*, 127–131. https://doi.org/10.1016/j.ecolmodel.2012.06.035

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, *293*(5538), 2248-2251.

Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for community interactions under climate change. *Trends in Ecology & Evolution, 25*(6), 325-331. https://doi.org/10.1016/j.tree.2010.03.002

Glazier, D. S. (2015). Is metabolic rate a universal ‘pacemaker’ for biological processes? *Biological Reviews, 90*(2), 377-407. https://doi.org/10.1111/brv.12115

Glazier, D. S. (2020). Activity alters how temperature influences intraspecific metabolic scaling: Testing the metabolic-level boundaries hypothesis. *Journal of Comparative Physiology B, 190*(4), 445-454. https://doi.org/10.1007/s00360-020-01279-0

Glazier, D. S., Clusella-Trullas, S., & Terblanche, J. S. (2016). Sexual dimorphism and physiological correlates of horn length in a South African isopod crustacean. *Journal of Zoology*, *300*(2), 99-110. https://doi.org/10.1111/jzo.12338

Gonçalves-Souza, T., Omena, P. M., Souza, J. C., & Romero, G. Q. (2008). Trait-Mediated Effects on Flowers: Artificial Spiders Deceive Pollinators and Decrease Plant Fitness. *Ecology*, *89*(9), 2407-2413. https://doi.org/10.1890/07-1881.1

González, A. M. M., Dalsgaard, B., Ollerton, J., Timmermann, A., Olesen, J. M., Andersen, L., & Tossas, A. G. (2009). Effects of Climate on Pollination Networks in the West Indies. *Journal of Tropical Ecology*, *25*(5), 493-506.

González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R. A., & Villalobos, F. (2020). Insect responses to heat: Physiological mechanisms, evolution and ecological implications in a warming world. *Biological Reviews, 95*(3), 802-821. https://doi.org/10.1111/brv.12588

Gooley, Z. C., & Gooley, A. C. (2020). Exposure to field realistic concentrations of imidacloprid at different ambient temperatures disrupts non-flight metabolic rate in honey bee (*Apis mellifera*) foragers. *Bulletin of Insectology, 73*(2), 161-170.

Goulson, D. (2009). *Bumblebees: Behaviour, Ecology, and Conservation*. Oxford University Press. http://ebookcentral.proquest.com/lib/waikato/detail.action?docID=472276

Goulson, D., Stout, J. C., & Kells, A. R. (2002). Do exotic bumblebees and honeybees compete with native flower-visiting insects in Tasmania? *Journal of Insect Conservation, 6*(3), 179-189.

Greenleaf, S. S., Williams, N. M., Winfree, R., & Kremen, C. (2007). Bee foraging ranges and their relationship to body size. *Oecologia*, *153*(3), 589–596. https://doi.org/10.1007/s00442-007-0752-9

Groh, C., Tautz, J., & Rössler, W. (2004). Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. *Proceedings of the National Academy of Sciences, 101*(12), 4268-4273.

Gudowska, A., Schramm, B. W., Czarnoleski, M., Kozłowski, J., & Bauchinger, U. (2017). Physical mechanism or evolutionary trade-off? Factors dictating the relationship between metabolic rate and ambient temperature in carabid beetles. *Journal of Thermal Biology*, *68*, 89-5. https://doi.org/10.1016/j.jtherbio.2016.11.009

Guiraud, M., Cariou, B., Henrion, M., Baird, E., & Gérard, M. (2021). Higher developmental temperature increases queen production and decreases worker body size in the bumblebee *Bombus terrestris*. *Journal of Hymenoptera Research*, *88*, 39-49. https://doi.org/10.3897/jhr.88.73532

Halsey, L. G., Matthews, P. G. D., Rezende, E. L., Chauvaud, L., & Robson, A. A. (2015). The interactions between temperature and activity levels in driving metabolic rate: Theory, with empirical validation from contrasting ectotherms. *Oecologia, 177*(4), 1117-1129. https://doi.org/10.1007/s00442-014-3190-5

Harder, L. D. (1983). Flower handling efficiency of bumble bees: morphological aspects of probing time. *Oecologia, 57*(1), 274-280.

Harrison, J. F. (2018). Approaches for testing hypotheses for the hypometric scaling of aerobic metabolic rate in animals. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *315*(5), R879-R894. https://doi.org/10.1152/ajpregu.00165.2018

Harrison, J. F., & Fewell, J. H. (2002). Environmental and genetic influences on flight metabolic rate in the honey bee, *Apis mellifera*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 133(*2), 323-333. https://doi.org/10.1016/S1095-6433(02)00163-0

Harrison, J. F., Klok, C., & Waters, J. S. (2014). Critical PO2 is size-independent in insects: Implications for the metabolic theory of ecology. *Current Opinion in Insect Science*, *4*, 54-59. https://doi.org/10.1016/j.cois.2014.08.012

Heath, A. (2021). Climate change and its potential for altering the phenology and ecology of some common and widespread arthropod parasites in New Zealand. *New Zealand Veterinary Journal*, *69*(1), 5-19. https://doi.org/10.1080/00480169.2020.1787276

Hegland, S. J., Nielsen, A., Lázaro, A., Bjerknes, A.-L., & Totland, Ø. (2009). How does climate warming affect plant-pollinator interactions? *Ecology Letters*, *12*(2), 184-195. https://doi.org/10.1111/j.1461-0248.2008.01269.x

Herbertsson, L., Khalaf, R., Johnson, K., Bygebjerg, R., Blomqvist, S., & Persson, A. S. (2021). Long-term data shows increasing dominance of Bombus terrestris with climate warming. *Basic and Applied Ecology, 53,* 116-123. https://doi.org/10.1016/j.baae.2021.03.008

Herrmann, J. D., Beye, H., de la Broise, C., Hartlep, H., & Diekötter, T. (2019). Positive effects of the pollinators *Osmia cornuta* (Megachilidae) and *Lucilia sericata* (Calliphoridae) on strawberry quality. *Arthropod-Plant Interactions*, *13*(1), 71-77. https://doi.org/10.1007/s11829-018-9636-7

Higginson, A. D., & Houston, A. I. (2015). The influence of the food-predation trade-off on the foraging behaviour of central-place foragers. *Behavioral Ecology and Sociobiology*, *69*(4), 551-561. https://doi.org/10.1007/s00265-014-1866-9

Hoover, S. E. R., Ladley, J. J., Shchepetkina, A. A., Tisch, M., Gieseg, S. P., & Tylianakis, J. M. (2012). Warming, CO2, and nitrogen deposition interactively affect a plant-pollinator mutualism. *Ecology Letters*, *15*(3), 227-234. https://doi.org/10.1111/j.1461-0248.2011.01729.x

Howlett, B. G., Butler, R. C., Nelson, W., & Donovan, B. J. (2013). *Impact of climate change on crop pollinator in New Zealand*. Wellington: Ministry for Primary Industries.

Howlett, B. G., & Gee, M. (2019). The potential management of the drone fly (*Eristalis tenax*) as a crop pollinator in New Zealand. *New Zealand Plant Protection*, *72*, 221-230. https://doi.org/10.30843/nzpp.2019.72.304

Huey, R. B., & Kingsolver, J. G. (2019). Climate Warming, Resource Availability, and the Metabolic Meltdown of Ectotherms. *The American Naturalist*, *194*(6), E140-E150. https://doi.org/10.1086/705679

Hui, D., & Jackson, R. B. (2007). Uncertainty in allometric exponent estimation: A case study in scaling metabolic rate with body mass. *Journal of Theoretical Biology*, *249*(1), 168-177. https://doi.org/10.1016/j.jtbi.2007.07.003

Hutchings, M. J., Robbirt, K. M., Roberts, D. L., & Davy, A. J. (2018). Vulnerability of a specialized pollination mechanism to climate change revealed by a 356-year analysis. *Botanical Journal of the Linnean Society*, *186*(4), 498-509. https://doi.org/10.1093/botlinnean/box086

Ilse, D. (1949). Colour discrimination in the dronefly, *Eristalis tenax*. *Nature, 163*(4137), 255-256.

Ings, T. C., & Chittka, L. (2009). Predator crypsis enhances behaviourally mediated indirect effects on plants by altering bumblebee foraging preferences. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1664), 2031-2036. https://doi.org/10.1098/rspb.2008.1748

IPCC (2022). Climate Change 2022: Impacts, Adaptation, and Vulnerability. In H. O. Pörtner, D. C. Roberts, M. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (Eds.), *IPCC Sixth Assessment Report*. Cambridge University Press. In Press.

Irlich, U. M., Terblanche, J. S., Blackburn, T. M., & Chown, S. L. (2009). Insect Rate-Temperature Relationships: Environmental Variation and the Metabolic Theory of Ecology. *The American Naturalist, 174*(6), 819-835. https://doi.org/10.1086/647904

Jerbi-Elayed, M., Lebdi-Grissa, K., Le Goff, G., & Hance, T. (2015). Influence of Temperature on Flight, Walking and Oviposition Capacities of two Aphid Parasitoid Species (Hymenoptera: Aphidiinae). *Journal of Insect Behavior*, *28*(2), 157-166. https://doi.org/10.1007/s10905-015-9490-8

Jerbi-Elayed, M., Tougeron, K., Grissa-Lebdi, K., & Hance, T. (2022). Effect of developmental temperatures on *Aphidius colemani* host-foraging behavior at high temperature. *Journal of Thermal Biology*, *103*, 103140. https://doi.org/10.1016/j.jtherbio.2021.103140

Jerde, C. L., Kraskura, K., Eliason, E. J., Csik, S. R., Stier, A. C., & Taper, M. L. (2019). Strong Evidence for an Intraspecific Metabolic Scaling Coefficient Near 0.89 in Fish. *Frontiers in Physiology*, *10*, 1166. https://doi.org/10.3389/fphys.2019.01166

Jones, B. R., & Bulbert, M. W. (2020). Directed chemical spray of the peppermint stick insect (*Megacrania batesii*) is induced when predation risk is at its highest. *Journal of Ethology, 38*(1), 51-59. https://doi.org/10.1007/s10164-019-00619-0

Jones, E. I., & Dornhaus, A. (2011). Predation risk makes bees reject rewarding flowers and reduce foraging activity. *Behavioral Ecology and Sociobiology*, *65*(8), 1505-1511. https://doi.org/10.1007/s00265-011-1160-z

Kaefer, H., Kovac, H., & Stabentheiner, A. (2012). Resting metabolism and critical thermal maxima of vespine wasps (*Vespula* sp.). *Journal of Insect Physiology*, *58*(5), 679-689. https://doi.org/10.1016/j.jinsphys.2012.01.015

Kawecki, T. J. (2009). Evolutionary ecology of learning: Insights from fruit flies. *Population Ecology, 52*(1), 15. https://doi.org/10.1007/s10144-009-0174-0

Keil, P., Simova, I., & Hawkins, B. A. (2008). Water-energy and the geographical species richness pattern of European and North African dragonflies (Odonata). *Insect Conservation and Diversity*, *1*(3), 142-150. https://doi.org/10.1111/j.1752-4598.2008.00019.x

Kenna, D., Pawar, S., & Gill, R. J. (2021). Thermal flight performance reveals impact of warming on bumblebee foraging potential. *Functional Ecology*, *35*(11), 2508–2522. https://doi.org/10.1111/1365-2435.13887

Kenny, G. (2011). Adaptation in Agriculture: Lessons for Resilience From Eastern Regions of New Zealand. *Climatic Change, 106*, 441-462. https://doi.org/10.1007/s10584-010-9948-9

Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., Rasmont, P., Schweiger, O., Colla, S. R., Richardson, L. L., Wagner, D. L., Gall, L. F., Sikes, D. S., Pantonja, A. (2015). Climate change impacts on bumblebees converge across continents. *Science 349,* 177-180.

Kingsolver, J. G., & Huey, R. B. (2008). Size, temperature, and fitness: three rules. *Evolutionary Ecology Research*, *10*(2), 251-268.

Kleiber, M. (1932). Body size and metabolism. *Hilgardia*, *6*(11), 315-353.

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the royal society B: biological sciences, 274*(1608), 303-313.

Kozłowski, J., & Konarzewski, M. (2004). Is West, Brown and Enquist's model of allometric scaling mathematically correct and biologically relevant? *Functional Ecology*, *18*(2), 283-289.

Kozłowski, J., & Konarzewski, M. (2005). West, Brown and Enquist's Model of Allometric Scaling Again: The Same Questions Remain. *Functional Ecology*, *19*(4), 739-743.

Kozłowski, J., Konarzewski, M., & Gawelczyk, A. T. (2003). Cell size as a link between noncoding DNA and metabolic rate scaling. *Proceedings of the National Academy of Sciences, 100*(24), 14080-14085.

Krčmar, S., Kučinić, M., Durbešić, P., & Benović, A. (2010). Insects from the middle of the Adriatic Sea. *Entomologia Croatica, 14*(3-4), 75-84.

Kremen C., Williams N. M., Aizen M. A., Gemmill-Herren B., LeBuhn G., Minckley R., Packer L., Potts S. G., Roulston T., Steffan-Dewenter I., Vázquez D. P., Winfree R., Adams L., Crone E. E., Greenleaf S. S., Keitt T. H., Klein A. M., Regetz J., & Ricketts T. H. (2007). Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecology Letters 10,* 299–314.

Kuhlmann, M., Guo, D., Veldtman, R., & Donaldson, J. (2012). Consequences of warming up a hotspot: species range shifts within a centre of bee diversity. *Diversity and Distributions*, *18*(9), 885-897.

Kühsel, S., & Blüthgen, N. (2015). High diversity stabilizes the thermal resilience of pollinator communities in intensively managed grasslands. *Nature communications, 6*(1), 1-10.

Kumar, P. (2012). *The economics of ecosystems and biodiversity: ecological and economic foundations*. Routledge.

Labocha, M. K., Sadowska, E. T., Baliga, K., Semer, A. K., & Koteja, P. (2004). Individual variation and repeatability of basal metabolism in the bank vole, *Clethrionomys glareolus*. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1537), 367-372.

Lalouette, L., Williams, C. M., Hervant, F., Sinclair, B. J., & Renault, D. (2011). Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *158*(2), 229-234. https://doi.org/10.1016/j.cbpa.2010.11.007

Larsen, T. H., Williams, N. M., & Kremen, C. (2005). Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecology Letters, 8*(5), 538-547. https://doi.org/10.1111/j.1461-0248.2005.00749.x

Le Conte, Y., & Navajas, M. (2008). Climate change: impact on honey bee populations and diseases. *Revue Scientifique et Technique-Office International des Epizooties, 27*(2), 499-510.

Lemaître, J. F., Müller, D. W. H., & Clauss, M. (2014). A test of the metabolic theory of ecology with two longevity data sets reveals no common cause of scaling in biological times. *Mammal Review, 44*(3-4), 204-214. https://doi.org/10.1111/mam.12023

Lighton, J. R. B. (2017). Limitations and requirements for measuring metabolic rates: A mini review. *European Journal of Clinical Nutrition*, *71*(3), 301-305. https://doi.org/10.1038/ejcn.2016.265

Lighton, J., Brownell, P., Joos, B., & Turner, R. (2001). Low metabolic rate in scorpions: Implications for population biomass and cannibalism. *The Journal of Experimental Biology*, *204*, 607-613. https://doi.org/10.1242/jeb.204.3.607

Lighton, J. R. B., & Halsey, L. G. (2011). Flow-through respirometry applied to chamber systems: Pros and cons, hints and tips. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *158*(3), 265-275. https://doi.org/10.1016/j.cbpa.2010.11.026

Llandres, A. L., De Mas, E., & Rodríguez-Gironés, M. A. (2012). Response of pollinators to the tradeoff between resource acquisition and predator avoidance. *Oikos*, *121*(5), 687-696. https://doi.org/10.1111/j.1600-0706.2011.19910.x

Löffler, F., Poniatowski, D. & Fartmann, T. (2019). Orthoptera community shifts in response to land-use and climate change - Lessons from a long-term study across different grassland habitats. *Biological Conservation, 236*, 315-323.

López-Urrutia, Á., San Martin, E., Harris, R. P., & Irigoien, X. (2006). Scaling the metabolic balance of the oceans. *Proceedings of the National Academy of Sciences, 103*(23), 8739-8744. https://doi.org/10.1073/pnas.0601137103

Loy, X., & Brosi, B. J. (2022). The effects of pollinator diversity on pollination function. *Ecology, 103*(4), e3631. https://doi.org/10.1002/ecy.3631

MacLean, H. J., Kristensen, T. N., Overgaard, J., Sørensen, J. G., & Bahrndorff, S. (2017). Acclimation responses to short-term temperature treatments during early life stages causes long lasting changes in spontaneous activity of adult *Drosophila melanogaster*. *Physiological Entomology*, *42*(4), 404-411. https://doi.org/10.1111/phen.12212

Maksym, M., Pakhomov, O., & Brygadyrenko, V. (2021). Effect of increased ambient temperature on seasonal generation number in *Lucilia sericata* (Diptera, Calliphoridae). *Folia Oecologica*, *48*, 191-198. https://doi.org/10.2478/foecol-2021-0019

Ma, G., & Ma, C. S. (2012). Climate warming may increase aphids' dropping probabilities in response to high temperatures. *Journal of Insect Physiology, 58*(11), 1456-1462. https://doi.org/10.1016/j.jinsphys.2012.08.012

Mänd, M., & Karise, R. (2015). Recent insights into sublethal effects of pesticides on insect respiratory physiology. *Open Access Insect Physiology*, 31. https://doi.org/10.2147/OAIP.S68870

Maryanski, M., Kramarz, P., Laskowski, R., & Niklinska, M. (2002). Decreased energetic reserves, morphological changes and accumulation of metals in carabid beetles (*Poecilus cupreus L.*) exposed to zinc-or cadmium-contaminated food. *Ecotoxicology*, *11*(2), 127-139.

Massel, K., Lam, Y., Wong, A. C. S., Hickey, L. T., Borrell, A. K., & Godwin, I. D. (2021). Hotter, drier, CRISPR: The latest edit on climate change. *Theoretical and Applied Genetics*, *134*(6), 1691-1709. https://doi.org/10.1007/s00122-020-03764-0

McCallum, K. P., McDougall, F. O., & Seymour, R. S. (2013). A review of the energetics of pollination biology. *Journal of Comparative Physiology B, 183*(7), 867-876. https://doi.org/10.1007/s00360-013-0760-5

Meehl, G. A., & Tebaldi, C. (2004). More Intense, More Frequent, and Longer Lasting Heat Waves in the 21st Century. *Science*, *305*(5686), 994-997. https://doi.org/10.1126/science.1098704

Memmott, J., Carvell, C., Pywell, R. F., & Craze, P. G. (2010). The potential impact of global warming on the efficacy of field margins sown for the conservation of bumble-bees.

Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1549), 2071-2079.

Memmott, J., Craze, P. G., Waser, N. M., & Price, M. V. (2007). Global warming and the disruption of plant–pollinator interactions. *Ecology Letters*, *10*(8), 710-717. https://doi.org/10.1111/j.1461-0248.2007.01061.x

Metcalf C. L. (1913). The Syrphidae of Ohio. *Ohio Biological Survey* (10-99). Ohio State University.

Miller, L. P., Matassa, C. M., & Trussell, G. C. (2014). Climate change enhances the negative effects of predation risk on an intermediate consumer. *Global Change Biology, 20*(12), 3834-3844. https://doi.org/10.1111/gcb.12639

Miller-Struttmann, N. E., Geib, J. C., Franklin, J. D., Kevan, P. G., Holdo, R. M., Ebert-May, D., Lynn, A. M., Kettenbach, J. A., Hedrick, E., & Galen, C. (2015). Functional mismatch in a bumble bee pollination mutualism under climate change. *Science*, *349*(6255), 1541-1544. https://doi.org/10.1126/science.aab0868

Ministry for the Environment, (2016). *Climate Change Projections for New Zealand: Atmospheric Projections based on Simulations undertaken for the IPCC Fifth Assessment*. Prepared by A.B. Mullan, A. Sood, and S. Stuart. NIWA Client Report WLG2015-31.

Moiroux, J., Boivin, G., & Brodeur, J. (2015). Temperature influences host instar selection in an aphid parasitoid: Support for the relative fitness rule. *Biological Journal of the Linnean Society*, *115*(4), 792-801. https://doi.org/10.1111/bij.12545

Molnár, P. K., Sckrabulis, J. P., Altman, K. A., & Raffel, T. R. (2017). Thermal performance curves and the metabolic theory of ecology—a practical guide to models and experiments for parasitologists. *Journal of Parasitology, 103*(5), 423-439.

Moran, E. V., Hartig, F., & Bell, D. M. (2016). Intraspecific trait variation across scales: Implications for understanding global change responses. *Global Change Biology, 22*(1), 137-150. https://doi.org/10.1111/gcb.13000

Muljar, R., Karise, R., Viik, E., Kuusik, A., Williams, I., Metspalu, L., Hiiesaar, K., Must, A., Luik, A., & Mänd, M. (2012). Effects of Fastac 50 EC on bumble bee *Bombus terrestris* L. respiration: DGE disappearance does not lead to increasing water loss. *Journal of Insect Physiology*, *58*(11), 1469-1476. https://doi.org/10.1016/j.jinsphys.2012.08.014

Muntzer, A., Montagne, C., Ellse, L., & Wall, R. (2015). Temperature-dependent lipid metabolism in the blow fly *Lucilia sericata*. *Medical and Veterinary Entomology*, *29*(3), 305–313. https://doi.org/10.1111/mve.12111

Murren, C. J., Maclean, H. J., Diamond, S. E., Steiner, U. K., Heskel, M. A., Handelsman, C. A., Ghalambor, C. K., Auld, J. R., Callahan, H. S., Pfennig, D. W., Relyea, R. A., Schlichting, C. D. & Kingsolver, J. (2014). Evolutionary change in continuous reaction norms. *The American Naturalist*, *183*(4), 453-467.

Musolin, D. L., & Saulich, A. Kh. (2012). Responses of insects to the current climate changes: From physiology and behavior to range shifts. *Entomological Review*, *92*(7), 715-740. https://doi.org/10.1134/S0013873812070019

Nagy, K.A. (1987). Field metabolic rate and food requirement scaling in mammals and birds. *Ecological monographs, 57*(2), 111-128.

Niven, J. E., & Scharlemann, J. P. W. (2005). Do insect metabolic rates at rest and during flight scale with body mass? *Biology Letters*, *1*(3), 346-349. https://doi.org/10.1098/rsbl.2005.0311

Nock, C. A., Vogt, R. J., & Beisner, B. E. (2016). Functional Traits. In ELS (1–8). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470015902.a0026282

Nyamukondiwa, C., & Terblanche, J. S. (2010). Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies *Ceratitis capitata* and *Ceratitis rosa*: Thermal history affects short-term responses to temperature. *Physiological Entomology, 35*(3), 255-264. https://doi.org/10.1111/j.1365-3032.2010.00736.x

O'Connor, M. I., Gilbert, B., & Brown, C. J. (2011). Theoretical Predictions for How Temperature Affects the Dynamics of Interacting Herbivores and Plants. *The American Naturalist, 178*(5), 626-638. https://doi.org/10.1086/662171

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, *120*(3), 321-326.

Osman, M. A. M., & Shebl, M. A. (2020). Vulnerability of Crop Pollination Ecosystem Services to Climate Change. In E.-S. Ewis Omran & A. M. Negm (Eds.), *Climate Change Impacts on Agriculture and Food Security in Egypt: Land and Water Resources—Smart Farming—Livestock, Fishery, and Aquaculture* (223-247). Springer International Publishing. https://doi.org/10.1007/978-3-030-41629-4_11

Oyen, K. J., Giri, S., & Dillon, M. E. (2016). Altitudinal variation in bumble bee (*Bombus*) critical thermal limits. *Journal of Thermal Biology*, *59*, 52-57. https://doi.org/10.1016/j.jtherbio.2016.04.015

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual review of ecology, evolution, and systematics, 37*, 637-669.

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*(6918), 37-42.

Pettersen, A. K., White, C. R., & Marshall, D. J. (2016). Metabolic rate covaries with fitness and the pace of the life history in the field. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1831), 20160323.

Perry, C. J., & Barron, A. B. (2013). Neural mechanisms of reward in insects. *Annual Review of Entomology, 58(*543), 62.

Phillips, R. D., Hopper, S. D., & Dixon, K. W. (2010). Pollination ecology and the possible impacts of environmental change in the Southwest Australian Biodiversity Hotspot. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1539), 517–528. https://doi.org/10.1098/rstb.2009.0238

Piccolomini, A. M., Whiten, S. R., Flenniken, M. L., O'Neill, K. M., & Peterson, R. K. D. (2018). Acute Toxicity of Permethrin, Deltamethrin, and Etofenprox to the Alfalfa Leafcutting Bee. *Journal of Economic Entomology*, *111*(3), 1001-1005. https://doi.org/10.1093/jee/toy014

Pierce, C. L. (1988). Predator avoidance, microhabitat shift, and risk-sensitive foraging in larval dragonflies. *Oecologia, 77*(1), 81-90.

Prather, R. M., Roeder, K. A., Sanders, N. J., & Kaspari, M. (2018). Using metabolic and thermal ecology to predict temperature dependent ecosystem activity: A test with prairie ants. *Ecology*, *99*(9), 2113-2121. https://doi.org/10.1002/ecy.2445

Price, C. A., Weitz, J. S., Savage, V. M., Stegen, J., Clarke, A., Coomes, D. A., Dodds, P. S., Etienne, R. S., Kerkhoff, A. J., McCulloh, K., Niklas, K. J., Olff, H., & Swenson, N. G. (2012). Testing the metabolic theory of ecology. *Ecology Letters*, *15*(12), 1465-1474. https://doi.org/10.1111/j.1461-0248.2012.01860.x

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in ecology & evolution*, *25*(6), 345-353.

Rader, R., Cunningham, S. A., Howlett, B. G., & Inouye, D. W. (2020). Non-Bee Insects as Visitors and Pollinators of Crops: Biology, Ecology, and Management. *Annual Review of Entomology*, *65*(1), 391-407. https://doi.org/10.1146/annurev-ento-011019-025055

Rafferty, N. E., & Ives, A. R. (2011). Effects of experimental shifts in flowering phenology on plant–pollinator interactions. *Ecology Letters*, *14*(1), 69-74. https://doi.org/10.1111/j.1461-0248.2010.01557.x

Rall, B. C., Brose, U., Hartvig, M., Kalinkat, G., Schwarzmüller, F., Vucic-Pestic, O., & Petchey, O. L. (2012). Universal temperature and body-mass scaling of feeding rates. *Philosophical Transactions of the Royal Society B: Biological Sciences, 367*(1605), 2923-2934.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reddy, P. V. R., Verghese, A., & Rajan, V. V. (2012). Potential impact of climate change on honeybees (*Apis* spp.) and their pollination services. *Pest Management in Horticultural Ecosystems 18*(2), 121-127.

Regaudie-de-Gioux, A., & Duarte, C. M. (2012). Temperature dependence of planktonic metabolism in the ocean. *Global Biogeochemical Cycles*, *26*(1). https://doi.org/10.1029/2010GB003907

Reid, W. V., Mooney, H. A., Cropper, A., Capistrano, D., Carpenter, S. R., Chopra, K., Dasgupta, P., Dietz, T., Duraiappah, A. K., Hassan, R., Kasperson, R., Leemans, R., May, R. M., McMichael, A. J., Pingali, P., Samper, C., Scholes, R., Watson, R. T., Zakri, A. H., Shidong, Z., Ash, N. J., Bennett, E., Kumar, P., Lee, M. J., Raudsepp-Hearne, C., Simons, H., Thonell, J., Zurek, M. B. (2005). *Ecosystems and human well-being-Synthesis: A report of the Millennium Ecosystem Assessment*. Island Press.

Réveillon, T., Rota, T., Chauvet, É., Lecerf, A., & Sentis, A. (2019). Repeatable inter-individual variation in the thermal sensitivity of metabolic rate. *Oikos*, *128*(11), 1633-1640. https://doi.org/10.1111/oik.06392

Riveron, J., Boto, T., & Alcorta, E. (2009). The effect of environmental temperature on olfactory perception in Drosophila melanogaster. *Journal of Insect Physiology, 55*(10), 943-951. https://doi.org/10.1016/j.jinsphys.2009.06.009

Roberts, S. P., Harrison, J. F., & Dudley, R. (2004). Allometry of kinematics and energetics in carpenter bees (*Xylocopa varipuncta)* hovering in variable-density gases. *Journal of Experimental Biology*, *207*(6), 993-1004. https://doi.org/10.1242/jeb.00850

Romero, G. Q., Antiqueira, P. A. P., & Koricheva, J. (2011). A Meta-Analysis of Predation Risk Effects on Pollinator Behaviour. *PLoS One*, *6*(6), e20689. https://doi.org/10.1371/journal.pone.0020689

Sahli, H. F., & Conner, J. K. (2007). Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany*, *94*(2), 203-209. https://doi.org/10.3732/ajb.94.2.203

Salvucci, M. E., & Crafts-Brandner, S. J. (2000). Effects of temperature and dietary sucrose concentration on respiration in the silverleaf whitefly, *Bemisia argentifolii*. *Journal of Insect Physiology*, *46*(11), 1461-1467. https://doi.org/10.1016/S0022-1910(00)00070-6

Savage, V. M., Allen, A. P., Brown, J. H., Gillooly, J. F., Herman, A. B., Woodruff, W. H., & West, G. B. (2007). Scaling of number, size, and metabolic rate of cells with body size in mammals. *Proceedings of the National Academy of Sciences, 104*(11), 4718-4723. https://doi.org/10.1073/pnas.0611235104

Savage, V. M., Gillooly, J. F., Woodruff, W. H., West, G. B., Allen, A. P., Enquist, B. J., & Brown, J. H. (2004). The predominance of quarter-power scaling in biology. *Functional Ecology, 18*(2), 257-282. https://doi.org/10.1111/j.0269-8463.2004.00856.x

Schaffer, M. J. (1997). *Spatial aspects of bumble bee (Bombus spp. Apidae) foraging in farm landscapes*. Doctoral dissertation, Lincoln University.

Sentis, A., Binzer, A., & Boukal, D. S. (2017). Temperature-size responses alter food chain persistence across environmental gradients. *Ecology Letters, 20*(7), 852–862. https://doi.org/10.1111/ele.12779

Scaven, V. L., & Rafferty, N. E. (2013). Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. *Current Zoology*, *59*(3), 418-426.

Scholes, R. J. (2016). Climate change and ecosystem services. *WIREs Climate Change*, *7*(4), 537-550. https://doi.org/10.1002/wcc.404

Schweiger, O., Settele, J., Kudrna, O., Klotz, S., & Kühn, I., (2008). Climate change can cause spatial mismatch of trophically interacting species. *Ecology 89*, 3472-3479.

Sgolastra, F., Kemp, W. P., Buckner, J. S., Pitts-Singer, T. L., Maini, S., & Bosch, J. (2011). The long summer: Pre-wintering temperatures affect metabolic expenditure and winter survival in a solitary bee. *Journal of Insect Physiology*, *57*(12), 1651-1659. https://doi.org/10.1016/j.jinsphys.2011.08.017

Shah, A. A., Woods, H. A., Havird, J. C., Encalada, A. C., Flecker, A. S., Funk, W. C., Guayasamin, J. M., Kondratieff, B. C., Poff, N. L., Thomas, S. A., Zamudio K. R., & Ghalambor, C. K. (2020). Temperature dependence of metabolic rate in tropical and temperate aquatic insects: support for the climate variability hypothesis in mayflies but not stoneflies. *Global Change Biology, 27*(2), 297-311.

Sharma, T., & Singla, A. (2022). Potential Impacts of Climate Change on Insect Behavior. *Journal of Life Sciences, 11*(3), 29-35.

Shimomae, K., Sato, T., YuichiYoshida, Shane Naing, S., & Miyatake, T. (2022). Longevity of *Lucilia sericata* (Meigen, 1826) used as pollinator. *Journal of Asia-Pacific Entomology*, *25*(4), 101999. https://doi.org/10.1016/j.aspen.2022.101999

Silvola, J. (1984). Respiration and Energetics of the Bumblebee *Bombus terrestris* Queen. *Holarctic Ecology*, *7*(2), 177-181.

Slessor, K. N., Winston, M. L., & Le Conte, Y. (2005). Pheromone Communication in the Honeybee (*Apis mellifera L.*). *Journal of Chemical Ecology*, *31*(11), 2731-2745. https://doi.org/10.1007/s10886-005-7623-9

Somjee, U., Powell, E. C., Hickey, A. J., Harrison, J. F., & Painting, C. J. (2021). Exaggerated sexually selected weapons maintained with disproportionately low metabolic costs in a single species with extreme size variation. *Functional Ecology*, *35*(10), 2282-2293. https://doi.org/10.1111/1365-2435.13888

Soroye, P., Newbold, T., & Kerr, J. (2020). Climate change contributes to widespread declines among bumble bees across continents. *Science, 367*, 685-688.

Stabentheiner, A., Vollmann, J., Kovac, H., & Crailsheim, K. (2003). Oxygen consumption and body temperature of active and resting honeybees. *Journal of Insect Physiology, 49*(9), 881-889. https://doi.org/10.1016/S0022-1910(03)00148-3

Stabentheiner, A., & Kovac, H. (2016). Honeybee economics: Optimisation of foraging in a variable world. *Scientific Reports, 6*(1), Article 1. https://doi.org/10.1038/srep28339

Stelzer, R. J., Chittka, L., Carlton, M., & Ings, T. C. (2010). Winter active bumblebees (*Bombus terrestris*) achieve high foraging rates in urban Britain. *PLoS One*, *5*(3), e9559.

Stenseth, N. Chr., & Mysterud, A. (2002). Climate, changing phenology, and other life history traits: Nonlinearity and match–mismatch to the environment. Proceedings of the National Academy of Sciences, 99(21), 13379-13381. https://doi.org/10.1073/pnas.212519399

Steffan-Dewenter, I., Potts, S. G., & Packer, L. (2005). Pollinator diversity and crop pollination services are at risk. *Trends in ecology & evolution*, *20*(12), 651-652.

Suttle, K. B. (2003). Pollinators as mediators of top-down effects on plants. *Ecology Letters*, *6*(8), 688-694. https://doi.org/10.1046/j.1461-0248.2003.00490.x

Suverkropp, B. P., Bigler, F., & Van Lenteren, J. C. (2001). Temperature influences walking speed and walking activity of *Trichogramma brassicae* (Hym., Trichogrammatidae). *Journal of Applied Entomology*, *125*(6), 303-307. https://doi.org/10.1046/j.1439-0418.2001.00546.x

Symonds, M. R. E., & Elgar, M. A. (2002). Phylogeny Affects Estimation of Metabolic Scaling in Mammals. *Evolution*, *56*(11), 2330-2333. https://doi.org/10.1111/j.0014-3820.2002.tb00156.x

Tan, K., Hu, Z., Chen, W., Wang, Z., Wang, Y., & Nieh, J. C. (2013). Fearful Foragers: Honey Bees Tune Colony and Individual Foraging to Multi-Predator Presence and Food Quality. *PLoS One*, *8*(9), e75841. https://doi.org/10.1371/journal.pone.0075841

Tarahovsky, Y. S., Khrenov, M. O., Kovtun, A. L., & Zakharova, N. M. (2020). Comparison of natural and pharmacological hypothermia in animals: Determination of activation energy of metabolism. *Journal of Thermal Biology*, *92*, 102658. https://doi.org/10.1016/j.jtherbio.2020.102658

Tebaldi, C., Arblaster, J. M., & Knutti, R. (2011). Mapping model agreement on future climate projections. *Geophysical Research Letters*, *38*(23).

Terblanche, J. S., & Anderson, B. (2010). Variation of foraging rate and wing loading, but not resting metabolic rate scaling, of insect pollinators. *Naturwissenschaften*, *97*(8), 775-780. https://doi.org/10.1007/s00114-010-0693-8

Terblanche, J. S., & Chown, S. L. (2007). The effects of temperature, body mass and feeding on metabolic rate in the tsetse fly *Glossina morsitans centralis*. *Physiological Entomology*, *32*(2), 175-180.

Terblanche, J. S., Janion, C., & Chown, S. L. (2007). Variation in scorpion metabolic rate and rate–temperature relationships: Implications for the fundamental equation of the metabolic theory of ecology. *Journal of Evolutionary Biology*, *20*(4), 1602-1612. https://doi.org/10.1111/j.1420-9101.2007.01322.x

Terblanche, J. S., Klok, C. J., & Chown, S. L. (2004). Metabolic rate variation in *Glossina pallidipes* (Diptera: Glossinidae): gender, ageing and repeatability. *Journal of Insect Physiology*, *50*(5), 419-428. https://doi.org/10.1016/j.jinsphys.2004.02.009

Tewksbury, J. J., Huey, R. B., & Deutsch, C. A. (2008). Putting the heat on tropical animals. *Science*, *320*(5881), 1296-1297.

Thompson, J. N., Segraves, K. A., & Althoff, D. M. (2021). Coevolution and Macroevolution. In L. Nuño de la Rosa & G. B. Müller (Eds.)*, Evolutionary Developmental Biology* (193-205). Springer International Publishing. https://doi.org/10.1007/978-3-319-32979-6_125

Thomson, D. M. (2019). Effects of long-term variation in pollinator abundance and diversity on reproduction of a generalist plant. *Journal of Ecology, 107*(1), 491-502. https://doi.org/10.1111/1365-2745.13055

Thomsen, P. F., Jørgensen, P. S., Bruun, H. H., Pedersen, J., Riis-Nielsen, T., Jonko, K., Słowinska, I., Rahbek, C., & Karsholt, O. (2016). Resource specialists lead local insect community turnover associated with temperature–analysis of an 18-year full-seasonal record of moths and beetles. *Journal of Animal Ecology, 85*, 251-261.

Tomlinson, S., Dixon, K. W., Didham, R. K., & Bradshaw, S. D. (2017). Landscape context alters cost of living in honeybee metabolism and feeding. *Proceedings of the Royal Society B: Biological Sciences, 284*(1848), 20162676. https://doi.org/10.1098/rspb.2016.2676

Traill, L. W., Lim, M. L. M., Sodhi, N. S., & Bradshaw, C. J. A. (2010). Mechanisms driving change: Altered species interactions and ecosystem function through global warming.

Journal of Animal Ecology, *79*(5), 937-947. https://doi.org/10.1111/j.1365-2656.2010.01695.x

Tylianakis, J. M., Didham, R. K., Bascompte, J., & Wardle, D.A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecology Letters, 11*, 1351-1363.

Umbers, K. D. L., & Mappes, J. (2015). Postattack deimatic display in the mountain katydid, *Acripeza reticulata*. *Animal Behaviour*, *100*, 68-73. https://doi.org/10.1016/j.anbehav.2014.11.009

Vanbergen, A. J., & Initiative, the I. P. (2013). Threats to an ecosystem service: Pressures on pollinators. *Frontiers in Ecology and the Environment*, *11*(5), 251-259. https://doi.org/10.1890/120126

van Klink, R., Bowler, D. E., Gongalsky, K. B., Swengel, A. B., Gentile, A., & Chase, J. M. (2020). Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. *Science*, *368*(6489), 417-420.

Vannote, R. L., & Sweeney, B. W. (1980). Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. *The American Naturalist*, *115*(5), 667-695.

Varnon, C. A., Vallely, N., Beheler, C., & Coffin, C. (2021). The disturbance leg-lift response (DLR): an undescribed behavior in bumble bees. *PeerJ, 9*, e10997.

Velthuis, H. H., & Van Doorn, A. (2006). A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie, 37*(4), 421-451.

Visser, M. E., & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1581), 2561-2569.

Vucic-Pestic, O., Ehnes, R. B., Rall, B. C., & Brose, U. (2011). Warming up the system: Higher predator feeding rates but lower energetic efficiencies. *Global Change Biology*, *17*(3), 1301-1310. https://doi.org/10.1111/j.1365-2486.2010.02329.x

Warren, M. S., Hill, J. K., Thomas, J. A., Asher, J., Fox, R., Huntley, B., Roy, D. B., Telfer, M. G., Jeffcoate, S., Harding, P., Jeffcoate, G., Willis, S. G., Greatorex-Davies, J. N., Moss D., & Thomas, C. D. (2001). Rapid responses of British butterflies to opposing forces of climate and habitat change. *Nature, 414*(6859), 65-69.

Weibel, E. R., Bacigalupe, L. D., Schmitt, B., & Hoppeler, H. (2004). Allometric scaling of maximal metabolic rate in mammals: Muscle aerobic capacity as determinant factor. *Respiratory Physiology & Neurobiology*, *140*(2), 115-132. https://doi.org/10.1016/j.resp.2004.01.006

Welcome to the Climate Database. (n.d.). Retrieved October 11, 2022, from https://cliflo.niwa.co.nz/

West, G. B., & Brown, J. H. (2005). The origin of allometric scaling laws in biology from genomes to ecosystems: Towards a quantitative unifying theory of biological structure and organization. *Journal of Experimental Biology*, *208*(9), 1575-1592. https://doi.org/10.1242/jeb.01589

West, G. B., Brown, J. H., & Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science, 276*, 122-126.

West, G. B., Brown, J. H., & Enquist, B. J. (1999). The Fourth Dimension of Life: Fractal Geometry and Allometric Scaling of Organisms. *Science*, *284*(5420), 1677-1679. https://doi.org/10.1126/science.284.5420.1677

West, G. B., Woodruff, W. H., & Brown, J. H. (2002). Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals. *Proceedings of the National Academy of Sciences, 99*(suppl_1), 2473-2478. https://doi.org/10.1073/pnas.012579799

White, C. R., & Seymour, R. S. (2003). Mammalian basal metabolic rate is proportional to body mass2/3. *Proceedings of the National Academy of Sciences*, *100*(7), 4046-4049. https://doi.org/10.1073/pnas.0436428100

Wilson, R. J., & Fox, R. (2021). Insect responses to global change offer signposts for biodiversity and conservation. *Ecological Entomology*, *46*(4), 699-717. https://doi.org/10.1111/een.12970

Williams, K. A., & Villet, M. H. (2014). Morphological identification of *Lucilia sericata*, *Lucilia cuprina* and their hybrids (Diptera, Calliphoridae). *ZooKeys*, *420*, 69-85. https://doi.org/10.3897/zookeys.420.7645

Willis Chan, D. S., & Raine, N. E. (2021). Population decline in a ground-nesting solitary squash bee (*Eucera pruinosa*) following exposure to a neonicotinoid insecticide treated crop (*Cucurbita pepo*). *Scientific Reports, 11*(4241), 1-11. https://doi.org/10.1038/s41598-021-83341-7

Willmer, P., & Finlayson, K. (2014). Big bees do a better job: intraspecific size variation influences pollination effectiveness. *Journal of Pollination Ecology, 14*, 244-254.

Woods, W. A., Heinrich, B., & Stevenson, R. D. (2005). Honeybee flight metabolic rate: Does it depend upon air temperature? *Journal of Experimental Biology*, *208*(6), 1161-1173. https://doi.org/10.1242/jeb.01510

Wu, G. M., Barrette, M., Boivin, G., Brodeur, J., Giraldeau, L. A., & Hance, T. (2011). Temperature influences the handling efficiency of an aphid parasitoid through body size-mediated effects. *Environmental entomology, 40*(3), 737-742.

Yamahira, K., Kawajiri, M., Takeshi, K., & Irie, T. (2007). Inter- and Intrapopulation Variation in Thermal Reaction Norms for Growth Rate: Evolution of Latitudinal Compensation in Ectotherms with a Genetic Constraint. *Evolution*, *61*(7), 1577-1589. https://doi.org/10.1111/j.1558-5646.2007.00130.x

Xie, Q., Hou, B., & Zhang, R. (2008). Thermal responses of oriental fruit fly (Diptera: Tephritidae) late third instars: mortality, puparial morphology, and adult emergence. *Journal of economic entomology, 101*(3), 736-741.

Zaragoza-Trello, C., Vilà, M., Botías, C., & Bartomeus, I. (2021). Interactions among global change pressures act in a non-additive way on bumblebee individuals and colonies. *Functional Ecology*, *35*(2), 420-434. https://doi.org/10.1111/1365-2435.13703

Zhang, Y. (2013). Likelihood-based and Bayesian Methods for Tweedie Compound Poisson Linear Mixed Models. *Statistics and Computing, 23*, 743-757.