The role of thermal tolerance in biological invasion

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Abstract

Biological invasion and climate change are among the greatest threats to biodiversity currently. Several factors influence what makes a successful invasive species. Among these is the thermal tolerance of the invasive species. An organism's thermal physiology underpins many aspects of its life, including the habitats in which it can survive, its reproduction and its development. Climate change is increasing global average temperatures at an unprecedented pace, which is increasing thermal stress on virtually all life around the world. These effects are particularly pronounced in ectotherms, which are reliant on environmental temperature to set their body temperature and drive their metabolism. Insects comprise a significant proportion of globally important invasive species. As well as being ectothermic, they have a complex life cycle with a number of developmental stages, all of which may be influenced by environmental temperatures. Invasive species can often tolerate or adapt to a broad range of environmental conditions. They may therefore be expected to have a broader thermal tolerance than their native counterparts, and than other invasives that have a narrower invaded range.

This research used a blowfly model to investigate how thermal tolerance differed across temperatures, using a static temperature assay to measure thermal knockdown time. Key foci included the effects of both developmental heat acclimation and adult heat tolerance on thermal performance. In Chapter 2, variation in adult heat tolerance was assessed across three temperatures (41℃, 42℃, 43℃) in five blowfly species invasive to New Zealand. I found that the two more globally invasive species in the study exhibited higher heat tolerance than the three less invasive *Calliphora* species, whose native ranges are generally more temperate. In Chapter 3, the effect of developmental temperature on adult knockdown time in a single species (*Calliphora stygia*) was assessed. I showed that *C. stygia* reared at the lowest temperature (18℃) had the highest knockdown time and the smallest body size, while those reared at 26℃

exhibited the shortest knockdown time and an intermediate body size, with those reared at 22℃ being the largest.

Collectively, my results indicate that adult heat tolerance varies between invasive blowfly species, but that developmental acclimation temperature may not have a significant effect on adult heat tolerance. Given the increasing global temperatures seen with climate change, the effects of invasive species are likely to increase, with some species likely to do better than others in a warming world. Thermal performance will undoubtedly play a role in filtering out the ‘winners’ and ‘losers’, therefore further research into both heat and cold tolerance in both laboratory and field settings are crucial.

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Chapter 1 - Thesis introduction

1.1 Climate change and invasive species

Climate change is one of the defining ecological challenges of the 21st Century (Keegan et al., 2021; Shivanna, 2022). The frequency and intensity of extreme weather events has been increasing dramatically across the globe over recent decades. Climate change has led to a global average temperature increase of 1.1°C since pre-industrial times (1850–1900), with land heating up by ~1.59°C and sea temperatures rising ~0.88°C (International Panel on Climate Change (IPCC), 2023). Global surface temperature increased faster between 1970 and 2020 than over any other 50-year period for at least the last 2,000 years, and between 3.3-3.6 billion people now live in areas considered to be highly vulnerable to climate change effects (IPCC, 2023). Similar temperature patterns have also been seen in New Zealand (Figure 1.1).

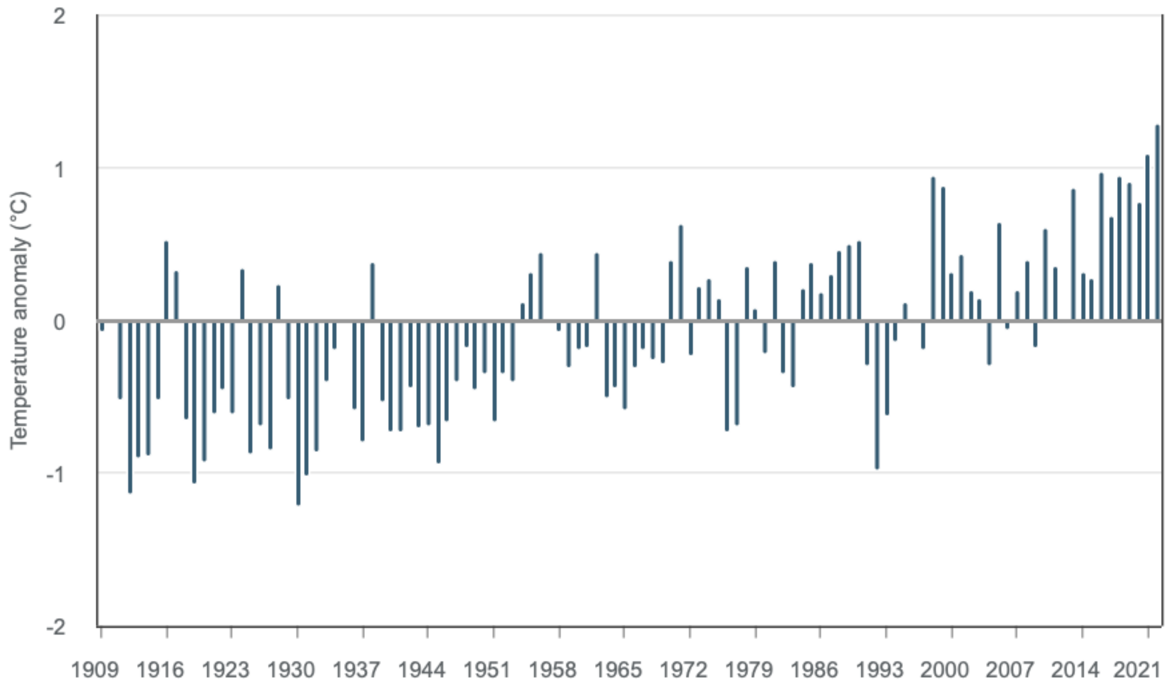

Figure 1.1 1909-2022 annual temperature anomaly in New Zealand against a baseline period of 1961-1990. Adapted from Stats NZ.

Global warming results in alterations in the hydrological cycle, with each 1°C of warming allowing the atmosphere to store 7% more water vapour according to the laws of thermodynamics (Ren et al., 2024). Changes to global and local hydrological changes go on to produce both more intense and more frequent rainfall events and periods of drought (NIWA, n.d.). These increasingly common extreme weather events are producing significant physical and economic costs to communities and ecosystems.

As well as altering weather events, climate change impacts ecosystems and biodiversity globally (Malhi et al., 2020; Weiskopf et al., 2020). Some species have been displaced and their distributions shifted in response to new challenges imposed by changing environmental conditions (Kerr, 2020; Rubenstein et al., 2023), whilst others have gone locally (IPCC, 2023) or completely (Ripple et al., 2025) extinct. Climate change associated extinction may occur through various abiotic and biotic factors, with important biotic factors including negative impacts on beneficial species, temporal mismatch between interacting species due to alteration in response to environmental cues, and the increased negative impacts of harmful species - such as increased predation, competition, or introduction of novel pathogens (Cahill et al., 2012).

Extinction events, either local or widespread, are more common in species that are already struggling, such as in small, isolated populations (Macinnis-Ng et al., 2021). This often corresponds to endemic species (Manes et al., 2021), those who inhabit specialised niches (Weinhäupl & Devenish-Nelson, 2024), including islands (Bender et al., 2019), or those that have a narrow thermal tolerance band, such as in marine (Manes et al., 2021), montane (Bender et al., 2019), or polar environments (Regehr et al., 2016). Understanding how species respond to climate events and stresses is important in the context of biodiversity and conservation, as well for predicting how these responses may alter the functioning of ecosystems in the future (Finch et al., 2021).

Increases in global temperatures may have significant impacts on insect populations. Insects are important economically and ecologically because of the ecosystem functions they provide (Barton & Evans, 2017; Verma et al., 2023), and are also important pest species (Goldson et al., 2020; Stathas et al., 2023). As ectotherms, insects rely on environmental temperatures to generate their own body heat for metabolism and risk severe negative effects from exposure to extreme temperatures due to disruption of their metabolism and population dynamics (Harvey et al., 2022). Insects also have complex life cycles with multiple developmental life stages, any of which may be affected by increasing ambient temperatures (Cui et al., 2018). These factors mean that climate change may have serious consequences for insects, although their small size and ability to be transported globally (Venette & Hutchison, 2021) might enable rapid adaptation to, or avoidance of, environmental change - espeicially in the context of biological invasion.

1.2 Invasive species

Invasive species are organisms introduced outside their native distribution that have spread beyond their initial points of introduction and established self-sustaining populations with negative effects on native ecosystems, economic activities, or human health (Lymbery et al., 2014). When non-native species arrive, they often take many years to establish, and only a minority will become invasive due to population growth lags after introduction, the need to overcome various environmental pressures (both abiotic and biotic), and/or a lack of evolutionary responses to their new conditions (Mooney & Cleland, 2001). However, climate change alters local abiotic conditions and can facilitate the introduction of invasive species into new regions as warming temperatures enable species to extend their range (Lai et al., 2021).

Invasive species straddle the microbial, animal, and plant kingdoms, and within the animal kingdom, both ectothermic and endothermic lifestyles, with examples being found in both aquatic and terrestrial environments (Haines et al., 2024). An important factor in the success of these diverse invasive groups is that they tend to share some important traits that make invading and spreading within their new environments a success (Cuthbert et al., 2025). Invasive species generally have efficient dispersal mechanisms, both to reach new environments and then spread from the original point of invasion (Finch et al., 2021); and invaded ranges often lack the threats that invasive species' face in their original habitats, such as natural predators (Hong et al., 2024). Their typical fast growth and reproduction rates, generalist diets, and rapid adaptive potential also facilitate successful invasion (Zhao et al., 2023).

Once invasive species are established, they may affect native species through hybridisation and introgression, leading to hybrids with expanded ranges (Croft et al., 2024) and the possible formation of new taxa (Vallejo-Marín & Hiscock, 2016), which can then spread more widely (Ellstrand & Schierenbeck, 2000). Invasive species can have negative impacts at multiple tropic levels (Figure 1.2) including changes in behaviour and traits of native species, niche displacement (Wauters et al., 2023), competition for resources (Nagano et al., 2023), predation (Ims et al., 2017), altered mutualistic relationships (Devenish et al., 2024), and the introduction of co-invading parasites that are more virulent in native species (Lymbery et al., 2014). These factors may contribute to extinctions at local, regional, or global scales (Bellard et al., 2021; Dueñas et al., 2021). Climate change may amplify these effects by modifying season length, habitat composition, and temperature, potentially increasing stress and disrupting native ecosystems and/or reducing their resistance to invasion (Weiskopf et al., 2020).

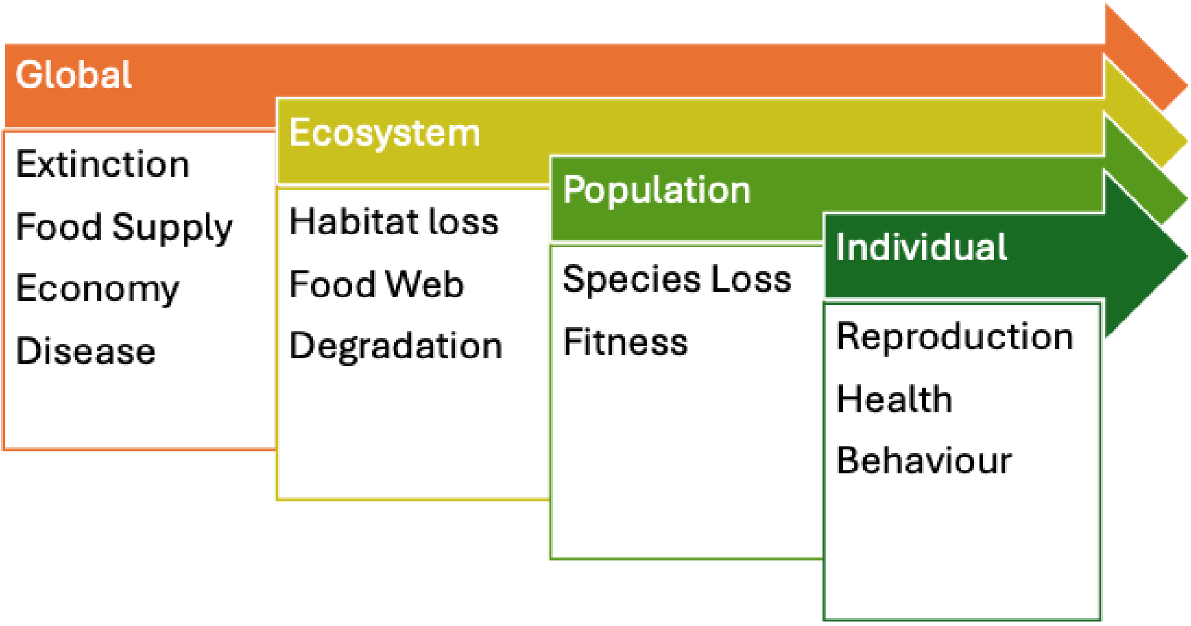

Figure 1.2 Negative impacts invasive species cause on differing ecological levels. Adapted from Carneiro et al., *2025.*

Invasive species are typically introduced either intentionally, for purposes such as agriculture, or inadvertently through transportation, ocean currents, floods, or high winds. Climate change is facilitating range expansion in potentially invasive species, as warming habitats reduce cold temperature barriers below critical thermal minimum thresholds (Hesselschwerdt & Wantzen, 2018), and some invasive species develop improved thermal tolerance following acclimation (Kelley, 2014). Invasive species may fill unoccupied ecological niches or out-compete native species (Mooney & Cleland, 2001). After invasive species establish, competition becomes more challenging for native species, particularly when climate change diminishes their competitiveness (Nagano et al., 2023) or imposes increased levels of thermal stress (Castillo-Pérez et al., 2025; Harvey et al., 2020). The effects of invasive species can intensify when ecosystem resilience is reduced by climate change, and when invasive species exacerbate climate-related stresses, potentially causing profound and widespread ecological impacts.

Invasive insects represent some of the most significant ecological, economic, and health threats globally (Abram et al., 2024). While their direct impacts, particularly on food production and economies, are well documented (Renault et al., 2022), indirect effects such as their role as vectors for human and agricultural disease transmission, the environmental consequences of control efforts, and trade barriers are often underestimated (Venette & Hutchison, 2021). Invasive insects affect many terrestrial and aquatic ecosystems and are a significant issue in urban and cultivated areas (Abram et al., 2024; Gippet et al., 2022). Many interventions have been trialled to address the impact of invasive insect species globally (McLaughlin & Dearden, 2019; Venette & Hutchison, 2021) and in New Zealand (Goldson et al., 2020; McLaughlin & Dearden, 2019), each with their own potential benefits and harms (Abram et al., 2024). Understanding insect invasiveness is therefore vital for developing timely and effective responses to these challenges, while understanding how and which invasive species will succeed in a warming word is a key factor in managing invasive insects.

1.3 Thermal physiology

Thermal physiology plays an important role in the adaptive responses of organisms to a world of increasing global temperature and habitat shifts (Dong, 2023), with intraspecific variation in thermal tolerance being an important component of potential adaptation (Herrando-Pérez et al., 2023). Thermal extreme limits are dependent on a multitude of factors and are defined as the points an organism or system can tolerate before experiencing detrimental effects (Leclerc et al., 2022). Thermal limits are set genetically, but often reflect a species' long-term thermal history, with thermal tolerance ranges being more narrow in tropical and aseasonal environments (Madeira et al., 2012), and physiological boundaries imposed by biochemical constraints (Bennett et al., 2021), and environmental factors such as salinity (Fernandes et al., 2023), oxygen (Fusi et al., 2024), and water supply (Anderson & Andrade, 2017; Youngblood et al., 2025). Thus, thermal limits are known to vary along latitudinal and altitudinal gradients (Sunday et al., 2019).

There is extensive interspecific variation in cold tolerance limits among animal species (Hoffmann et al., 2012) compared to extreme heat tolerance limits, where variation is more constrained (Bennett et al., 2021). Thus, variation in thermal tolerance is a key driver in the biogeographical distribution of species around the globe, and heat tolerant species typically have differing habitats or niches than cold tolerant species (Schou et al., 2022). For example, species adapted to significant seasonal environmental change are typically excluded from tropical climates by other biotic and non-biotic factors (Moore et al., 2023).

Thermal stress is an important component of climate change, with species increasingly becoming exposed to temperatures close to their upper thermal limits (Van Heerwaarden et al., 2024), particularly in tropical climates (Fernandes et al., 2023; Hoffmann et al., 2012). At this point, thermoregulation becomes a challenge, and vital functions start to become affected, with humidity (Li et al., 2024), temperature exposure duration (Ørsted et al., 2024), and the ability of a species to adapt via behavioural and physiological responses (Woodruff et al., 2024) ultimately working together to determine individual outcomes.

1.4 Thermal tolerance in invasive insects

Thermal tolerance is defined as the range where an organism or ecosystem can function, survive, and grow, with wider thermal tolerances enabling species to inhabit a broader geographic extent (Sunday et al., 2012). Thus, invasive species may be expected to have broader thermal tolerances due to their native habitats having broader temperatures than those of their invaded habitats. In addition, invasive species can show increased plasticity, which allows function across a wider thermal breadth (Da Silva et al., 2021).

Importantly for this thesis, invasive species are expected to have a broad environmental tolerance, that helps them to adapt to new habitats with differing environmental conditions, especially those warmer than their native range (Da Silva et al., 2021; Kelley, 2014). Thermal tolerance is particularly important for invasive insect species due to their complex life history, with multiple juvenile developmental stages. Thermal stress (i.e., hot or cold) can have impacts on each individual stage and acclimation and other phenotypic responses in each of these stages can potentially have impacts on heat tolerance in the other life stages (Pottier et al., 2022; Rebolledo et al., 2021).

1.5 Measuring thermal tolerance

There are two key ways in which thermal tolerance of insects can be measured: static and dynamic assays (Jørgensen et al., 2019). Static assays expose individuals to a set temperature, typically at one end of the species' presumed thermal range. They test for the length of time an individual is able to withstand the test temperature before experiencing a predefined deleterious effect, such as loss of mobility or death (Bak et al., 2020). Dynamic assays expose individuals to changing temperatures, including assessment of the critical thermal maximum (Ct_{max}) or critical thermal minimum (Ct_{min}) and dynamic (heat shock or heatwave) experiments (Kingsolver & Umbanhowar, 2018). Ct_{max} determination experiments expose individuals to increasing temperatures until they reach the critical pre-determined end point. The same can be done to measure

Ct_{min}, using decreasing temperatures, while fluctuating temperatures are used to mimic heatwaves (Scharf et al., 2022).

While field studies that observe and measure individuals in the natural environment are a valuable tool to study species responses to real world conditions and interactions, studies looking at thermal tolerance across differing geographic regions or over time are rare because of the inability to control for numerous factors (such as temperature variability, humidity, and ecosystem resources) in the field (Sasaki et al., 2025). In addition, unknown variables, such as age, diet, and previous thermal history of field-caught individuals can introduce variability in results that cannot be accounted for, thus making it harder to isolate the effect of a specific trait such as thermal tolerance (Noer et al., 2022). Conversely, laboratory experiments on colony-reared individuals allow for the control of extrinsic factors that are unable to be accounted for in field experiments, traits can be manipulated independently, and conditions can be tightly controlled and easily replicated (Calisi & Bentley, 2009). However, laboratory studies are often said to lack real world ecological variation (Binning et al., 2025; Ross et al., 2018) and thus require careful interpretation before extrapolation to the real world (Huho et al., 2007).

An alternative approach involves studies that combine these methods using wild-caught individuals that have undergone a brief acclimation period in a laboratory before testing. The period of acclimation allows for some experimental control without losing all real-world variation. It is an important method for assessing thermal tolerance as it allows for stabilisation of the health and behaviour of the study individuals following potentially stressful collection and transport and provides more reproducible results because of a more stable physiological baseline (Obernier & Baldwin, 2006). Acclimation at a stable temperature for all individuals dampens the effects of their prior thermal history and may facilitate phenotypic plastic responses, such as heat hardening, that would be a part of their natural response to heat stress and therefore can provide a better indicator of the individual or species heat tolerance.

Field studies are generally more appropriate if the aim is to look at physiological differences or how a species evolves in relation to an environmental change under natural conditions. On the other hand, if the aim is to look at acclimation, specific limits, or plastic responses to an environmental stress then laboratory studies are a more commonly chosen and reasonable approach.

1.6 Blowfly system

Blowflies include a group of successful invasive ectothermic species found globally (Figure 1.3) that, like most insects, undergo metamorphosis. Blowfly larvae feed predominantly on carrion or sometimes other decomposing organic matter, with adult female blowflies laying their eggs on suitable material. Blowflies are decomposers and scavengers and thus contribute to nutrient cycling but, outside of their native range, they can also cause significant health risks to humans, wildlife, and livestock. In New Zealand, some species of invasive blowfly are involved in myiasis (flystrike), which affects 2-10% of the national sheep flock annually (Beef + Lamb New Zealand, 2022). There is no recent published data from New Zealand on the economic impacts of flystrike, with Heath & Bishop (1995) estimating it at around ~ $37 million. However, a more recent study from Australia suggested that losses to the Australian sheep industry related to flystrike were around ~$AUD175 million annually (Kotze & James, 2021).

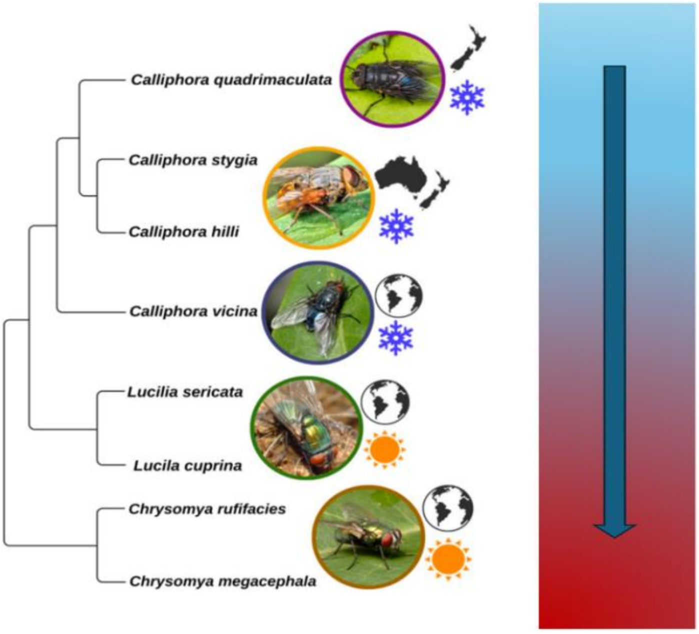

Figure 1.3. Model system of seven invasive and one native New Zealand blowfly species, with their phylogenetic relationship, habitat preference (cooler or warmer), and geographic distribution (New Zealand, Australia and cosmopolitan) displayed. A single image for each sister species is used. Image from Invasomics Lab, University of Waikato. Gradients on the right hand side show increasing invasiveness, and predicted thermal tolerance, respectively.

Blowflies are likely introduced to new environments via occasional long-distance dispersal (they have a high dispersal ability, with adults recorded as travelling kilometres to search for carrion; Lee et al., 2023), and/or via transport on cargo, animal hide, and food waste (Croft et al., 2024; Sherman et al., 2006). They are found in all continents, except Antarctica, though some species have established in the sub-Antarctic (Daly et al., 2023). Their global success is likely the result of their rapid life cycles, competitive ability, dispersal rates, and high capacity for adaptation (Croft et al., 2024).

Blowflies are easy to rear under laboratory conditions and are relatively easy to identify (e.g., Dear, 1986). Temperature is an important factor in their development and survival. For example, blowfly larvae often show a species-specific temperature preferences for development (Day et al., 2021), though the optimal temperature may vary between different populations of the same species (Kotzé et al., 2016; Voss et al., 2014; Zhang et al., 2019). Invasive blowfly species have been shown to have a broader thermal tolerance window than their native competitors (Kelley, 2014), as well as having higher thermal limits generally, giving them an advantage in hot climates and urban heat islands (Pinto et al., 2021; Rusch et al., 2020). Collectively, their overall adaptability to environmental change and general invasion success make blowflies an excellent model species to study variation in thermal tolerance of invasive species.

1.7 Thesis overview

Following this introductory chapter, Chapter 2 explores thermal tolerance of five wild-caught blowfly species after a brief period of acclimation, to assess variation in thermal tolerance among species. Chapter 3 explores first generation *Calliphora stygia* blowflies reared at three different temperatures under laboratory conditions to assess how developmental temperature affects thermal tolerance in a single species. Finally, Chapter 4 provides insights into the findings of this thesis, as well as future research directions and implications. Invasive species are an escalating biological and economic issue worldwide. By examining various aspects of thermal performance variation in adult invasive blowflies, this thesis contributes to knowledge regarding the potential for spread and adaptation of invasive insects in new environments in the context of changing climates.

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Chapter 2 - Do invasive species differ in their thermal tolerance?

2.1 Abstract

Organisms are increasingly being exposed to environmental temperatures outside their typical range due to climate change, often requiring rapid adaptive responses via behavioural, genetic, and phenotypic plasticity. Invasive species are expected to be among the most successful species at showing such responses to changing conditions, yet limited work has focused on variation in thermal tolerance among multiple invasive species.

Here, we captured invasive blowflies from rural and urban locations and exposed them to three static temperature assays to test knockdown time after a brief period of laboratory acclimation.

We found that *Chrysomya* spp. exhibited the highest heat tolerance among the five studied species, whilst *C. vicina* had the lowest. We also found that males had higher knockdown times than females for all species. The higher thermal performance of *Chrysomya* spp. aligned with its widespread and thermally diverse geographic origins. However, we expected females to show longer knockdown times based on previous findings.

Overall, our study expands on previous research considering the importance of thermal tolerance and its potential role in invasion success while providing suggestions for further research into the additional factors that may interact with thermal tolerance to influence invasive species and the rate at which they are able to adapt.

2.2 Introduction

Environmental temperatures are changing around the world in response to anthropogenic climate change, with average increases of at least 1.1°C since 1880 and the majority of warming (increases in both average temperatures and extreme weather days and events) occurring since 1975 (Lindsey & Dahlman, 2025). Understanding how species may respond to these rapid changes is critical, because temperature is a major abiotic factor influencing animal and plant physiology and distributions globally (Christensen et al., 2020; Seebacher et al., 2023).

Temperature extremes can cause evolutionary changes and alter species distributions and ecosystem stability (Dee et al., 2020), for example by causing habitat loss, extinction (Duffy et al., 2022), and the disruption of ecosystem functioning (Staudt et al., 2013). In some species, these effects are already being felt. For example, bats in Israel and Italy are suffering climate-related adverse effects, including increasing mortality and heat stress events (Russo et al., 2025; Salinas-Ramos et al., 2023). Australian stingless bees are also increasingly experiencing periodic heat events that exceed their thermal limits (Nacko et al., 2023). Thus, predicting species responses to thermal extremes in particular, is important for determining how at-risk various types of species may be in the near future (Buckley & Huey, 2016; White et al., 2024) and for understanding how species distributions will change in the coming decades (Bridle & Hoffmann, 2022; Rubenstein et al., 2023).

In response to temperature variation, species can display behavioural, physiological, and genetic changes (Bonamour et al., 2019; Freudiger et al., 2021). For example, in response to increasing atmospheric temperatures, the European hare exhibits reduced activity during its normally active nocturnal periods and increased activity during the day - likely as a way to reduce energy expenditure at night, while requiring more energy in the daytime to find shade and avoid over-heating (Steigler et al., 2023). Meanwhile, heat hardening is an example of a phenotypic plasticity response to elevated temperatures that results in short term acclimation to heat stress via production of heat shock proteins (Dahlgaard et al., 1998), and is seen in a variety of ectothermic species (White et al., 2024; Woodruff et al., 2024).The rapidity of onset and persistence of the heat hardening response varies between species (Dahlgaard et al., 1998; Sørensen et al., 2019). Genetic changes in response to temperature are also common, especially along climactic gradients (Chen et al., 2021; Miñano et al., 2022; Yang et al., 2025). For example, Stonehouse et al. (2023) identified 36 genes in the European great tit likely linked to climate adaptations. Evolutionary adaptation has also been documented in some species over a human lifespan (Martin et al., 2023). Examples of this include the green anole lizard (*Anolis carolinensis),* which developed persistent improved cold tolerance in a southern United States population that had been exposed to severe winter storms, with genomic changes underlying this adaptation and suggesting evolutionary selection (Campbell-Staton et al., 2017); and the development of pesticide resistance in *Drosophila* due to a single allele change (Daborn et al., 2002). Despite the different ways species can potentially respond to warming temperatures, many - particularly those with longer life cycles or later breeding ages - may struggle to keep up with the pace of climate change (Radchuk et al., 2019).

Smaller ectothermic animals with shorter generation times and higher fecundity, such as insects and fish, are potentially best suited to adapt to rising temperatures - because faster generation times increase the potential speed of adaptation (Radchuk et al., 2019; Thomas et al., 2010). Research shows evidence of rapid adaptation to temperature change leading to improved heat and cold tolerance for several invasive insects. For example, Ma et al. (2024) performed field and greenhouse experiments on the thrips species, *Thrips palmi,* which has expanded its range northwards from the subtropics to temperate climates. These authors found evidence of genomic and phenotypic adaptation to new climates, with southern and greenhouse populations showing increased heat tolerance and individuals in the northern open-air population showing better cold tolerance. Meanwhile, Sherpa et al. (2024) studied the Asian tiger mosquito (*Aedes albopictus*), which has extended its range into areas of Asia and shows associated impacts on thermal tolerance, resulting in three evolutionary clades with temperature-associated differentiation in several candidate genes. Finally, Dudaniec et al. (2018) also noted rapid genomic change among damselfies (*Ischnura* *elegans*) as a result of significant increases in maximum summer temperatures in their northern ranges.

A key and concerning theme here is that invasive insect species seem to adapt particularly quickly to climate-induced temperature changes. Thus, with a changing climate, invasive species can be expected to become more successful. Indeed, insect invasions have increased at pace with climate change (Comte et al., 2024; Schneider et al., 2022) and insects are among the most highly represented invasive species globally (e.g., making up 17% of the IUCN worst invasive species list; Venette & Hutchison, 2021) due to characteristic traits that enable their establishment and spread. Insects often show rapid adaptation (Dudaniec et al., 2018; McCulloch & Waters, 2022), a higher CT_{max} compared to many other species (Katzenberger et al., 2021), and the ability to easily disperse to new habitats in response to climate change (McCain & Garfinkel, 2021; Ojija et al., 2025). Thus, understanding how invasive insects tolerate and adapt to new environments is important for developing management strategies that will help to reduce economic, as well as environmental, impacts. In the context of climate change, understanding insect thermal responses to warming environments and unpredictable heat waves will be especially important given their role in ecosystem functions and the physiological constraints resulting from their ectothermy (Burc et al., 2025; Harvey et al., 2020).

Interspecific variation in thermal tolerance is seen across insect species within particular habitats (Franken et al., 2017; Katzenberger et al., 2021). Thermal tolerance can vary widely even in closely related species, as noted by Li et al. (2023) in their study of aphids. However, some insect species exhibit intraspecific differences among individuals of different sex or size, or among populations from different habitats imposing variable selective pressures (Garfinkel & McCain, 2025; Rivera-Rincón et al., 2024). Thus, a key aspect of understanding how invasive insects have adapted to changing thermal regimes involves looking at differences in thermal tolerance between related clades or species. Multiple different families of insects show examples of invasive species with improved thermal tolerance compared to closely related species (Boher et al., 2016; Comeault et al., 2020; Da Silva et al., 2021; Janion-Scheepers., et al

2017; Xiao et al., 2016), suggesting that improved thermal tolerance has developed independently in multiple invasive insect clades.

Blowflies are some of the most successful insects worldwide, with several invasive blowflies showing rapid adaptation in response to new environments (Croft et al., 2024a; Hwang & Turner, 2009) - including thermal challenges (Daly et al., 2023) - and increasing distributions in response to climate change (Valdez-Espinoza et al., 2025). Blowflies occupy a diverse range of environments, including urban and rural, and are often found near animal waste, compost, exposed flesh, and carrion (Kavazos & Wallman, 2012). They are very good invaders - dispersing readily and exhibiting several features associated with successful invasion, including rapid generation times, high fecundity, an inconspicuous nature (allowing long-distance transport), and an ability to undergo hybridisation with its associated gene flow (Carmo et al., 2018; Croft et al., 2024b; Oliveira & Vasconcelos, 2019). Different invasive species can have different thermal niches, for example, *Calliphora vicina* is cold-adapted and expanding towards Antarctica, while species from the genera *Lucilia* and *Chrysomya* are warm-adapted and generally inhabit temperate and tropical habitats, while showing some limitation by overwinter temperatures (Cammack & Nelder, 2009; Rusch et al., 2020). Thus, invasive blowflies provide an ideal system to understand the thermal drivers of insect invasion dynamics.

Here, we assessed whether thermal performance (measured as knockdown time in response to three experimental temperatures) varied across differentially invasive blowfly species (i.e., blowflies found sympatrically in New Zealand, but that differ in their global spread and thus potentially differ in ‘invasiveness’). We predicted that the more invasive species in our study (i.e., those with broader global distributions) would show a higher tolerance to increased temperatures reflecting a broader thermal range of habitat suitability, and that increasing experimental temperatures would decrease thermal knockdown times in all species due to the increasing thermal stress.

2.3 Methods

2.3.1 Study species

We focused on six blowflies in this study, including the native blowfly *Calliphora quadrimaculata*. All of the studied species are sympatric in distribution in New Zealand, with the whole group occupying similar niches and performing similar ecological roles as primary decomposers (Dear, 1986). Among the invasive blowflies in this study, *Calliphora hilli* and *Calliphora stygia* are native to Australia and only found within Australasia (Johnston et al., 2025), whilst *C. vicina* is native to Europe but invasive globally in countries such as the US, Australia, and New Zealand (Limsopatham et al., 2018). *Chrysomya rufifacies* is believed to have originated in Asia but is now found throughout the Americas as well as in Asia, Australia, and New Zealand, while *Lucilia spp.* are found in tropical and temperate habitats in Europe, Australia, and Asia (Badenhorst & Villet, 2018; Picard & Wells, 2009). Given their global distribution and habitat preference differences, we may expect to see variation in thermal tolerance that reflects these potential differences in invasiveness.

2.3.2 Collection sites

The five invasive blowfly species and the native species *C. quadrimaculata* were collected from four sites (Figure 2.1a) over a 15-month study period, ranging from March 2023 to May 2024. Three sites were considered rural, including Ed Hillary Hope Reserve (private conservation reserve), Kaniwhaniwha (public conservation land) - both large stands of native bush with adjacent grassy areas located next to freshwater streams - and Whatawhata (a rural farm setting, with paddocks near private dwellings, farm buildings, and farm animals). The University of Waikato campus was considered to be an urban location, with much greater human disturbance.

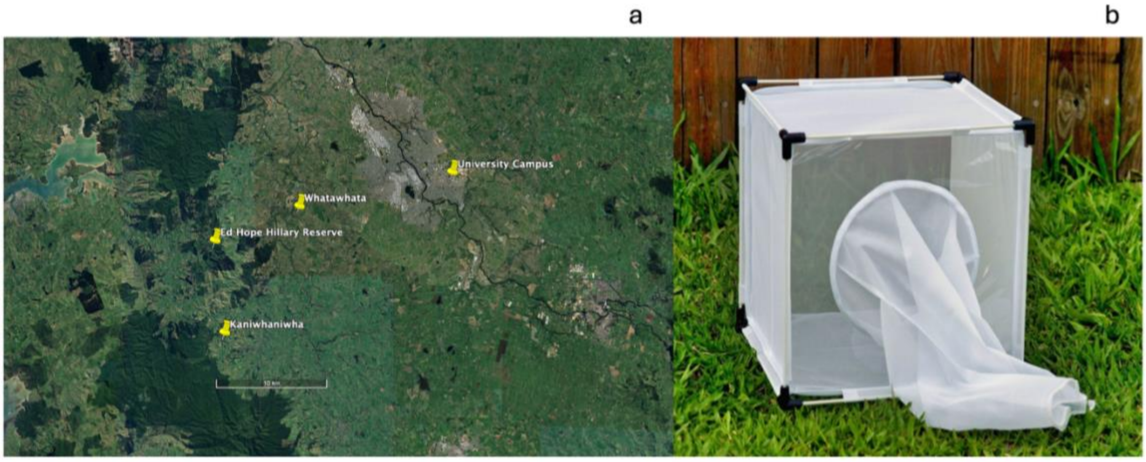

Figure 2.1. a) Map of collection sites; b) Mesh cage used for housing flies both in the field and during acclimation.

2.3.3 Collection methods

To attract blowflies, raw mincemeat was prepared as bait by leaving it to spoil in a semi-shaded outdoor environment, out of reach of mammal and bird disturbance. Once the bait was emitting a strong odour (2-3 days), it was taken to the desired collection site and the container lid removed. Blowflies were captured using a handheld insect net as they landed on the bait container, in or hovering over the bait. Collection days were random throughout the summer-to-autumn sampling periods, but collections occurred during peak blowfly activity hours (mid-morning to early afternoon).

Captured blowflies were transferred from the net into mesh holding cages (Figure 2.1b), which housed a water container to reduce the risk of dehydration, and were kept in the shade during the capture process to reduce stress on the captured blowflies before transportation back to the university laboratory on the same day.

2.3.4. Acclimation

To standardise blowfly thermal history immediately prior to the thermal tolerance assay, all blowflies were acclimated to the laboratory environment in the same mesh insect cages that were used for collection. Acclimation was carried out at 22 °C +/- 1°C in a temperature-controlled room for 48 hours under a 12:12 light: dark cycle. During acclimation, the flies had access to raw sugar provided in weight boats and *ad libitum* water, provided in plastic containers with a wick to allow for water absorption but prevent drowning.

2.3.5 Knockdown assays

Three experimental temperatures were selected (41°C, 42°C, 43°C), with these temperatures found to be at or near the upper thermal limits for the studied species (data not published). For each assay, groups of ~30 blowflies were placed individually into 15 mL clear plastic tubes (Falcon tubes; Greiner). The assay was then carried out in an incubator (Ratek orbital mixer incubator) that had been preheated to the desired test temperature as confirmed by a digital temperature display. Tubes were placed vertically in a rack inside the incubator to allow for assessment of knockdown time (deemed to occur when the fly lost the ability to climb up the tube walls, and was unable to self-correct), with flies continuously monitored for up to 3 hr (blowflies still active at 3 hr were recorded as having a knockdown time of 180 mins).

2.3.6 Identification and body sizing

Taxonomic identification of assayed individuals was conducted by placing a pin into the underside of the thorax of the blowfly and viewing specimens under a 20x microscope (Olympus SZH10, Research Stereo). The taxonomic key of Dear (1986) was then used to identify blowflies to genus (*Lucilia* and *Chrysomya* spp.) or species level, with sex also determined based on eye morphology and genitalia.

During the identification process, individuals were also photographed (EOS 600D, Canon) for body size measurement. A ruler was placed alongside each fly, and a photograph was taken from above the pinned specimen. Images were then evaluated in imageJ ver. 1.54g (Schneider et al., 2012), with head width, thorax length, and thorax width measured.

2.3.7 Data analysis

All statistical analyses were performed in R v 4.2.3 (R Core Team, 2025).

Generalised linear mixed models (package glmmTMB, ver. 1.1.9; Brooks et al., 2017) were used to explore how knockdown time varied with species, experimental temperature, and sex. Models included thorax length as a covariate (given that thorax length was highly correlated with the other body size measurements, it was used as the single measure of body size; Figure S2.1), and location and month/year of sampling as random effects. A negative binomial error distribution was used for all models. An initial glmmTMB was run with both sexes combined, following which the model was run separately for males and females to assess sex-specific differences. ANOVA type III was then used to assess the significance of the main model effects and interactions. Models were also run for individual species to assess the effects of sex and temperature on knockdown time. To investigate model-predicted knockdown times and test for significant differences between variables (i.e., sex, temperature, species), estimated marginal means (EMMs) and pairwise comparisons were conducted using the emmeans package ver. 1.10.7 (Searle et al., 1980). Results were visualised using ggplot2 ver. 3.5.1 (Wickham et al., 2007).

2.4 Results

2.4.1 Collection breakdown

A total of 759 blowflies (672 females and 87 males) were caught and assayed for analysis in this study. A total of two *C. quadrimaculata* were captured over the study period and were therefore removed from the dataset before analysis. Among the invasive species, *Chrysomya spp.* was caught most frequently (35.05%), with *Lucilia* spp. next most common (30.96%) (Figure 2.2a). *C. vicina* was the least frequently caught (4.87%). Flies were collected in relatively even numbers at rural (50.07%) and urban (49.93%) sites, with the greatest number of flies captured at Kaniwhaniwha (Figure 2.2b). Each species was generally caught at both rural and urban sites, though *Chrysomya spp.* was more numerous at rural sites and *Lucilia* spp. was only caught at urban sites, and at the rural Whatawhata site (Figure S2.2). Flies were assayed in relatively even numbers at the three different experimental temperatures (Figure 2.2c), and most of the flies were captured during March (n=260) and April (n=245) months, with higher overall numbers caught in 2023 (n=539) versus 2024 (n=220) (Figure S2.3; Table S2.1).

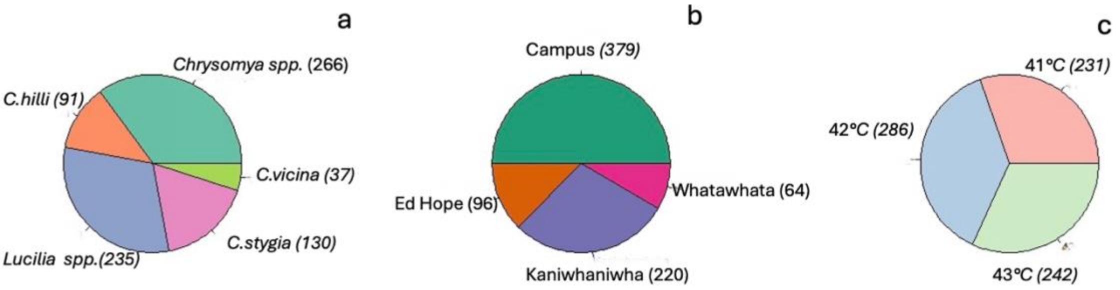

Figure 2.2: Proportion of blowflies caught by species and location, and the number of replicates for each experimental temperature. a) Relative proportions of different species, with Lucilia spp. (31%) and Chrysomya spp. (24%) being the most common; b) Collection sites of samples, showing Kaniwhaniwha (29%) and Campus (41.9%) as the most represented locations; c) Percentage of assays conducted at each experimental temperature: 42°C (37.4%), 43°C (31.9%), and 41°C (30.4%).

2.4.2 Variation in knockdown time with species and sex

The full GLMM revealed significant differences in knockdown time among species ($\chi^2_4 = 16.20$, $p < 0.01$), temperatures ($\chi^2_2 = 37.10$, $p < 0.001$), sex ($\chi^2_1 = 8.40$, $p < 0.01$), and thorax length ($\chi^2_1 = 15.67$, $p < 0.001$). Significant interactions between species and temperature ($\chi^2_8 = 112.34$, $p < 0.001$) and temperature and sex ($\chi^2_2 = 9.37$, $p < 0.01$) were also detected, whilst species and sex ($\chi^2_4 = 6.21$, $p = 0.184$) and the three way interaction of sex*species* temperature ($\chi^2_8 = 11.73$, $p = 0.164$) were not significant (Table 2.1).

Due to the significant differences in knockdown time found between sexes, sex-specific GLMMs were run to understand how species differences were structured among males and females separately (Figure 2.3a). The female-only model (Figure 2.3b) revealed that knockdown time differed significantly among species ($\chi^2_4 = 15.52$, $p < 0.01$), temperature ($\chi^2_2 = 38.36$, $p < 0.001$), thorax length ($\chi^2_1 = 12.47$, $p < 0.001$), and the interaction between species and temperature ($\chi^2_8 = 110.79$, $p < 0.001$). However, the male-only model (Figure 2.3c) revealed significant effects of only species ($\chi^2_4 = 14.92$, $p < 0.01$) and thorax length ($\chi^2_1 = 4.11$, $p = 0.043$) on knockdown time, with temperature (χ^2_2 = 3.33, *p* = 0.189) and the interaction of species and temperature (χ^2_8 = 8.31, *p* = 0.404) non-significant (Table 2.1).

Table 2.1 Results of the analysis of deviance (type III) for the effects of species (five species), temperature (41°C,42°C,43°C), sex (male, female) and thorax length on knockdown time (negative binomial generalised linear model). The reported degrees of freedom is equal to the number of parameters associated with the effect. Significant values are indicated in bold.

	χ^2	**df**	**p**
Full Model			
Species	16.201	4	**<0.010**
Temperature	37.097	2	**<0.001**
Sex	8.399	1	**<0.010**
Thorax length	15.673	1	**<0.001**
Species*Temperature	112.336	8	**<0.001**
Species*Sex	6.208	4	0.184
Temperature*Sex	9.370	2	**<0.010**
Species*Temperature*Sex	11.728	8	0.164
Female model			
Species	15.516	4	**<0.010**
Temperature	38.362	2	**<0.001**
Thorax length	12.468	1	**<0.001**
Species*Temperature	110.793	8	**<0.001**
Male model			
Species	14.925	4	**<0.010**
Temperature	3.330	2	0.189
Thorax length	4.110	1	**0.043**
Species*Temperature	8.306	8	0.404

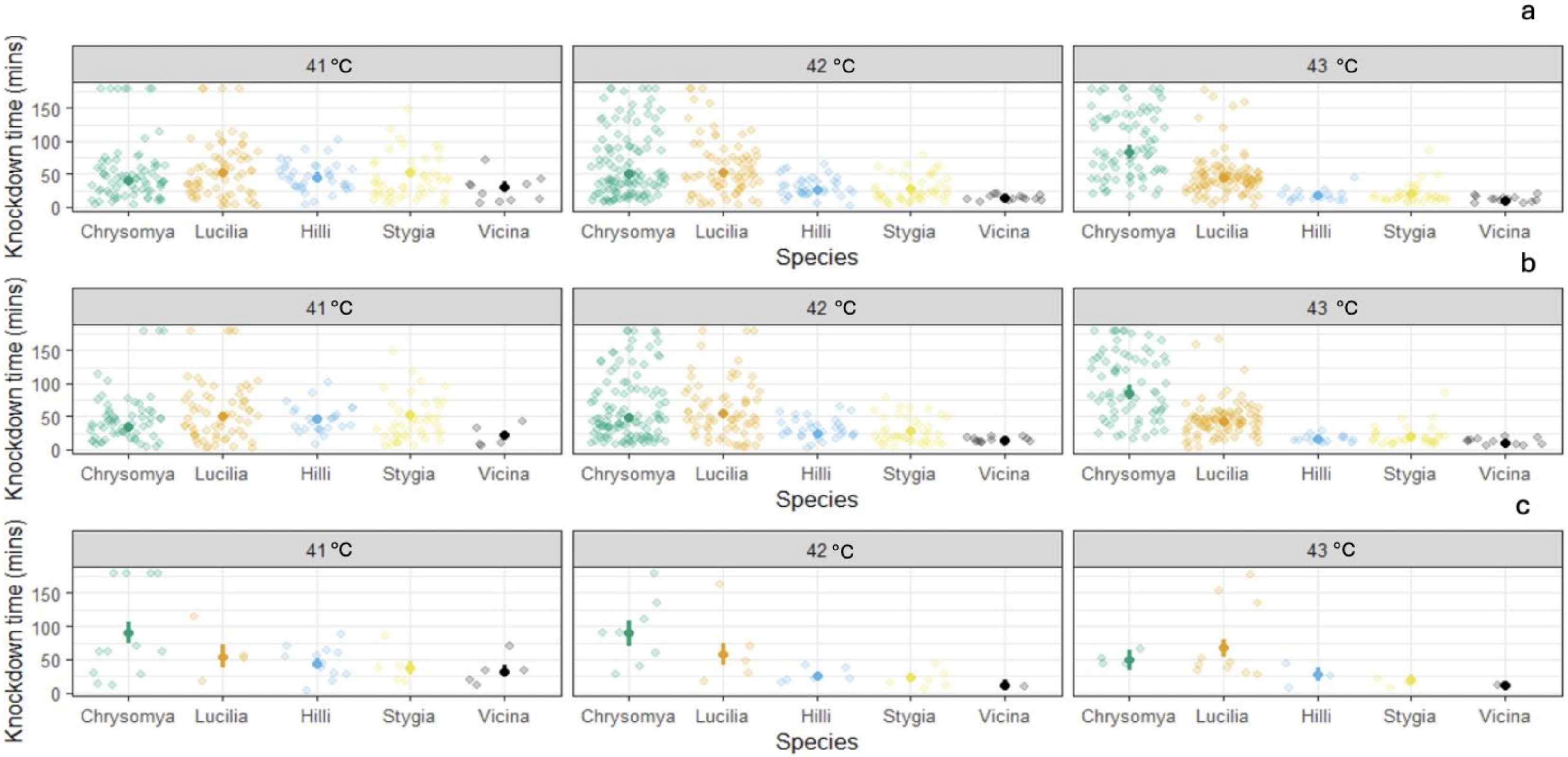

Figure 2.3. Variation in knockdown time with standard error bars for the five studied species (each point displays data for an individual fly), with data shown separately for: a) The combined glmm model; b) Female data only; and c) Male data only.

To investigate how experimental temperature affects knockdown time in a sex-specific manner, pairwise comparisons of EMMs were performed for both females and males (Table 2.2). These revealed no significant differences in knockdown time between species at 41°C, with the exception of male *Chrysomya* spp. compared to *C. vicina* (Table 2.2). At both 42°C and 43°C, female *Chrysomya* spp. had significantly higher knockdown times than all other females (Table 2.2), while female *Lucilia* spp. exhibited higher knockdown times than all other females except *Chrysomya* spp.. Female *C. vicina* consistently displayed the lowest knockdown times (Table 2.2). At 42°C, male *Chrysomya* spp. exhibited significantly higher knockdown times than *C. hilli* (p=0.0001), *C. stygia (p*=<0.0001*)* and *C. vicina (p*=0.0045) males, but only displayed a higher knockdown time than *C. vicina (p*=0.0006) males at 43°C. Male *C. hilli* showed higher knockdown times compared to *C. vicina* males at 43°C, while male *Lucilia* spp. had higher knockdown times than male *C. stygia* at 42°C and male *C. vicina* at 43°C (Table 2.2).

Table 2.2 Pairwise comparisons of sex and temperature from estimated marginal means models. Estimates are derived from a negative binomial linear model testing the effects of species and thorax length on knockdown time. Significant p values are in bold.

	Female 41°C				**Male 41°C**			
	Estimate	**SE**	**Z Ratio**	**P Value**	**Estimate**	**SE**	**Z ratio**	**P Value**
Chrysomya spp. - *C. hilli*	-0.114	0.214	-0.534	0.984	0.715	0.280	2.554	0.079
Chrysomya spp. - *Lucilia* spp.	-0.132	0.154	-0.858	0.912	0.524	0.405	1.294	0.695
Chrysomya spp. - *C. stygia*	-0.107	0.184	-0.580	0.978	0.879	0.366	2.403	0.115
Chrysomya spp. - *C. vicina*	0.678	0.384	1.765	0.394	1.033	0.364	2.840	**0.037**
C. hilli - *Lucilia* spp.	-0.018	0.238	-0.076	1.000	-0.192	0.397	-0.483	0.989
C. hilli - *C. stygia*	0.008	0.213	0.036	1.000	0.163	0.362	0.452	0.991
C. hilli - *C. vicina*	0.792	0.389	2.034	0.250	0.318	0.362	0.879	0.905
Lucilia spp. - *C. stygia*	0.026	0.225	0.114	1.000	0.355	0.458	0.776	0.938
Lucilia spp. - *C. vicina*	0.810	0.387	2.092	0.224	0.509	0.459	1.109	0.802
C.*stygia* - *C. vicina*	0.785	0.381	2.057	0.239	0.154	0.430	0.359	0.997
	Female 42°C				**Male 42°C**			
	Estimate	**SE**	**Z ratio**	**P value**	**Estimate**	**SE**	**Z ratio**	**P value**

Chrysomya spp. - C.*hilli*	0.731	0.146	5.003	**<0.001**	1.270	0.293	4.328	**<0.001**
Chrysomya spp. - *Lucilia* spp.	-0.145	0.125	-1.154	0.778	0.391	0.318	1.228	0.735
Chrysomya spp. - C.*stygia*	0.485	0.148	3.286	**0.009**	1.415	0.302	4.689	**<0.001**
Chrysomya spp. - C. *vicina*	1.235	0.235	5.258	**<0.001**	2.187	0.628	3.483	**0.005**
C.*hilli* - *Lucilia* spp.	-0.875	0.172	5.102	**<0.001**	-0.879	0.332	-2.646	0.062
C.*hilli* - C.*stygia*	-0.246	0.183	-1.342	0.665	0.145	0.331	0.439	0.992
C.*hilli* - C. *vicina*	0.504	0.262	1.923	0.305	0.917	0.648	1.416	0.618
Lucilia spp. - C.*stygia*	0.630	0.160	3.939	**<0.001**	1.024	0.370	2.765	**0.045**
Lucilia spp. - C. *vicina*	1.380	0.226	6.113	**<0.001**	1.796	0.680	2.642	0.063
C.*stygia* - C.*vicina*	0.750	0.252	2.973	**0.025**	0.772	0.596	1.294	0.695
	Female 43°C				**Male 43°C**			
	Estimate	**SE**	**Z ratio**	**P value**	**Estimate**	**SE**	**Z Ratio**	**P Value**
Chrysomya spp. - C.*hilli*	1.593	0.158	10.063	**<0.001**	-0.489	0.588	-0.833	0.921
Chrysomya spp. - *Lucilia* spp.	0.624	0.101	6.148	**<0.001**	0.010	0.309	0.033	1.000
Chrysomya spp. - C.*stygia*	1.410	0.121	11.672	**<0.001**	0.447	0.371	1.207	0.748
Chrysomya spp. - C. *vicina*	2.003	0.164	12.201	**<0.001**	1.209	0.303	3.985	**<0.001**
C.*hilli* - *Lucilia* spp.	-0.970	0.157	-6.191	**<0.001**	0.500	0.664	0.752	0.944
C.*hilli* - C.*stygia*	-0.183	0.167	-1.096	0.809	0.936	0.435	2.155	0.197
C.*hilli* - C.*vicina*	0.410	0.214	1.914	0.310	1.698	0.609	2.789	**0.042**
Lucilia spp. - C.*stygia*	0.787	0.135	5.843	**<0.001**	0.437	0.456	0.957	0.874
Lucilia spp. - C.*vicina*	1.380	0.167	8.248	**<0.001**	1.199	0.330	3.627	**0.003**
C.*stygia* - C.*vicina*	0.593	0.192	3.087	**0.017**	0.762	0.398	1.913	0.310

Overall, males and females showed generally similar knockdown time patterns, with the same species ranks (*Chrysomya* spp./*Lucilia* spp. highest > *C. stygia*/*C. hilli* > *C. vicina*), except that *Chrysomya* spp. and *Lucilia* spp. were only significantly different for females at 43°C ($p<0.0001$) when *Chrysomya* spp. knockdown time was highest.

Examining pair-wise relationships within species in more detail, we found that female *Chrysomya* spp. had significantly higher knockdown times than *C. hilli* at both 42°C ($p < 0.0001$) and 43°C ($p < 0.0001$), than *C. stygia* at 42°C ($p = 0.0090$) and 43°C ($p < 0.0001$), and *C. vicina* at 42°C ($p < 0.0001$) and 43°C ($p < 0.0001$). Female *Chrysomya* spp. also exceeded *Lucilia* spp. in knockdown time ($p < 0.0001$). Meanwhile, female *Lucilia* spp. out-performed most of the other species at most test temperatures, female *C. stygia* had higher knockdown times than female *C. vicina* at 42°C and 43°C, and female *C. vicina* displayed the lowest knockdown times for all comparisons (Table 2.2). Male *Chrysomya* spp. showed similar patterns at 42°C, but only displayed a higher knockdown time than C. *vicina* ($p=0.0006$) males at 43°C . However, *C. hilli* males again showed higher knockdown times than *C. vicina* males at 43°C (Table 2.2).

Females were, on average, larger than males for all species, but these differences were not significant in any of the species.

2.4.3 Species-specific analyses

Individual GLMM models per species (Table 2.3) which revealed that the knockdown time of *Chrysomya* spp. was significantly impacted by sex ($\chi^2_1=6.57$, $p=0.01036$), with males having a higher thermal tolerance and a positive relationship with temperature ($\chi^2_2 = 31.23$, $p < 0.001$), whilst *Lucilia* spp. only showed a marginal effect of sex ($\chi^2_1 = 3.91$, $p = 0.048$), with males more thermally tolerant and showing no significant effect of temperature ($\chi^2_2 = 4.74$, $p = 0.093$). Thorax length had a significant negative effect on knockdown time in *C. stygia* ($\chi^2_1 = 18.62$, $p < 0.001$) and there was a negative trend for temperature on knockdown time which neared significance ($\chi^2_2 = 5.67$, $p = 0.059$). Finally, temperature was the only variable with a significant negative effect on knockdown time for *C. hilli* ($\chi^2_2 = 17.67$, $p < 0.001$) (Table 2.3).

Table 2.3 Results of the analysis of deviance (type III) for the effects of sex (male and female), temperature (41°C,42°C,43°C) and thorax length on knockdown time of individual species (negative binomial generalised linear model). Significant values are indicated in bold.

	χ^2	**df**	**P**
***Chrysomya* spp.**			
Sex	6.572	1	**0.010**
Temperature	31.234	2	**<0.001**
Thorax Length	0.949	1	0.330
***Lucilia* spp.**			
Sex	3.906	1	**0.048**
Temperature	4.744	2	0.093
Thorax Length	0.108	1	0.743
C. stygia			
Sex	1.647	1	0.199
Temperature	5.675	2	0.059
Thorax Length	18.623	1	**<0.001**
C. hilli			
Sex	0.420	1	0.517
Temperature	17.669	2	**<0.001**
Thorax Length	3.268	1	0.071

Overall, males had higher knockdown times in both *Chrysomya* spp. and *Lucilia* spp., whilst there was no significant difference in knockdown time between sexes for *C. hilli* and *C. stygia* (Figure 2.4).

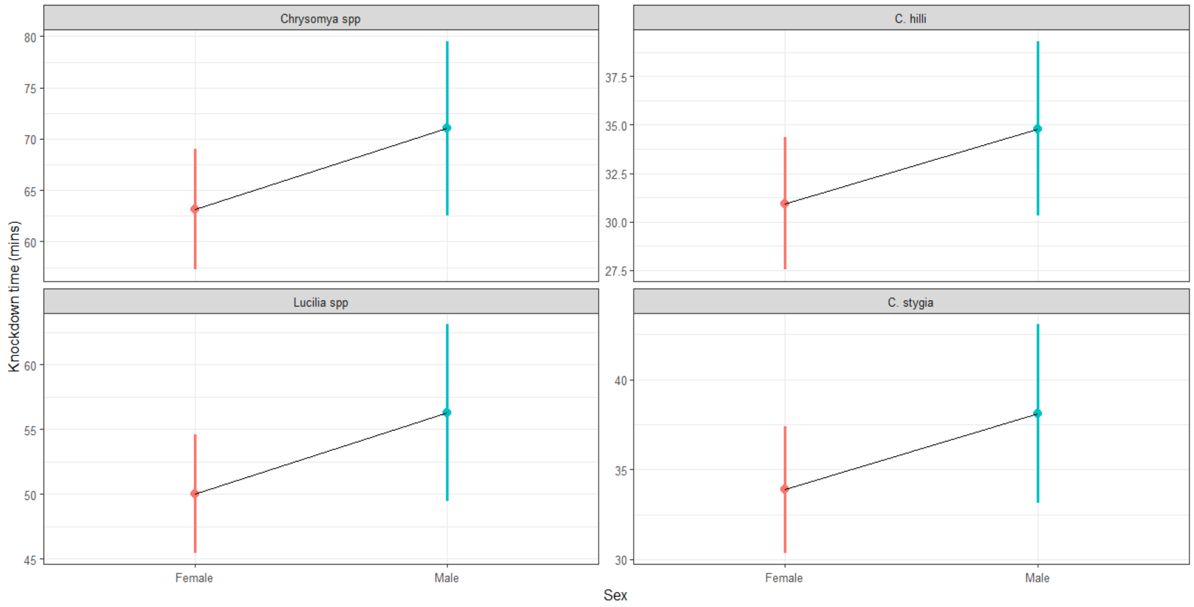

Figure 2.4. Sex differences in knockdown time (mean ± SE) across four blowfly taxa. In Chrysomya spp. *and* Lucilia spp.*, males exhibited significantly longer knockdown times than females, indicating greater heat tolerance. Error bars represent standard errors of the mean.*

2.5 Discussion

We measured heat tolerance in blowflies and found that knockdown time was species-specific, strongly temperature-dependent in females, and comparatively stable across temperatures in males. The study confirmed our hypothesis that invasive species pre-adapted to warm conditions in their native ranges would have a higher thermal tolerance than the other species. Findings were also in line with our second hypothesis that all species would show lower knockdown times at higher temperatures, this being confirmed in four out of the five species investigated, with *Chrysomya* spp. uniquely showing an increase in knockdown time at the highest test temperature. The pronounced species × temperature interaction we identified in females suggests that sex-specific physiological mechanisms may influence upper thermal limits for the studied species.

Our findings are consistent with previous research showing that thermal tolerance varies between blowfly species, affecting their geographic distribution (Pinto et al., 2021), larval development and pupation (Johnson et al., 2022), and adult survival (Okpara & VanLaerhoven, 2023). In our study *Chrysomya* spp. and *Lucilia* spp. had the highest knockdown times, whilst *C. vicina* had the lowest, with these differences between species increasing with increasing temperature. We found no difference in knockdown times between any of the studied species at 41°C, which suggests that the thermal tolerance range of the whole group likely exceeds this temperature, consistent with other blowfly studies of thermal critical and lethal maximum. For example, thermal analysis found that 50% of *Lucilia sericata* died at 41°C whilst 50% mortality was seen in *Phormia regina* at 42°C (Monzon et al., 2022). Meanwhile, Rusch et al. (2020) studied knockdown time and lethality in response to elevated temperatures in *Ch. rufifacies* and found that ~50% of individuals had been knocked down at 43°C.

Our study findings are likely driven by differences in thermal tolerance between species, but other factors that may have affected our results include prior native range adaptation, sex, and body size. The original native ranges of the studied species could have a significant impact on knockdown time. For example, *Chrysomya* spp. originate from Asia (Badenhorst & Villet, 2018), where summer temperatures often exceed 40°C, and the native range of *Lucillia* spp. includes the tropics (Whitworth, 2014). Both species may therefore have intrinsic genetic adaptations, phenotypically plastic mechanisms, such as the use of heat shock proteins and antioxidants, and/or behavioural strategies (Bodlah et al., 2023), such as temperature avoidance, to withstand extreme environmental heat (Bodlah et al., 2017; Diniz et al., 2012). Meanwhile, *C. vicina* had the lowest heat tolerance among the assessed species, which is consistent with its origin in the temperate climates of Europe, where it is active in cooler seasons (Hodecek & Jakubec, 2022; López-García & Martín-Vega, 2024) and shows an upper developmental temperature limit of around 30 °C. (Hodecek & Jakubec, 2022; Limsopatham et al., 2018). Environmental conditions in the native range may also explain the imbalance in species distributions we found between urban and rural populations, with *Lucilia* spp. being predominantly found at the warmer urban sites - consistent with prior research (Langer et al., 2019) and possibly due to urban areas acting as heat sinks (Hwang & Turner, 2009) - whilst the various Calliphora species were mostly found at rural sites.

Differences in body morphology and physiology between sexes can significantly influence heat tolerance and response to thermal stress. For example, females were found to have higher thermal tolerance than males in other blowfly studies (Monzon et al., 2022; Rusch et al., 2025), and this is a common trend among invertebrates because females tend to be larger so more able to withstand higher temperatures for longer periods than smaller individuals (Peralta-Maraver & Rezende 2021), expend more energy in reproduction (Mbande et al., 2023), and often have longer lifespans than males (Pottier et al., 2021). In contrast, we found that males of both *Chrysomya* spp. and *Lucilia* spp. had higher knockdown times than their female conspecifics. Although these findings may have been impacted by the much smaller number of males captured and assayed at 42°C and 43°C (n=49) compared to females (n=479) in our study, finding that males have higher thermal limits than females is not unprecedented (Bedulina et al., 2017; Missionário et al., 2021). In some cases, males have been suggested to have larger home ranges than females - facilitating broader thermal variation (Todd & Nowakowski, 2020); they can also inhabit warmer microhabitats (Logan et al., 2020), have a faster pace of life in order to reproduce at high rates (Pottier et al., 2021), and be smaller, allowing easier heat loss (Missionário et al., 2021) and better tolerance of short-term temperature elevations (Peralta-Maravdr & Rezende, 2021). Further research into sex-related thermal tolerances of the studied species would be valuable to explore these questions further.

As well as species and sex effects, we found that thorax length had a significant effect on knockdown time. On average, females had longer thorax lengths than males in all studied species, but these differences were not significant, suggesting that differences in knockdown time related to thorax length are not due to sexual size dimorphism. However, in our species-specific data, thorax length was only a significant factor in decreasing knockdown time for one species (*C. stygia)*, although it was marginally non-significant in *C. hilli*. Prior research is inconsistent as to the effect of body size on thermal tolerance (e.g., Chanthy et al., 2012; Leiva et al., 2019). Over multiple ectothermic species, thermal tolerance is generally lower in larger ectotherms, though this is strongly impacted by phylogeny (Leiva et al., 2023). In our study, *C. stygia* had the largest average thorax length (6.6 mm) followed by *Chrysomya* spp. (5.8 mm), while *Lucilia* spp. was the smallest (5.4 mm). These findings therefore do not support the argument that average body size is linearly associated with increasing heat tolerance between species.

Whilst *C. vicina* showed no significant difference in knockdown time compared to the other species at 41°C, our results suggest that its thermal critical maximum falls in the 42-43°C range, with its knockdown times being as short as ~6 minutes for some individuals. The overall trend for *C. hilli*, *C. stygia* and *C. vicina* saw knockdown time decreasing with as temperature increased for all three species, potentially consistent with their closer ancestry and more temperate origins (Dear, 1986). In contrast, the average knockdown time of *Lucilia* spp. remained relatively stable across the three temperatures, whilst that of *Chrysomya* spp. increased with increasing temperature, with a large increase in knockdown time of females at 43 °C. While these findings are consistent with the widespread global success of these latter species, and their regular occurrence in warmer climates, the increased knockdown time with increasing temperatures in female *Chrysomya* spp. was unexpected. The prior thermal and life history of the wild-captured flies was unknown in our study and we can only speculate that these may have impacted knockdown times due to variation in fly age, reproductive status, and/or prior thermal exposures. Although we lack specific data for *Chrysomya* spp., thermal tolerance is known to be at its maximum at emergence in several insect species and to then decline over the next several days (Mbande et al.,2023; Neven, 2015). Similarly, reproductive status can affect thermal tolerance in insect, with mated females generally having the highest thermal tolerance (Mbande et al., 2023; Mutamiswa et al., 2022). Finally, prior heat exposure may promote field-based acclimation. Disproportionate assaying of younger, mated, and/or acclimated *Chrysomya* spp. compared to other species in our study may therefore have affected the results. Alternatively, *Chrysomya* spp. may genuinely be the most plastic species in our studied group, with its wide geographic range driving large intraspecific variation of thermal tolerance even in the absence of these other factors.

We found that thermal tolerance varied drastically across the five differentially invasive species with varying global extents, particularly at the highest test temperatures. Despite these differences, all of these species have successfully invaded New Zealand and *C.vicina* in particular, matches the geographic extent of *Chrysomya* spp, suggesting that average environmental temperature does not necessarily constrain invasive potential for this group. While our results suggest that the more invasive *Chrysomya* spp. and *Lucilla* spp. are best placed to cope with the ongoing progressive thermal stresses of climate change,invasive species with differing thermal tolerance may be able to co-exist by exploiting different microhabitats and leveraging phenotypic and behavioural plasticity.

Although we found compelling evidence for variation in knockdown time among species and sex, there are caveats to consider. First, we only studied species in their invasive New Zealand ranges - these populations have likely adapted to New Zealand thermal regimes and seasons and may not reflect ancestral physiology. Obtaining comparative data on native ranges of many of these species would therefore be beneficial. Second, it is not possible in field studies to control for diet, age, prior thermal history, parasite status, and mating status; yet all of these factors can affect knockdown times (Coleman et al., 2015; Mbande et al., 2023; Rusch et al., 2020). Third, our sampling strategy was geared towards the collection of females, because males have less tendency to land on baits and are less frequently caught. Thus, collection of additional males might help to confirm differences in thermal tolerance between sexes seen in the wild population.

Future research could take a genetic focus to explore mechanistic underpinnings to the trait variation data revealed here. Thermal tolerance could also be explored further by exposing flies to sub-lethal heat stress events and assessing thermal tolerance under a range of different acclimation conditions to better represent the likely nature of periodic heat episodes in the wild, and the role of prior thermal history in determining heat responses.

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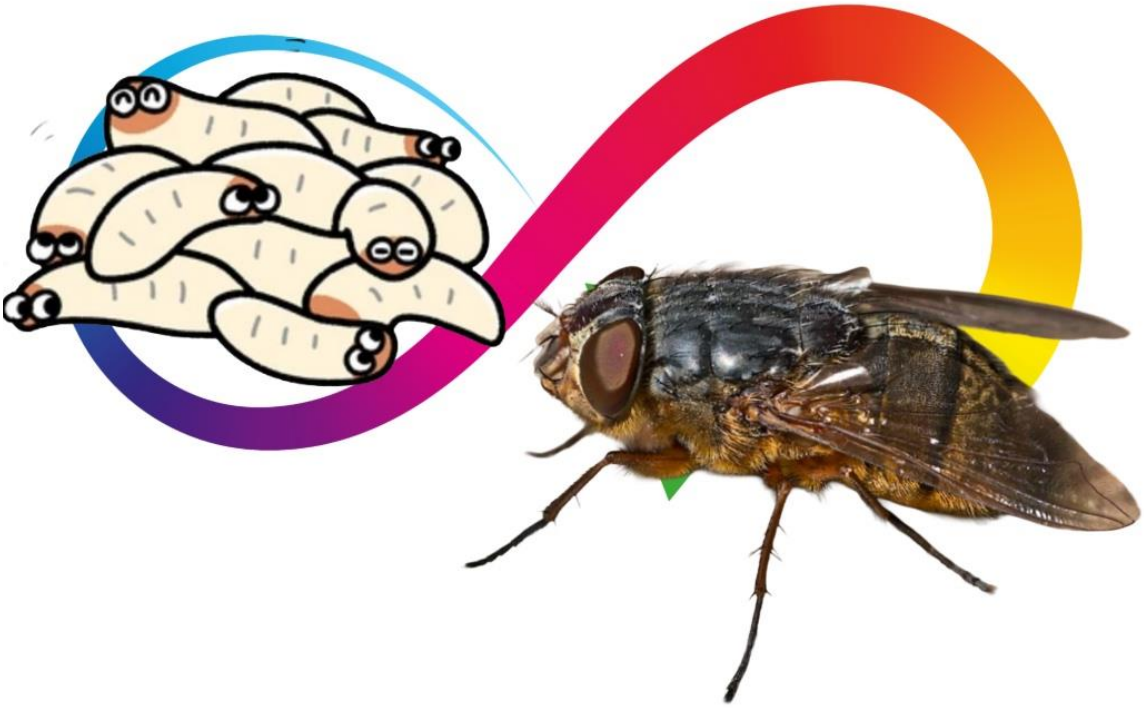
Chapter 3 – Does thermal tolerance differ with developmental temperature?



3.1 Abstract

Climate change and invasive species are two of the biggest challenges facing biodiversity globally. There are several factors that make a species successful at becoming invasive, including their ability to tolerate temperature extremes, thus climate change is likely to have key impacts on invasive species distributions. Developmental temperature plays a key role in the function, survival and physiology particularly of ectothermic species, and is an important component of overall heat tolerance. Yet, limited research has focused on the effects of developmental temperature on adult thermal tolerance.

Here, we examined the effects of developmental heat acclimation on adult heat tolerance using an invasive blowfly, *Calliphora stygia*. We exposed eggs of wild caught blowflies to differential developmental temperatures and the tested thermal knockdown response of adults five days post-emergence using a static temperature assay set at 42°C.

We found that *C. stygia* reared at 18°C had a higher knockdown time than those reared at 22°C or 26°C. In addition, individuals reared at 22°C had the largest average body size (measured as thorax length). Thus, in contrast to our expectations, our overall results indicated limited effects of developmental temperature on adult heat tolerance and a non-typical pattern of sexual size dimorphism. These results add new nuance to our existing knowledge on the impacts of developmental temperature on adult thermal performance, suggesting it may not be a significant driver of invasive potential in some insect species.

3.2 Introduction

Temperature is one of the most biologically important factors that organisms experience (Radchuk et al., 2012; Schou & Cornwallis, 2024). All organisms have an optimal thermal range, in which they are able to survive and successfully reproduce without consequences or trade-offs. Outside of this, species have to adapt or shift to a more tolerable temperature range in order to survive or thrive (Harvey et al., 2020; Woodruff et al., 2024). Thus, upper and lower thermal limits are a major factor influencing habitat choice (Hoffmann et al., 2012). Climate stability also has an important role in an organism's thermal physiology, with narrower thermal ranges seen in species which inhabit environments with minimal seasonal variability (White et al., 2024). Variable temperatures may thus be an important factor impacting an organism's thermal physiology, as they require wider thermal tolerances or supplementary mechanisms to enable appropriate species responses (Clifton & Refsnider, 2022; Madeira et al., 2012).

Higher temperatures are a major outcome of climate change, and thus pose a major threat to global biodiversity and ecosystem stability (Pfenning-Butterworth et al., 2024; Shivanna, 2022). Many species are already being exposed to persistent temperatures outside of their thermoneutral zone (Pottier et al., 2025), with these sometimes exceeding critical thermal limits (e.g., during increasingly frequent extreme heat events) (Russo et al., 2025). Biodiversity loss and sharp declines in ecosystem function are likely to occur due to the combined impacts of environmental warming (García et al., 2018), including alterations in water and nutrient cycling, predation, decomposition, and pollination alongside temperature (Menzel & Feldmeyer, 2021).

Development in differing and fluctuating environments can lead to variable physiological responses in a range of species (Greiser et al., 2022; Kern et al., 2015; Paaijmans et al., 2013). Climate change thus may have important effects on both the adult and developmental responses of organisms to increased ambient temperatures (Uehling et al., 2019). These effects are putting many species at risk of local extinction, but climate change does potentially allow for invasive species with a wider thermal tolerance range to successfully invade new habitats (Kelley, 2014; Titelboim et al., 2019). In particular, persistent species will need to be able to reproduce and survive in changing conditions. However, a species' ability to respond to temperature is not static.

Responding to increasing ambient temperatures can involve a range of mechanisms. Genetic mechanisms can enable species to adapt to climate change via natural selection, but the process is generally slow and limited by standing genetic variation within the population (Hamann et al., 2020; Araya-Donoso et al., 2021; Weiskopf et al., 2020). As a result, many species will not be able to adapt fast enough to keep up with the extreme challenges produced by climate change (Bell & Collins, 2008), with the potential result being local or global extinction (Catullo et al., 2019). Often more effective means of coping with extreme heat episodes and heat stress include the use of behavioural mechanisms (Beever et al., 2017) and phenotypic plasticity (Bonamour et al., 2019; Rodrigues & Beldade, 2020). Behavioural responses can be important in allowing species to move to more tolerable conditions, and are particularly important in species for which environmental temperatures determines the sex of offspring. For example, nesting earlier or at differing sites can overcome potential sex imbalances due to increasing temperature (Refsnider & Janzen, 2012). Phenotypic plasticity describes the ability of an individual to change physical and biochemical characteristics in response to differing environmental conditions, and thermal acclimation is a key example, where exposure to sub-lethal temperatures results in the development of physiological changes, which allow organisms to survive in more extreme temperatures (Lü & Liu, 2017).

Invasive species are established non-native species that are a threat to native biodiversity (Venette & Hutchison, 2021). They can result in ecological, environmental, or economic damage, though classification can be controversial in terms of domesticated animals (Home et al., 2017; Trouwborst et al., 2020). Key traits found across successful invasive species include high dispersal, rapid growth and reproduction, increased phenotypic plasticity, absence of natural predators in the new environment, and high tolerance for a wide range of environmental conditions (Bates et al., 2013). Among these, the ability of a species to live in environments in which the climate is different from that of their native range is a key feature of invasiveness (Sun et al., 2023).

Invasive species often have the ability to out-compete native species due to generally greater physiological tolerance to a wider range of temperatures and better acclimation to warmer temperatures (Kelley, 2014), both of which can enable a broader geographic distribution range (Nagano et al., 2023). Because upper thermal limits are generally narrower among species, whilst thermal minima tend to be broader in range, invasive species are increasingly able to withstand winter conditions where they may not have previously been able to survive due to warming temperatures, while also being able to tolerate hotter summers compared to native species that are not heat-adapted (Finch et al., 2021). Elucidating the thermal and developmental limits of invasive species is thus important for understanding their future impacts on global biodiversity (Day et al., 2021; De Jong et al., 2025; MacLean et al., 2017).

Much of the research to date looks at short-term thermal acclimation of invasive species, and less is known about how developmental temperatures affect and interact with adult heat tolerance (Willot et al., 2021). This is probably in part due to the challenges associated with studying thermal tolerance in wild populations. For example, wild individuals have unknown thermal histories, meaning unknown acclimation effects might drive measured thermal performance (Raby et al., 2025). Similarly, confounding environmental factors, such as resource availability, disease, or chemical exposure might impact thermal performance (Farias et al., 2024; Mitchell et al., 2017; Verberk et al., 2015). Using laboratory-reared species can allow for more accurate assessment of thermal tolerance whilst controlling for unknown confounding variables. However, such experiments may lack ecological realism (Binning et al., 2025).

The temperature at which an organism develops is an important variable that can have persistent effects on its ability to tolerate and adapt to changing environmental temperature, due to profound and lasting effects on physiology and performance (De Jong et al., 2023). These effects are particularly important in ectotherms, with temperature variation during the development period also known to shape such outcomes as sex, size, foraging behaviour, wing size and shape, cognition, and fecundity (Huang et al., 2020; Agyekum et al., 2022; Lavin et al., 2022; Gérard et al., 2022; Soravia et al., 2023). For example, Klepsatel and Gáliková (2021) found that *Drosophila* reared at higher temperatures exhibit lower locomotor activity than those reared at lower temperatures.

Insects that develop at higher temperatures generally have higher thermal tolerance as adults (Kellermann et al., 2017; Slotsbo et al., 2016), as seen in the fall army worm (Mbande et al., 2023) and *Drosophila* (Kellermann et al., 2017). This sets up the expectation that invasive species may have a better developmental heat acclimation response, with increased adult heat tolerance compared to native and less invasive species. Meanwhile, increased developmental temperatures typically reduce body size and can also increase mortality rates (Soravia et al., 2023). This is based on a shortening of the development period in response to higher temperatures (Dillon & Frazier, 2013), whereas lower temperatures allow a prolonged developmental period and larger body size in a process termed the ‘temperature size rule’ (Matsumura et al., 2024).

Because female insects tend to be larger than males, body size dimorphism and sex-specific thermal tolerance under different developmental temperatures may be expected. However, in a meta-analysis of thermal acclimation, Pottier et al. (2021) found that 44 different ectotherm species showed no significant difference between sexes in their thermal limits following acclimation. Despite this, these authors did note that wild-caught females tended to exhibit higher thermal plasticity and tolerance,and some studies have found higher thermal tolerance in females, including Diptera, as a result of developmental temperature exposure (Mbande et al., 2023; Rusch et al., 2020; Xue & Ma, 2020). In contrast, others have shown higher larval thermal tolerance in males versus females (e.g., Mutamiswa et al., 2022). Given these findings, the relationship between developmental temperature, thermal performance, and sex is unclear.

Blowflies are a geographically widespread genus with important roles in decomposition and pollination (Arias-Robledo et al., 2018; Barton & Evans, 2017). As invasive species, blowflies can disperse long distances, adapt rapidly to new environments, and become agricultural pests, causing myiasis (Kotze & James, 2021). In New Zealand, *Calliphora stygia* is an invasive species introduced from Australia, while *Chrysomya* spp. is a more globally cosmopolitan species (Badenhorst & Villet, 2018). As a result, *Chrysomya* spp.

can be expected to have a broader thermal regime across its distribution, while *C. stygia* is more restricted to cooler areas (Johnston et al., 2025). Temperature plays a key role in blowfly development and fitness. For example, maggots typically cluster together in masses to optimise developmental temperatures and rates (Gbenonsi & Higley, 2025; Kotzé et al., 2016), and development rates of larvae correlate directly with temperature (Yan et al., 2024; Zhang et al., 2019). However, limited research has focused on the effects of developmental temperature on thermal tolerance in invasive blowflies.

Here, we investigated how developmental temperature influences adult heat tolerance in *Chrysomya* spp. and *C. stygia*. We predicted that developmental temperature would significantly influence adult knockdown time, so that blowflies reared at higher temperatures would show a better thermal tolerance due to acclimation. Our results provide new insights into how increasing environmental temperatures might affect the development and adult thermal performance of invasive species in future.

3.3 Methods

3.3.1 Sample collection

Bait was prepared by allowing ~500g of frozen beef mince to defrost in a plastic container (1.15L). The container was then placed outdoors in a fenced area free from bird or mammal access for 2-3 days, which allowed the mincemeat to begin to spoil without disruption by vertebrate scavengers. Once the bait was prepared, it was used to attract flies at Ed Hillary Hope Reserve (-37.844215, 175.071322), a rural site in Waikato New Zealand that adjoins native bush and is located close to a freshwater stream.

Sampling took place on a single day during March 2025. Flies were captured using a hand held insect net when they opportunistically landed on the bait. Fly species were identified in the field, with *C. stygia* and *Chrysomya* spp. kept and non-target fly species released. The captured flies were placed into species-specific mesh insect cages (27 L, BugDorm) and kept in the shade whilst to reduce overheating and mortality.

Following transport to the University of Waikato invertebrate facility, fly cages were placed in controlled conditions at 22°C, with a 12h:12h light:dark cycle. Flies were given access to *ad libitum* raw sugar and water and fresh beef mince was provided as a substance to allow for eggs to be laid. Eggs were collected daily and placed into smaller rearing boxes (1.5 L, BugDorm) inside environmental chambers (Nuve TK600) set to 18°C, 22°C, and 26°C, each operating on a 12-hour light:dark cycle. Approximately 100g of mincemeat was provided per insect rearing box to support larval development. Upon adult emergence, flies were moved to the larger insect mesh cages and supplied with fresh mincemeat and *ad libitum* sugar and water and maintained at the larval development temperature for five days prior to thermal tolerance testing.

3.3.2 Thermal Tolerance Assay

The heat tolerance assay was conducted by placing flies (n = ~15-25) individually in 15ml falcon tubes (Greiner) and then placing tubes in an incubator (Ratek, Orbital Mixer Incubator) at 42°C for a maximum of 3 h. Flies walked or sat on the sides of the tubes during the assay period, and knockdown time was recorded as the time it took for each fly to fall to the bottom of the tube and be unable to stand again thereafter.

Following the thermal tolerance assay, all flies were photographed (Canon EOS 600D) under a 20x microscope (Olympus SZH10, Research Stereo). Thorax length was then measured against a reference ruler (30cm) from each photo using ImageJ software (V. 1.54g) (Schneider et al., 2012).

3.3.3 Data Analysis

All data analysis was conducted in R v 4.2.3 (R Core Team, 2025). Analysis focused only on *C. stygia* because *Chrysomya* spp. unfortunately emerged at low numbers overall, with no emergence after rearing at 26°C.

To determine if knockdown time was associated with developmental temperature in *C. stygia*, a GLMM model from the glmmTMB package v. 1.1.9 (Brooks et al., 2017) was used. Developmental temperature, sex, and their interaction were included in the model as fixed effects, with thorax length included as a covariate and assay date included as a random effect. A negative binomial error distribution with a log link function was used to account for overdispersion of data. Model fit was assessed using simulated residuals from the DHARMa package v. 0.4.7 (Hartig, 2016). The significance of predictors was tested using the car v 3.1-2 (Fox et al., 2001) package and a Type III Wald chi test. Finally, estimated marginal means (EMMs) were obtained using emmeans v. 1.10.7 (Lenth, 2017) and the predictions visualised using ggplot2 v 3.5.1 (Wickham et al., 2007).

To determine if body size was associated with developmental temperature, a linear model was fitted, with thorax length as the response variable and developmental temperature, sex, and their interactions included as fixed effects. Model assumptions were assessed using the DHARMa package, significance of the predictors was tested using a Type III ANOVA (glmmTMB), and estimated marginal means were assessed using the emmeans package and visualised using ggplot2.

3.4 Results

3.4.1 Knockdown time

A total of 306 flies were assayed, of which 279 were *C. stygia* (93 at 18°C, 147 at 22°C and 39 at 26°C as shown in Figure 3.1), with 141 females and 138 males. Table 3.1 displays the model output for the effects of temperature, sex, thorax length and the temperature x sex interaction on knockdown time.

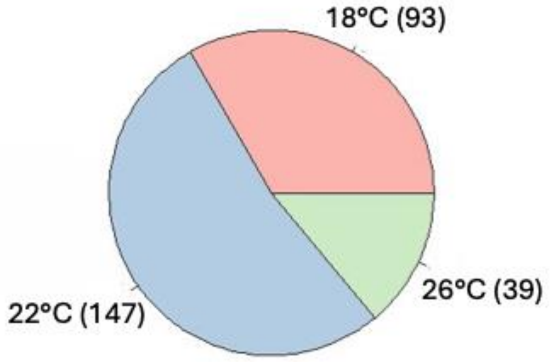


Figure 3.1 Number of Calliphora stygia individuals assayed at each developmental temperature.

Table 3.1 Results of the analysis of variance (type III) negative binomial generalised linear model, the effects of temperature, sex, thorax length and the interaction between temperature and sex on knockdown time. Significant values are indicated in bold.

	X^2	**Df**	**P**
Temperature	5.335	2	0.069
Sex	0.910	1	0.340
Thorax Length	16.909	1	**<0.001**
Temperature*Sex	5.282	2	0.071

Knockdown time displayed a decreasing trend, with increasing temperature (average knockdown time at 18°C = 131 minutes 30 seconds, 22°C = 81 minutes 4 seconds, 26°C =56 minutes 21 seconds), with the trend marginally significant (p=0.069) for both sexes. While sex had no significant effect on knockdown time, the temperature*sex interaction was marginally significant (p=0.071), with a male bias at 18 °C and a female bias at 26 °C (Figure. 3.2). Overall, males had an average knockdown time of 89 minutes 6 seconds, whilst females had an average knockdown time of 99 minutes 37 seconds. Thorax length was significantly related to knockdown time (p<0.001), with larger individuals showing reduced knockdown times (Figure 3.3).

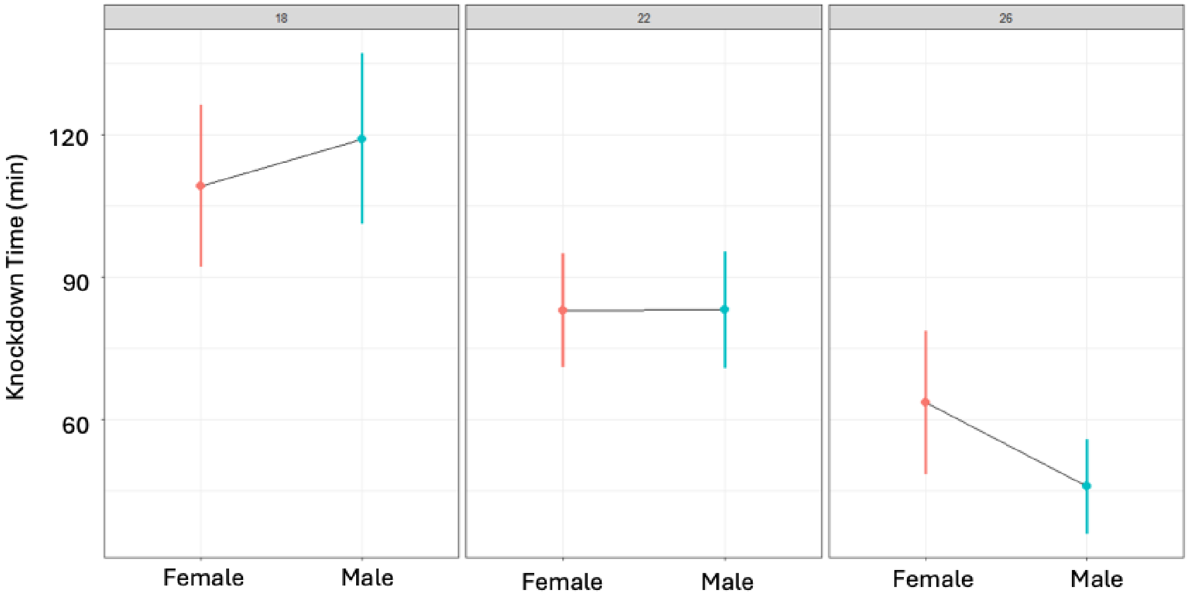


Figure 3.2 Mean knockdown time (mins) in Calliphora stygia at 42°C by sex (female vs. male) across three developmental temperatures (18 ℃, 22 ℃, and 26 ℃). Error bars represent standard errors.

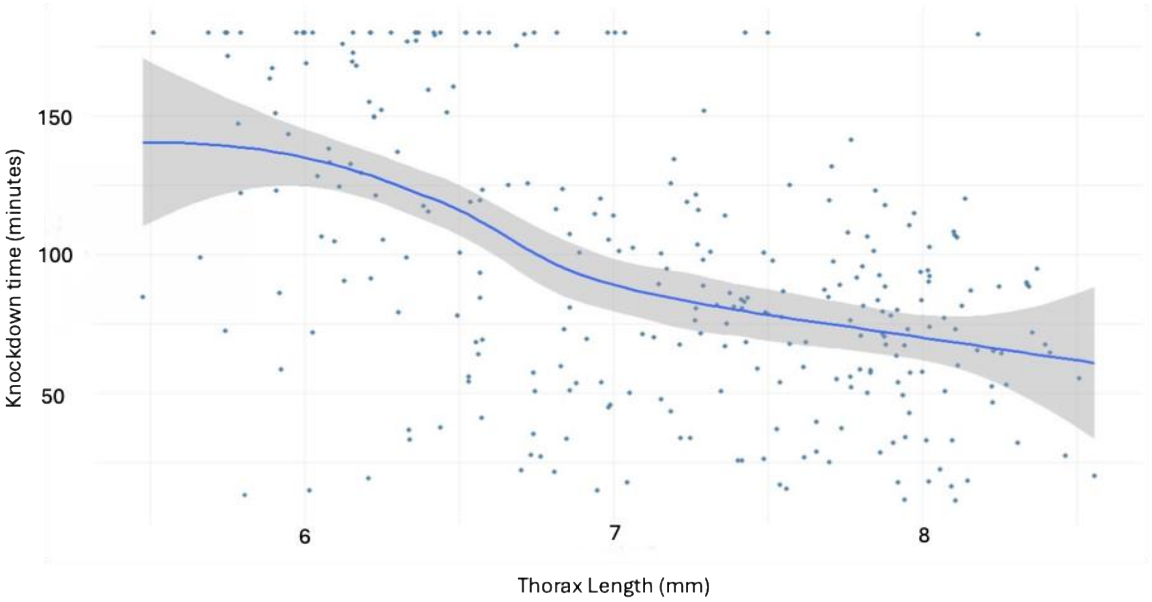


Figure 3.3 Trend of Calliphora stygia knockdown time by thorax length, displaying a decrease in knockdown time with increasing length.

3.4.2 Developmental temperature

Thorax length was significantly affected by developmental temperature ($p<0.001$) and sex ($p=0.019$) with no significant temperature*sex interaction (Figure. 3.3; Table 3.2).

Body size showed a unimodal relationship overall, with largest body sizes at 22°C (7.62mm), smallest body sizes at 18°C (6.42mm), and intermediate values at 26°C (7.02mm). Males were the larger sex across all three developmental temperatures (Figure. 3.4). The average thorax length for the entire male group was 7.62mm whilst females had an average length of 7.02mm.

Table 3.2 Results of the analysis of variance (type III) linear model, the effects of temperature, sex and the interaction on thorax length. Significant values are indicated in bold.

	Sum Sq	DF	F Value	P
Temperature	44.670	2	76.148	**<0.001**
Sex	1.630	1	5.546	**0.019**
Temperature*Sex	0.150	2	0.259	0.772

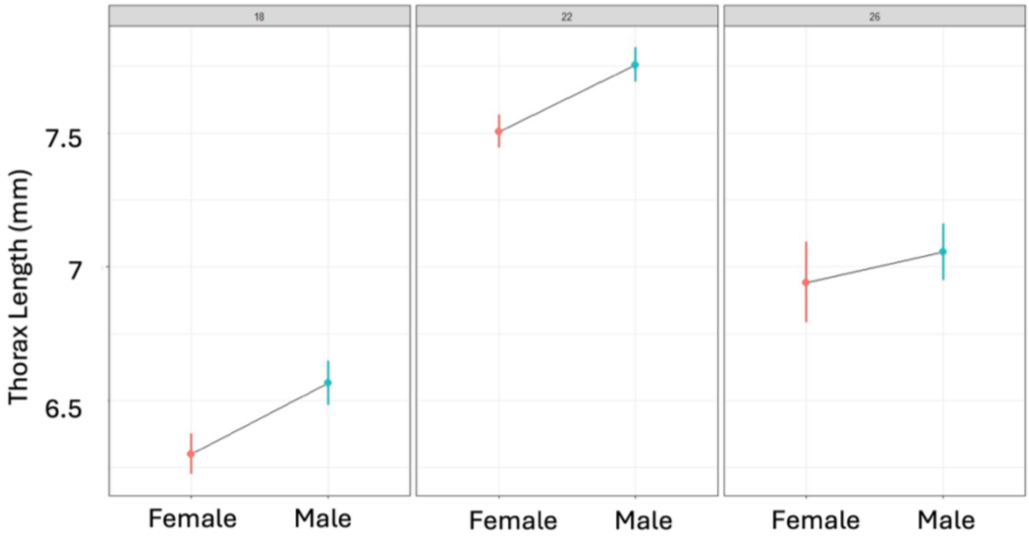


Figure 3.4 Mean thorax length (mm) of Calliphora stygia by sex (female vs. male) across the three developmental temperatures (18 ℃, 22 ℃, and 26 ℃). Error bars represent standard errors.

3.5 Discussion

We assessed the effects of developmental temperature on heat tolerance and found that *C. stygia* reared at lower developmental temperatures (18°C) had higher heat tolerance than those reared at higher temperatures (22°C and 26°C). In other words, and in contrast with expectations, heat tolerance declined with increasing developmental temperature in our assays. We also found that flies reared at intermediate temperatures (22°C) had the largest body sizes (as determined via thorax length measurements), while flies reared at 18°C were smallest - a finding that contrasts with the temperature-size rule often seen in ectotherm development (Forster & Hirst, 2012). Finally, males were the larger sex at all three development temperatures and sex-specific heat tolerance varied with development temperature, with males having better heat tolerance at 18°C and females at 26°C; this result contrasts with expected patterns of sexual size dimorphism commonly seen in insects. Thus, our findings provide surprising suggestions that developmental heat acclimation does not have strong effects on adult heat tolerance in *C. stygia* and, as such, may not be a significant driver of invasive potential in some insect species.

3.5.1 Knockdown decreased with increased developmental temperature

In our assays, *C. stygia* reared at 18°C showed a significantly longer knockdown times (mean ~131 mins) than those reared at 22°C and 26°C (mean of ~81 and ~56 mins, respectively), suggesting that a lower developmental temperature may improve adult heat tolerance. This contrasts with most previous research (Mueller et al., 2019; Podlesnik, 2025), which suggests that individuals reared at higher temperatures have improved heat tolerance in both marine (Ashlock et al., 2024) and terrestrial (Jerbi-Elayed et al., 2021; Willot et al., 2021) ectotherms. For example, warmer developmental temperatures have been shown to improve heat stress responses in adults of *Drosophila melanogaster*, with the effect thought to persist through adulthood but decline with increasing age (Kellermann et al., 2017; Slotsbo et al., 2016).

Most research to date has focused on larval and pupal thermal tolerance, development and survival, without assessing the strength and persistence of these effects into adulthood. However, developmental acclimation to heat has been shown to increase adult thermal tolerance in *Drosophila* - although this effect was only weak initially, the impact of developmental heat acclimation strengthened with the duration of heat exposure, suggesting that developmental temperatures can have additive or interactive effects with adult phenotypic plasticity (Willot et al., 2021). Meanwhile, Pottier et al.

(2022) found that ectotherms reared at higher temperatures showed only weak improvements in their heat tolerance. Willot et al. (2021) noted in their study of developmental acclimation in *D. melanogaster* over five generations that developmental acclimation had positive but weak effects on adult heat tolerance that increased with the duration of heat exposure. Similarly, Jerbi-Elayed et al. (2021) reared the aphid parasitoid (*Aphidus colemani*) for a single generation and found that developmental acclimation only produced weak improvements in adult thermal tolerance. Collectively, these findings suggest that the effects of development temperature on thermal performance of adults may not always form a positive linear relationship.

Differences among studies will undoubtedly be in some parts due to the way in which thermal performance is being measured. For example, Jerbi-Elayed et al. (2021) found that the developmental temperature that maximised adult heat tolerance varied with the assay type, with heat stupor (i.e., knockdown) thresholds being highest for individuals reared at 20°C, but heat coma and death thresholds highest for those reared at 28°C. The persistence of developmental acclimation to adult heat tolerance also varies between studies. For example, Ashlock et al. (2024) found that developmental heat acclimation improved copepod adult heat tolerance, with no improvement in tolerance over multiple generations beyond the initial plastic response. In a study of fly cold tolerance, developmental acclimation effects were greatest in newly hatched flies, with cold tolerance then declining over the next three days to a stable lower degree (Colinet et al., 2012). In our study, use of a static heat stress temperature well above the tested developmental temperatures may not have allowed sufficient time for the protective effects of developmental acclimation to be realised, with this reflected in a reduced ability to use plastic responses, such as heat hardening, in the flies raised at higher temperatures. Heat hardening involves a rapid process of acclimation where mild heat stress triggers protective physiological and biochemical responses such as heat shock protein production to significantly improve heat tolerance (Gu et al., 2019). Future research should therefore explore the effects of development temperature on heat tolerance in *C. stygia* using a range of different heat stress assays.

Adult heat tolerance is ultimately the result of interactions between developmental acclimation, evolutionary adaption, and adult phenotypic plasticity (Beaman et al., 2016). As a result, there is high potential for trade-offs between thermal tolerance, plasticity, and upper thermal limits (Ashlock et al., 2024). For example, Van Heerwaarden et al. (2024) found that species with higher heat tolerance have reduced heat hardening ability at higher developmental temperatures. Barley et al. (2021) also found in their review of thermal tolerance in ectotherms that individuals with higher innate heat tolerance had less acclimation ability. Consistent with this, Willot et al. (2021) found that knockdown recovery time was fastest for *D. melanogaster* reared at lower temperatures despite those individuals having lower heat thermal thresholds. In our study, higher knockdown times following development at 18°C may reflect a trade-off between developmental temperature and subsequent heat hardening as a phenotypic plastic response to heat stress. Thus, flies reared at 18°C may have been able to allocate greater resources to phenotypic plasticity than those reared at higher temperatures.

3.5.2 Developmental temperature had varied effects on body size

We found that individuals reared at 22°C had larger body sizes than those reared at 18°C and 26°C. Decreased body size at higher temperatures is very well-recognised in ectotherms and is believed to be as a result of increased metabolism and a faster developmental rate i.e., the temperature-size rule (Matsumura et al., 2024). This rule has proved accurate in multiple species, including *D. melanogaster* (Ghosh et al., 2013) and multiple beetle species (Tseng et al., 2018) and holds for over 80% of ectothermic species (Verberk et al., 2020), including blowfly species (Zhang et al., 2019). However, some insect species do not seem to have their size significantly impacted by developmental temperature (Daňková et al., 2023). For example, Voss et al. (2014) examined the effects of development temperatures between 12°C -30°C on *Calliphora varifrons* and found that peak body sizes occurred in flies reared at 18°C, with a subsequent decline in body size at higher temperatures. In our study, 18°C may have similarly been at or below the minimum temperature threshold for optimal insect development for *C. stygia*. Though we observed that most larvae hatched at rearing temperatures of 22°C, and a decline in hatching rate was noted at 26°C, we currently lack data on lower thermal limits for this species.

Another explanation for our findings may be that our body size measurements in *C. stygia* may not have adequately captured any developmental temperature effects. For example, in contrast to thorax length, developmental temperature in hoverflies (*Eristalis tenax*) was shown to be significantly associated with changes in wing length and the non-size related trait, adult lifespan (Daňková et al., 2023). Diet is also an important determinant of development time and body size in insects (Pullock et al., 2023). In particular, larvae developing at higher temperatures are generally more active (Zamudio et al., 1995), potentially resulting in increased competition for resources and a consequently slower development time (Gbenonsi & Higley, 2025), which may impact adult insect size. Similarly, decreased body size at cooler developmental temperatures in our study may have resulted from slower developmental rates at sub-optimal temperatures, while larger body sizes at 22°C may be reflective of optimal resource allocation and physiological growth.

3.5.3 Sexual dimorphism in body size and knockdown time

While some insect species have males that are typically larger than their female conspecifics, females generally tend to be the larger sex due to the reproductive advantages conferred via the ability to lay more eggs (Blanckenhorn, 2000; Del Castillo et al., 2023; Matsumura et al., 2024). However, sexual size dimorphism tends to generally decrease with increasing temperature and there is evidence that it may vary within a species in different populations (Matsumura et al., 2024). For example, Puniamoorthy et al. (2012) found that dung fly (*Sepsis punctum*) body size was male-biased in Europe, but female-biased in North America, due to stronger sexual selection pressures favouring larger males in the European population. In this study, males were consistently larger than females across all three developmental temperatures. In other studies on Calliphorid blowflies, body size has been shown to decrease as density increases, with males and females responding differently to increasing densities (Macedo et al., 2018). Thus, *C. stygia* may exhibit sexual dimorphism related to density and associated competition for resources or mates, alongside other sexual selection pressures. Though our result was generally unexpected, it may suggest that sexual size dimorphism in blowflies is variable and influenced by temperature growth regulation - a worthy area for future exploration.

Previous insect studies have generally shown an absence of sexual dimorphism in thermal tolerance, or that females have higher thermal tolerance than males (Stillwell et al., 2009; Weaving et al., 2023; Xue & Ma, 2020; Pottier et al., 2021). Here, we found that sex and developmental temperature showed a significant interactive effect on adult heat tolerance, with males exhibiting higher heat tolerance after rearing at 18°C and females exhibiting higher tolerance after rearing at 26°C. Overall, our results suggest that thermal sensitivity may be sex-specific and plastic across developmental temperatures in *C. stygia*, which may have important ecological implications. In particular, differing thermal tolerance in the wild could affect population sex ratios, as well as reproduction and fitness in the context of climate change (Santos et al., 2023).

3.5.4 Conclusions

Future research should explore the effects of developmental temperature on thermal tolerance using different thermal exposure assays, more species, and higher numbers of replicates to more fully determine the strength and persistence of the effects observed here. Testing offspring of wild-caught flies at differing times of the year may also be beneficial to help determine whether adult thermal history has an impact on the thermal performance under different developmental temperatures in the offspring - including a focus on both larvae and adults of different ages. Finally, our experiments did not explicitly control for larval density, which will be an important factor to investigate in future research.

3.6 References

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Chapter 4 - Thesis discussion

4.1 Research summary

This thesis examined thermal tolerance of invasive blowfly species, using static temperature assays to assess thermal knockdown times. Chapter 2 investigated whether invasive blowfly species differ in their thermal tolerance by comparing knockdown times among species at various assay temperatures. Chapter 3 considered how developmental temperature affects adult heat tolerance within a single species raised under different developmental temperatures.

The research conducted in Chapter 2 showed that invasive blowflies exhibit differing thermal tolerance, with the Calliphora species (*Calliphora stygia*, *Calliphora hilli*, and *Calliphora vicina*) being more sensitive than the globally more invasive *Lucilia* spp. and *Chrysomya* spp.. *Chrysomya* spp. had the greatest knockdown time at increased temperature, and males had higher heat tolerance across the two more globally invasive *Chrysomya* spp. and *Lucilia* spp.. These results suggest that globally more invasive species have higher thermal tolerance than those with a more restricted range. They are therefore perhaps more likely to cope with the effects of climate change, at least in the short term.

Chapter 3 showed that *C. stygia* reared at 18°C had the highest thermal tolerance, whilst those reared at 22°C exhibited the largest thorax length. This research adds to previous knowledge suggesting that thermal tolerance may be multifactorial, with developmental and acclimation temperatures among the many variables that should be considered when assessing thermal tolerance.

Overall, this research provides insight into differential heat tolerance of invasive species and provides a foundation for future work that evaluates the role of thermal tolerance in invasion success (Bellard et al., 2013; Finch et al., 2021) - particularly in the context of projected increases in temperature projected under climate change scenarios (Harvey et al., 2022; Madeira et al., 2012).

4.2 Broader implications

The thermal physiology of organisms is increasingly important in a world of global warming and environmental change, and has been a focus of much recent ecological research (Andreassen et al., 2025; Fernandes et al., 2023; Pottier et al., 2025).

Findings in Chapter 2 were consistent with the hypothesis that blowfly species that are more globally invasive would exhibit a greater heat tolerance than invasive species with a narrower global range. However, the finding that males had a higher thermal tolerance than females was unexpected, as several prior studies have suggested that females tend to have higher heat tolerance (Mbande et al., 2023).

Overall, my Chapter 2 findings highlight that thermal tolerance is an important factor underlying a species' ability to exploit ecological niches and suggests that interspecific variation in thermal tolerance may play an important part in determining the spread and impact of invasive species. This information adds to existing knowledge about how invasive blowflies may tolerate increased temperatures in future and may assist in determining which areas are at risk of blowfly invasion or increased local abundances.

The findings in Chapter 3 differed from expectations, given that prior research indicates that body size generally decreases with increasing heat, as per the temperature size rule (Ghosh et al., 2013). Meanwhile, individuals reared at higher temperatures are expected to have higher heat tolerance (Podlesnik, 2025), but I found the opposite pattern in my experiments.

The role of developmental acclimation in adult heat tolerance has been a focus of much ecological research, with study findings disagreeing on how developmental temperature affects the strength and persistence heat tolerance in response to environmental temperatures (Cooper et al., 2012; Haskett et al., 2024; Pottier et al., 2022). My research findings support a weak effect of development acclimation on adult heat tolerance, and suggest that phenotypic plasticity, prior thermal history, and other physiological factors are ultimately important in collectively determining adult heat tolerance.

4.3 Caveats

My Chapter 2 experiments were constrained by the seasonal availability of blowflies, with species capture limited to the times when various species were available in the wild (and this differed temporally). This prevented certain comparisons of wild-caught species to be assessed within the same time period, though temporal variation was controlled for in the statistical models. The opportunistic sampling strategy I used also encompassed relatively geographically separated sampling sites, with collection variation at any particular site further limiting spatial replication (but also accounted for in models).

The native New Zealand species *Calliphora quadrimaculata* was not found in high enough numbers at the collection sites to be included in the study. Therefore, no comparison was able to be made between invasive and native species to determine whether the former are able to exhibit significantly more heat tolerance than the latter.

As well as stochasticity in sampling rates across species, time, and space, the use of wild-caught individuals in Chapter 2 meant there was no way to control for the prior thermal exposure (or capacity for heat hardening) of tested individuals. Indeed, the temperature at which wild-caught flies emerging from pupation were exposed was also unknown, while the potential optimal egg-laying temperature in the wild is unknown. Although rearing temperatures can be controlled for in laboratory experiments, such as applied in Chapter 3, blowflies in the wild are able to use behavioural strategies to select optimal environments and/or avoid challenging ones (Aubernon et al., 2018; Gomes et al., 2018; Rusch et al., 2025).

In Chapter 3, the density of individuals at each rearing temperature was unfortunately not controlled for. Density has been reported to impact development, size, and stress responses in insects (Than et al., 2020) and also vertebrate ectotherms, such as salamanders (Charbonnier et al., 2018). Meanwhile, larval density is known to affect larval survival, growth, and development time, as well as adult size and reproduction in multiple insect species. For example, Evans et al. (2022) found that increasing density of *Aedes* spp. mosquito larvae at field sites was associated with decreased larval survival to adulthood, though environmental factors such as season and location also impacted larval survival. Diamantidis et al. (2019) similarly found decreased survival to adulthood with increased larval density in their study of the Mediterranean fruit fly (*Ceratitis capitata*), along with increased development time, and reduced larval and adult body mass and reproductive success. Finally, Reyes-Ramirez et al. (2023) noted that increasing larval density of invasive *Drosophila suzukii* reduced their development time, but at the cost of smaller and less fertile females being produced. These density related effects may have thus affected our study results.

In both research chapters, the assay design was useful for determining knockdown time, but the set-up is unlikely to have mirrored realistic environmental conditions beyond heatwaves. In particular, blowflies were exposed to temperatures near the presumed critical thermal maxima of the tested species, with temperatures above 40°C only occasionally experienced at the study collection sites. The world is already under considerable thermal stress, with hundreds of species having gone locally extinct with increasing global average surface temperatures since preindustrial levels (IPCC, 2023). Particularly among insects, elevated temperatures generally lead to smaller organisms that develop more rapidly and experience a myriad of negative effects (Harvey et al., 2022). Prolonged future elevated temperatures may produce significant thermal stress on organisms even in the absence of extreme heatwaves (Terblanche et al., 2007), and dehydration is known to worsen an organism's thermal tolerance (Youngblood et al., 2025). These factors mean that climate change is likely to continue to worsen thermal stress of invasive and native species.

On the other hand, climate change may allow species to invade new habitats as they warm (Harvey et al., 2022), or are no longer as cold in winter (Hesselschwerdt & Wantzen, 2018). Though invasive species often have better heat acclimation responses to improve thermal tolerance than native species (Kelley, 2014), as well as increased tolerance to dehydration and heat (Da Silva et al., 2021), climate change will ultimately likely increase temperatures beyond the upper thermal limits of many some invasive insects (Harvey et al., 2020), but provide new opportunities for others (Finch et al., 2021).

4.4 Future research / Next steps

My sample collection data showed variations in species prevalence across sampling locations and times, but due to the study design, was unable to determine whether this was due to the presence of a gradient in species distribution related to thermal tolerances and proximity to urban heat island effects. Thus, future research could use a more strictly regimented sampling strategy, collecting flies at regular intervals to better detect changes in thermal heat tolerance over time from a higher number of sites (to increase replication of urban versus rural sampling). Additional sampling by citizen scientists could be used to collect higher numbers of blowflies from different thermal environments over many months and a wide geographic area.

A second area for further investigation is to examine the role of genetic factors in thermal tolerance patterns. In particular, examining whether there are genetic differences at specific genes between the different species might provide mechanistic explanations for detected differences in thermal tolerance.

For extending Chapter 3, examining the effects of fluctuating temperature during acclimation or development is an important area for further study. In particular, use of a ramping temperature design in a laboratory setting would provide a complementary measure of how a species' thermal tolerance changes under different thermal scenarios, providing additional context for how this might impact invasive potential. Alternatively, flies could be exposed to large fluctuating temperatures that would better assess thermal tolerance in more realistic environmental conditions.

To further understand differences in thermal tolerance between wild-caught and laboratory-reared individuals, future studies could use field-based measurement devices to measure thermal tolerance directly in the field. These results could then be compared to those for field-collected individuals with short-term versus multi-generational laboratory acclimation, which would also allow comparison between natural and experimental conditions. The discovery of a strong correlation between field and laboratory findings would provide greater ecological realism to laboratory assays. Moreover, because prior research has reached differing conclusions as to the strength and persistence of developmental heat acclimation on adult heat tolerance, further research that determines the impact of developmental heat acclimation across different life stages would be beneficial.

Finally, the relationship between thermal tolerance and other factors should be explored to comprehensively examine species' invasive potential. In particular, the relationship between thermal tolerance and factors like fecundity and inter-specific competition across generations would allow assessment of how fitness-based phenotypic traits and behavioural plasticity together impact thermal tolerance. Though many species have increased mortality or are unable to effectively reproduce at high thermal extremes, being unable to adapt to low winter temperatures may also determine invasive success. Thus, an additional key phenotype to investigate is cold tolerance, with characterisation of lower thermal limits expanding our knowledge of the environments in which invasive species may be able to survive over winter.

Whilst thermal tolerance was the focus of this research, multiple other factors may contribute to the success of species' invasion into new habitats. These include prior geographic distribution (Wang et al., 2024), propagule size (Lonhart, 2008), reproductive output (Kelehear & Shine, 2020), vigour in the face of inter-specific competition (with both native species and other invasives) (Rivera-Estay et al., 2024), ability to adapt to new habitats (including fragmented and urban environments; Lozon & MacIsaac, 1997) and the degree of underlying behavioural and phenotypic plasticity present (Atallah et al., 2014; Pyper et al., 2024). Many of these factors may interact with and be influenced by the thermal tolerance of an invasive species, and ongoing research into their combined effects will be important for advancing our understanding of how invasive species successfully establish and spread in new environments. This information, particularly as it relates to thermal tolerance, will be key in formulating strategies to deal with spread of invasive species.

4.5 Conclusion

In my research, thermal tolerance varied between species and with developmental temperature in a mix of expected and unexpected ways. Understanding the relationship between a warming world and the ability of various species to adapt to higher temperatures is important for predicting and managing future biological invasions around the globe. Increasing understanding of invasiveness, and adaptation to climate change more generally, is important for supporting the survival of native and endangered species and determining how they may be affected by invasive species. As a changing climate encourages those species with ‘superior’ traits to adapt and thrive, there will be environmental, economic, and societal consequences as the current ecological balance is challenged. My work adds to the valuable and important body of knowledge around how climate change is impacting invasive species and their ability to tolerate and adapt to increasing temperatures. It also provides information which may assist in the mitigation of negative impacts of blowflies as they currently exist in the New Zealand environment.

4.6 References

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Appendix

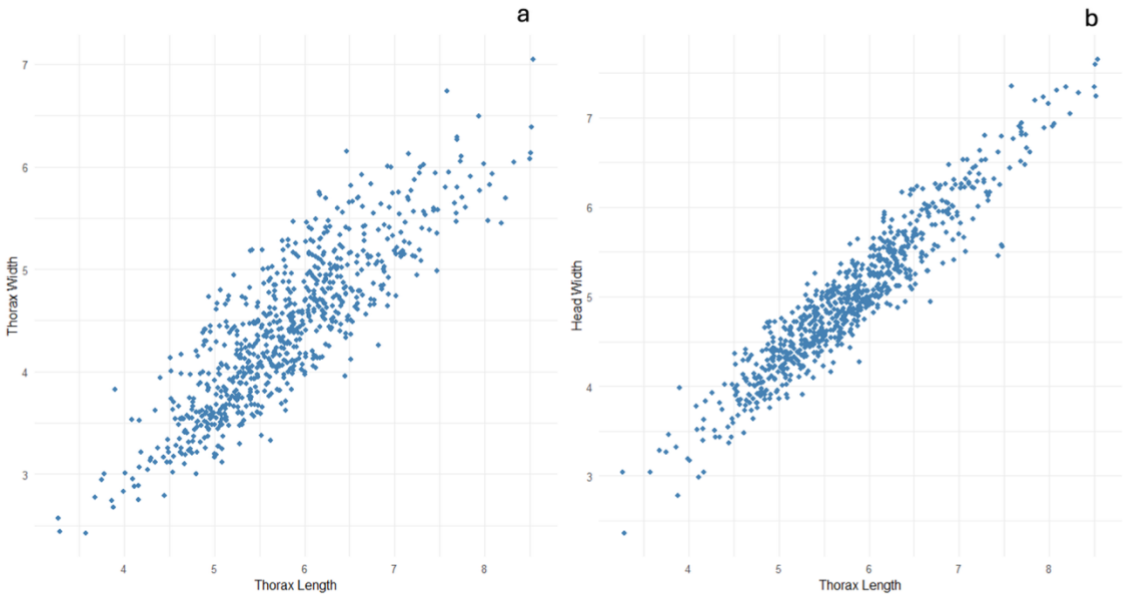

Figure S2.1. a) Relationship between thorax width and thorax length in blowflies. Each point represents an individual, displaying the overall positive correlation between body size traits (0.85). b) Relationship between thorax length and head width in blowflies. Points show individual measurements, with the correlation between these morphological traits displaying a high correlation (0.94).

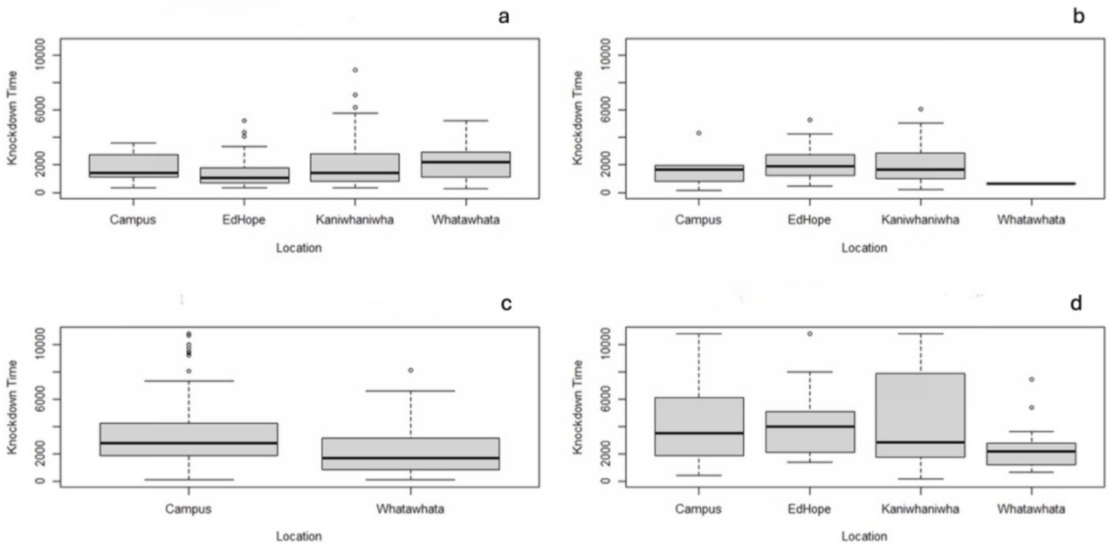

Figure S2.2. Boxplot of knockdown time for each species based on collection site: a) Calliphora stygia, b) Calliphora hilli, c) Lucilia spp., d) Chrysomya spp..

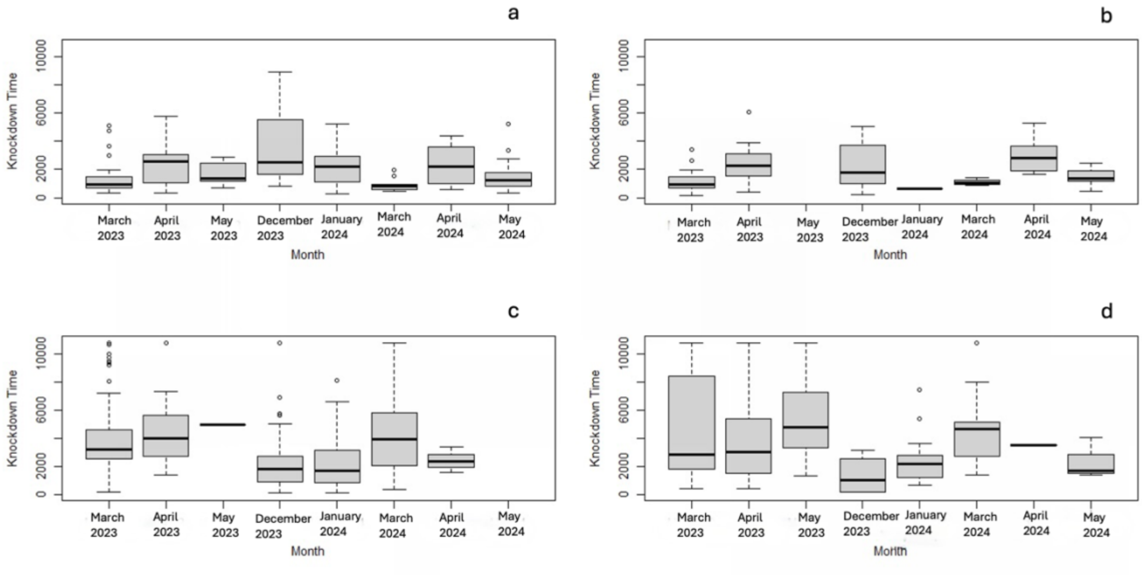

Figure S2.3. Boxplot of knockdown time for each species based on collection time a) Calliphora stygia, b) Calliphora hilli, c) Lucilia spp., d) Chrysomya spp..

Table S2.1. Total counts of each species collected at the study locations and at each time of capture, and then assayed at each experimental temperature.

		C. *stygia*	**C. *hilli***	**C. *vicina***	***Lucilia spp.***	***Chrysomya spp.***	**Total**
Location							
Ed Hillary Hope Reserve		45	38	1	0	12	96
Kaniwhaniwha		55	44	3	0	118	220
Whatawhata		13	1	7	30	13	64
Campus		17	8	26	205	123	379
Month/Year							
January	2024	13	1	7	30	13	64
March	2023	31	16	4	76	72	260
	2024	13	3	7	29	9	
April	2023	19	26	0	23	128	245
	2024	7	18	1	22	1	
May	2023	8	0	7	1	34	96
	2024	26	17	0	0	3	
December	2023	13	10	11	54	6	94
Assay temperature							
41°C		48	36	10	64	73	231
42°C		46	38	13	71	118	286
43°C		36	17	14	100	75	242
Total number of each species		**130**	**91**	**37**	**235**	**266**	**759**