Effects of temperature on the parasitoid *Eretmocerus warrae*

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ABSTRACT

Eretmocerus warrae (Hymenoptera: Aphelinidae) is a parasitoid of the greenhouse whitefly，*Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). It is suspected to be a better biological control agent at high temperatures than *Encarsia formosa* (Hymenoptera: Aphelinidae), a wasp which is widely sold for control of *T. vaporariorum*. Experiments were conducted to investigate how temperature affects the development and behaviour of *E. warrae*. It attained the highest estimated developmental rate at 31.5 °C and the optimum temperature for oviposition was 30.5 °C. Developmental times of *E. warrae* at fluctuating temperatures that simulate night-day patterns were similar to those predicted based on constant temperatures. Above the optimum temperature, *E. warrae* tolerated higher temperatures than *En. formosa* during development and as adults. Using a ramping temperature approach, the critical thermal maxima of *E. warrae* was 42.7 ± 0.5 °C, 0.6 °C higher than that of *En. formosa*. Thus, *E. warrae* is better adapted to high temperatures than *En. formosa*, and it could be a complementary or superior biological control agent during summer months in hot regions.

The fitness of parasitoids are substantially been influenced by temperature. Although the body-size of both this parasitoid and its host were influenced by temperature, *E. warrae* gained relatively more fitness in body-size at 20 °C. The predicted body volume index increment of body size from 32 to 20 °C of the parasitoids was 274.1 %, compared with its host at 91.1 %. The life-time oviposition of female adult of *E. warrae* that grew at immature developmental temperature of 20 °C was 86 ± 22, more than that of 26 and 32 °C at 66 ± 11 and 65 ± 23. Besides the influence on fecundity, temperature also influences the oviposition behaviour at the adult stage, more eggs were oviposited at 20 and 26 °C than those parasitoids at 32 °C. The relationship of life-time oviposition with immature and adult temperature was modelled, both immature and adult temperature play a role on life-time oviposition. Temperature negatively affected longevity in both adult stage and immature developmental stages. An adult female could live 8.9 ± 1.8 days at 20 °C, compared with 5.7 ± 1.7 days at 32 °C. Moreover, *E. warrae* conducts a quicker oviposition at 26 °C than at 20 °C. More eggs were laid in the first two days at 26 °C, while after two days, females oviposited more eggs at 20 °C. The body-size has a positive effect on both the life-time oviposition and longevity.

Experiments were conducted to investigate the effects of (1) host stage on the behaviour and development of parasitoids, and (2) the relative body-size of parasitoids on the host stage choice. In both no choice and choice tests, the parasitoid *E. warrae* preferred the second instar of *T. vaporariorum* for parasitism. The behavioural patterns of the parasitoid were observed when handling different instars of hosts. A significantly larger proportion of behaviour was devoted to stinging (40.7 %) when the second instar hosts were provided to parasitoids. The acceptance (84.1 ± 8.9 %) and emergence rates (83.2 ± 7.8 %) from second instar hosts were also the highest, which indicated that second instars are the preferred stage to attack.

The body size of *E. warrae* is affected by temperature during immature development. Wasps that develop at 20 ^{o}C are larger than those that develop at 32 ^{o}C. The relative body-size of the parasitoid compared to its host was shown to affect the host stage choice of the parasitoids. Smaller wasps preferred younger host instars while third instar hosts appeared to be too large to be parasitised by them. Larger wasps preferred older instars, while the relatively small first instar hosts were not attacked by larger wasps. However, both small and large wasps preferred second instar hosts. The behavioural patterns of small and large wasps handling second instar hosts were compared. Smaller wasps spent longer periods on antennating hosts than larger ones. Despite displaying similar proportions of the sting behaviour, the frequencies of stinging behaviour of larger parasitoids (35.4 ± 4.1 / h) were

much higher than those of smaller ones (17.4 ± 6.0 / h), which reflected the greater fecundity and oviposition activity of larger wasps. In addition, experience was shown to affect the efficiency stinging behaviour of *E. warrae*.

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PREFACE

This thesis includes three manuscripts that are written for publication in refereed journals.

Journal papers

Tao Wang, Katja Hogendoorn, Michael A. Keller. The effects of temperature on the development, fecundity and mortality of *Eretmocerus warrae*: Is *E. warrae* better adapted to high temperatures than *Encarsia formosa*? Submitted to: Biological Control

Tao Wang, Michael A. Keller. The effects of temperature on body-size, longevity and life-time oviposition of *Eretmocerus warrae*. For submit to: Bulletin of Entomological Research

Tao Wang, Michael A. Keller. Effects of relative body-size on the host stage choices of parasitoids: Developmental temperature affects interactions between *Eremocerus warrae* and its host. For submit to: Journal of Pest Science

Conferences papers based on the research presented in this thesis

28 September – 1 October, 2014, 45th Annual conference of the Australian Entomological Society in Canberra, <u>*oral presentation*</u> 'The effects of temperature on the development and mortality of *Eretmocerus warrae* (Nauman & Schmidt)'.

27 – 30 November, 2016, 47th Annual conference of the Australian Entomological Society in Melbourne, <u>*oral presentation*</u> 'Effects of relative body-size on the host stage choices of parasitoids: Developmental temperature affects interactions between *Eremocerus warrae* and its host'.

Note that references are included within each chapters.

CHAPTER ONE

Introduction and background

1. Introduction

This research was directed towards understanding the effects of temperature, especially extreme high temperature, on the biology of a parasitic wasp, *Eretmocerus warrae* (Nauman & Schmidt) (Hymenoptera: Aphelinidae). This species is a parasitoid that attacks the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae). This wasp has recently been developed as a commercial biological control agent by Biological Services (Loxton, South Australia) following its discovery in a commercial greenhouse in South Australia during peak summer temperatures (Biological Services, 2015). It is suspected to be a better biological control agent at hot temperatures than another wasp, *Encarsia formosa* (Hymenoptera: Aphelinidae), which is widely sold for control of *T. vaporariorum* (Hoddle and van Driesche, 1998). Therefore, series of studies were conducted to investigate how temperature affects the development and behaviour of *E. warrae.* Special attention was given to the effects of stressful high temperatures on survival and activity, and to an elucidation of oviposition behaviour.

First, I studied the effects of temperature on the developmental rate and reproduction of *E. warrae*. Its development and reproduction were studied across a range of constant temperatures. An experiment with fluctuating temperature was also undertaken to investigate if these conditions affected the developmental rate of *E. warrae* in a similar manner to constant temperature regimes. Moreover, the effects of temperature on the development and mortality of *E. warrae* and *En. formosa* were compared. Both static and ramping methods were used in studies involving to estimate critical lethal high temperatures.

Second, the effects of temperature on selected life history traits of *E. warrae* were investigated. These studies examined how temperature affects the body-size of *E. warrae* and its host during their immature stages, and the resulting effects on the life-time oviposition and longevity of adult parasitoids. Over the course of an adult female's lifetime, the number of eggs laid was recorded each day in order to determine the efficiency of oviposition at different temperatures. The differential effects of temperature on body-size, oviposition and longevity were also investigated.

Third, as body size was shown to be affected by temperature, the effects adult wasp size on the behaviour of *E. warrae* and parasitism of *T. vaporariorum* were investigated. Two experiments were conducted to investigate how the relative body-size of the wasp influences the parasitism of different host instars. I examined whether large wasps are likely to ignore small nymphs of greenhouse whitefly and, conversely, whether small wasps will attack large nymphs.

The results of these studies will provide a better understanding of the range of temperature conditions that affect *E. warrae* and likely effects on the control of *T. vaporariorum*. This should provide farmers with insights into ways to use *E. warrae* more effectively in biological control.

2. Greenhouse whitefly

Greenhouse whitefly is a damaging herbivorous insect that is a common pest in glasshouses (Byrne and Bellows, 1991, Hoddle and van Driesche, 1998). It attacks a wide variety of agricultural and horticultural plants. It has been found in many parts of the world and causes severe damage to greenhouse crops when present at high densities (Byrne and Bellows, 1991, Mckee et al., 2007). Three types of damage are caused by this pest (Byrne and Bellows, 1991; Mckee et al., 2007, Mckee and Zalom, 2009). First, direct feeding can reduce crop yield. Second, honeydew can contaminate crops and stimulate the growth of sooty moulds, both of which can reduce crop quality. Third, it can act as a vector of viruses that infect plants. Since greenhouse whitefly causes substantial damage to crops around the world and it has a history of evolution of resistance to insecticides (Gorman *et al*., 2007), its effective management requires ongoing attention.

3. Control of greenhouse whitefly

In spite of considerable research on the management of greenhouse whitefly, ongoing research is needed to maintain and improve the efficacy of control. For example, insecticides can suppress this pest. Some insecticides have been reported to kill more than 90% of adults and 80% of nymphs, respectively (Bi et al., 2002). But resistance of greenhouse whitefly to many insecticides has been demonstrated (Gorman, 2007). In addition, yellow sticky cards can contribute to the management of greenhouse whitefly (Sanderson and Roush, 1992), and some physical factors such as light conditions can be used to minimise whitefly activity in greenhouses (Johansen, 2009). Among the pest control options, biological control is preferable in most circumstances because it limits non-target effects on the environment, can prevent problems caused by insecticides, and possibly produces more cost-effective suppression of this pest. Many biological control agents have been used in the management of greenhouse whitefly throughout the world, including parasitoid wasps, predators, viruses and fungi.

3.1. Biological control of greenhouse whitefly

Predators

There have also been many studies of the species of the predators that attack greenhouse whitefly, such as the mirid bugs and the anthocorids (Montserrat et al., 2000; Gabarra et al., 2004). But single species of predators typically do not control greenhouse whitefly effectively (Gerling, 1990).

Pathogens

As an alternative to insecticides, viruses and fungi have also been used to control whiteflies (Faria and Wraight, 2001). However, pathogens are not so effective if pest densities are high (Faria and Wraight, 2001).

Parasitic wasps

Parasitoid wasps have been used to manage *T. vaporariorum* in greenhouses in Europe since the 1920s (Hoddle et al., 1998; van Lenteren et al., 2009). The release of parasitoid wasps has become increasingly extensive, and now many species of aphelinid wasps are used to control greenhouse whiteflies. Dozens of species of *Encarsia* and *Eretmocerus* attack whiteflies. Understanding the distribution, life history, bionomics and utilization of whitefly parasitoids gives insights into how parasitoids can be used in the control of whiteflies (Viggiani, 2003; Liu et al., 2015). Among parasitoid wasps, *En. formosa* is the most widely used and effective biological control agent in the world (Hoddle et al., 1998; van Lenteren et al., 2009).

Encarsia formosa

From the 1970s, the release of *En. formosa* as a whitefly regulating agent has increased substantially, from 100 hectares in 1970 to nearly 5000 in the year 1993 (van Lenteren et al., 1996). Its biology, population dynamics, methods for rearing and release, and efficacy have been extensively reported (van Lenteren et al., 1996; Hoddle et al., 1998; van Lenteren et al., 2009). However, there are some weaknesses that can reduce the efficacy of *En. formosa* in the control of greenhouse whitefly. For instance, greenhouse whitefly colonies still grow in the hot summer and cold winter when *En. formosa*’s activity and population growth are decreased by extreme temperatures, and thus it cannot control this pest effectively at both high and low temperatures extremes that occur in greenhouses (Gerling, 1990; Briere et al., 1999). Species that have a broader tolerance for extreme temperatures are needed to replace or complement *En. formosa.*

Eretmocerus species

Eretmocerus species have been used to control *T. vaporariorum* in greenhouses in many parts of the world. Previous research has demonstrated that *Eretmocerus* spp. may be more effective than *Encarsia* spp in the biological control of whiteflies (De Barro et al., 2000b). Parthenogenic parasitoids like *Eretmocerus* spp. are easier to establish in greenhouses because of their higher population grow rate than *Encarsia* spp. (Stouthamer, 1993). In some studies (Goolsby et al., 1998, De Barro et al., 2000b), *Eretmocerus* spp. performed better than *Encarsia* spp. in suppressing *Bemisia tabaci.* The effectiveness of *Eretmocerus* spp. may be the result of their parthenogenic nature (De Barro et al., 2000a), since they are more likely to increase their population when their density is low because parthenogenic species do not need to find a mate. Among the *Eretmocerus* species, *E. mundus* and *E. eremicus* are commercially sold agents for biological control of whiteflies (Hoddle et al., 2003). Although *Eretmocerus* spp. can be effective biological control agents, their use is not always cost-effective. The use of *E. eremicus* could be more expensive than using insecticides (van Driesche et al., 1999). Moreover, many species that could be used to suppress the greenhouse whitefly are still untried or have not been studied in detail. *E. warrae* is one such parasitoid of the greenhouse whitefly that warrants further investigation.

3.2. *Eretmocerus warrae*

E. warrae was first studied in Australia by De Barro et al. (2000b), who presented the morphological and molecular characteristics of this wasp. To date, *E. warrae* has been reared in commercial greenhouses in Loxton, SA, Australia by Biological Services. It has shown great potential for the control of greenhouse whiteflies. The investigation of De Barro et al.

(2000b) showed that *E. warrae* produces brood with sex ratio that is extremely female biased. James Altmann has confirmed this observation (Biological Services, 2015).

There has been limited research on the biology of *E. warrae* and its role in the biological control of whiteflies. Kumar et al. (2008, 2011) showed that *E. warrae* can be used together with selected insecticides like neem. The development, adult emergence, host-feeding, reproduction, and diurnal rhythms of this parasitoid were investigated by Hanan et al. (2009, 2012a). They found that its longevity and reproduction potential are affected by food supply, and drew the conclusion that the parasitism rate was higher when food like nectar and pollen are available than when only the host is present. Such food extends the longevity of *E. warrae* and increases the number of eggs laid (Hanan et al., 2010). Both host-feeding and parasitism rates declined as the density of hosts increased, whereas the numbers of eggs laid, and hosts killed by host-feeding, and the longevity of the parasitoid increased (Hanan et al., 2012b). However, effects of temperature on development and performance of *E. warrae* are unknown.

4. Effects of temperature on insects

General effects of temperature

For insects, temperature is a key ecological factor that affects all aspects of life, from physiology to behavioural patterns (Ludwig, 1928; McDougall and Mills, 1997). Insects are poikilothermic, i.e., their development and biology are influenced by the temperature of the environment. In the optimum range, insect metabolism can take place normally and the development of insects in this range shows a non-linear response to temperature (Briere et al., 1999) (Figure 1.). Rates of development are lower than the optimum temperature if the temperature is higher or lower than this optimum. Experiments have shown that longevity and fecundity are also affected by the temperature (McDougall and Mills, 1997; Greenberg et al., 2000; Zandi-Sohani et al., 2009). Longevity decreases as the temperature increases in most cases and fecundity also has an optimum temperature. Research relating temperature to the development of insects is one of the most important aspects of the study of many kinds of insects, including greenhouse whitefly and its parasitoids (Greenberg et al., 2000; Zandi-Sohani et al., 2009).

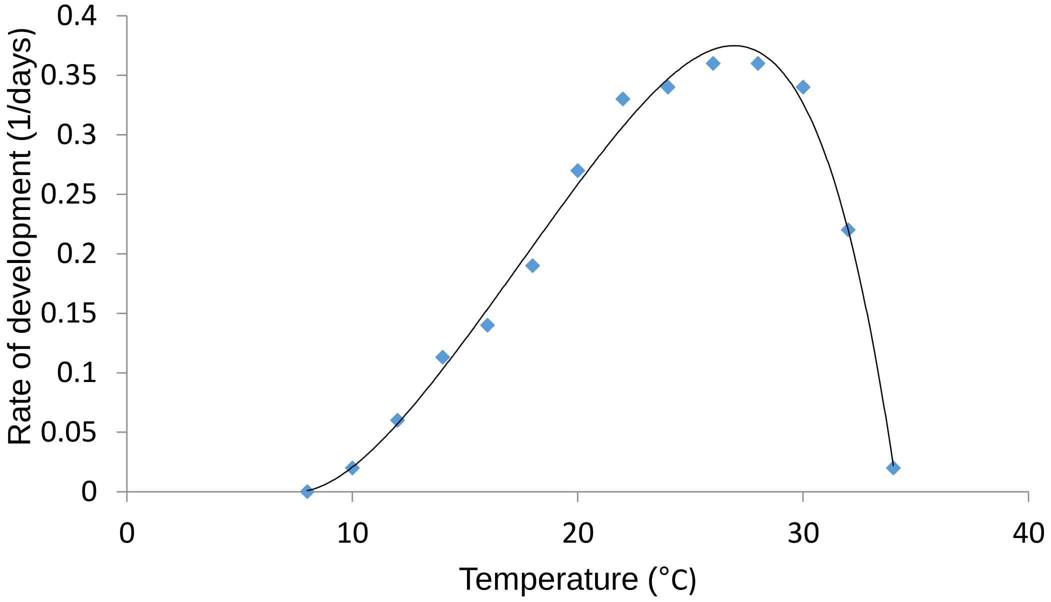

Figure 1. Typical temperature-dependent developmental rate curve of an insect. The curve is fitted to hypothetical data.

Effects of temperature on parasitoids

For parasitoids, temperature influence tri-trophic interactions, that is, plants and host insects are also influenced by temperature. The physical and chemical responses of plants to changes of temperatures affect both hosts and parasitoids (Berry and Bjorkman, 1980). The fitness of host insects is also influenced by temperature conditions. Hosts that offer better resources can increase the fitness of a parasitoid (Godfray, 1994). The resources associated with a host insect can be affected by temperature directly or indirectly through changes in the host plant.

Temperature in greenhouses

In general, there is a vertical temperature gradient in greenhouses, with the temperature at the canopy level about 5°C higher than on the ground level (Qian et al., 2010). Temperature is normally higher inside than outside the greenhouse. In hot summers, it can reach particularly high levels at which the insects in a greenhouse will be negatively influenced.

High temperature

High temperatures can severely limit the development and behaviour of insects. The mortality of insects increases when the temperature is relatively high, above the optimum temperature, and this complicates research on the effects of temperature on rates of development (Briere et al., 1999). Although there are many studies of the effects of temperature on insects, studies on the effects of high temperatures are relatively few (Chen et al., 2005). Because of the severe effects of high temperature on mortality, particularly in biological control systems in which high temperature might lead to failure of control, the effects of high temperature on development, mortality and behaviour of insects warrant investigation.

Development at fluctuating temperatures

Effects of fluctuating temperature have not been broadly investigated in insects. However, daily fluctuating of temperature is intrinsic in all ecosystems and this is different from many examples of scientific research in which temperatures are held constant. The development of some parasitoids can be faster under fluctuating temperature regimes than that at constant temperatures (Stenseth, 1975). The effects of fluctuating temperature on the development of parasitoids need to be investigated.

5. Investigation of the effects of temperature on *Eretmocerus warrae*

5.1. Effects of temperature on development, oviposition and mortality of *Eretmocerus warrae*

Developmental rate at constant temperatures

To date, the influence of temperature on the development and oviposition of *E. warrae* has not been investigated. How development, longevity and behaviour of this wasp are affected by temperature is significant in utilizing *E. warrae* in greenhouses. Knowing basic parameters, such as lower and upper developmental threshold temperature and developmental rate are useful for deploying it as a biological control agent and population stability of this parasitoid. The determination of these parameters will also enable an advanced release strategy to be developed in consideration of expected temperature conditions in greenhouses.

Development at fluctuating temperatures

In order to predict the development of *E. warrae* in environments where temperature is fluctuating, the effects of fluctuating temperature on development of this wasp need to be investigated. This investigation will also indicate whether there are differences between constant and fluctuating temperatures on rates of development of parasitoids.

Oviposition rate at constant temperatures

Besides effects on development of *E. warrae*, temperature also affects the behaviour of this wasp. Oviposition activity is affected by the ambient environment, notably temperature. Sometimes farmers need a quick solution for a pest problem, so the oviposition rate at different temperatures should be taken in to account.

Development and emergence of greenhouse whitefly at high temperatures

The development and survival of hosts influences parasitoids to a large extent, particularly at high temperatures. If the host dies in the early stage of development, then solitary parasitoid has little chance to survive. So in order to understand effects of high temperature on *E. warrae*, effects of extreme temperature on the hosts should be studied beforehand.

Development and emergence of *E. warrae* and *En. formosa* at high temperatures

When temperatures get close to the upper lethal threshold temperature, the developmental rate and survival of parasitoids are severely affected. Observations suggest that *E. warrae* actively parasitizes greenhouse whitefly at higher temperatures than *En. formosa* (Biological Services, 2015). If this proves to be true, then *E. warrae* should be a complementary biological control agent to *En. formosa*, and may be a better biological control agent when the temperature is very hot. Therefore, the developmental biology and parasitic behaviour of *E. warrae* and *En. formosa* should be compared at a range of high temperatures.

Mortality of *E. warrae* and *En. formosa* at high temperatures

Above the upper lethal temperature threshold, high temperatures can cause death or irreversible injury to insects. However, in the hot summer of some regions, temperature can be higher than the optimum range of insects, and thus cause the failure of biological control. Hence effects of high temperature on mortality of *E. warrae* and *En. formosa* need to be determined to compare their reliability at high temperatures.

In determination of the mortality of *E. warrae* and *En. formosa* at high temperatures, both static and dynamic methods can be used. The latter method uses ramping temperatures to assess the critical upper lethal threshold and it is thought to be more ecologically- relevant (Terblanche et al., 2007).

6. The effects of temperature on body-size, longevity and life-time oviposition of *E. warrae*

Effects of immature developmental temperature on body-size of parasitoids and hosts

Hotter temperatures generally lead to a smaller body-size of insects and conversely, relatively colder temperature normally bring about larger body-size (Nunney and Cheung, 1997, Angilletta et at., 2004). For parasitoids, the response to temperature is more complicated than their hosts since their nutritional levels also depend on hosts. But theoretically, effects of temperature on parasitoids are similar to those on hosts (Godfray, 1994). In addition, host plants affect both hosts and parasitoids because of physical and chemical changes in response to temperature (Berry and Bjorkman, 1980).

Effects of immature developmental temperature on fecundity and longevity of parasitoids

Generally, developmental temperature influences the fecundity and longevity of parasitoids. Parasitoids develop at lower ambient temperatures normally are normally fitter physically that they have more ovarioles and live longer (Honěk, 1993; Roff, 2002). However, some insects so not increase fecundity substantially in cold ambient environments since their overall grow rates are slower (Angilletta et at., 2004). There was no relationship between body-size and longevity of the solitary bee *Osmia cornuta* (Bosch and Vicens, 2006).

Effects of adult temperature on oviposition and longevity of parasitoids

Parasitoids have lower levels of metabolism in colder ambient environments should live longer and oviposit slower (Godfray, 1994). Slower oviposition behaviour does not necessarily lead to lower life-time oviposition. But in harsh environment, parasitoids cannot oviposit all eggs or absorb some eggs over time (Godfray, 1994), and thus in such instances slower oviposition results in lower life-time oviposition.

Relationships between body-size, oviposition and longevity of parasitoids

Body-size generally related to the fecundity and longevity of parasitoids. Larger individuals of a species are typically physically stronger and have a higher fecundity and a longer life-span. However, there is no relationship between body-size and longevity in some cases, e.g., the solitary bee *Osmia cornuta* (Bosch and Vicens, 2006). The realised level of fecundity has impact on the longevity of parasitoids since energy consumed by behaviours related to oviposition (Godfray, 1994). Conversely, longer longevity means more opportunities for ovipositing and a higher life-time oviposition (Blackburn, 1991).

7. Effects of relative body-size on the host stage choices of parasitoids

Host stage choice of parasitoids

The stage of a host influences the fitness of a parasitoid, and thus parasitoids should select the stages that can lead to higher levels of reproductive success (Godfray, 1994). Older stages of hosts often provide a higher level of nutrition and thus can be the theoretical best choice (Godfray, 1994; Da Rocha, 2007). However, other factors such as host defence affect the host-choice strategies of parasitoids (Godfray, 1994).

Searching behaviour of parasitoids according to different stages of hosts

When searching for hosts, parasitoids engage in different types of behaviour such as antennating the substrate, probing hosts with their ovipositor and stinging hosts. Understanding the searching behaviour of parasitoids gives insights into their relationships with hosts. Searching varies among species of parasitoids (Wang and Keller 2002), and comparative behavioural ecology can elucidate parasitoid-host relationships, as is exemplified by aphelinid parasitoids (Hunter and Woolley, 2001). The analytical method of

Field and Keller (1993) helps us to understand how searching behaviour is organized by parasitoids.

The searching behaviour of parasitoids is often different when handling different stages of hosts because they can differ in size, odours and defensive behaviour (Bell, 2012). Parasitoids depend on various cues to find hosts, and different stages of hosts are different in such cues (Mattiacci and Dicke, 1995). This will make some stages of hosts easier to find, e.g., a larger body-size should be detected easier to see. Moreover, the acceptance levels also vary in relation to different stages, since older stages of hosts can often display more vigorous levels of defence against their natural enemies (Godfray, 1994).

Effects of host stages on development of parasitoids

The fitness of a parasitoid is profoundly influenced by the stage of the host (Jervis, 2008). Parasitoids that develop in different stages of hosts should have varying levels of fitness, as indicated by varying survival rates, developmental times and body-sizes. For solitary species, host stage choices are more critical for the fitness of offspring.

Effects of temperature on interactions between parasitoid and its host

Temperature influences the development and behaviour of a parasitoid and its host. Both parasitoids and hosts are generally more active in warmer ambient environments, but development at warmer temperatures normally lead to a smaller body-size (Angilletta et al., 2004). The temperature-dependent body-size differences could affect the host stage choices of parasitoids.

Effects of relative body-size between parasitoids and hosts on host stage choice and searching behaviour of parasitoids

The body-sizes of parasitoids are generally related to their host stage choice strategies (Godfray, 1994). For parasitoids of different body-sizes, the relative body-size will be different if the body-size of hosts remains normal. The normal-sized hosts will be relatively larger for smaller parasitoids and smaller for larger wasps. The question occurs, is the host stage choice related to the relative body-size between parasitoids and their hosts? Also there might be some other reasons that affect the host stage choice of parasitoids. The mechanism of host stage choice strategies of parasitoids warrant investigating.

Body-size might also influences the searching behaviour of parasitoids. Larger in body-size of parasitoids normally means finding and handling a host easier. They might be able to handle older hosts of higher levels of defence behaviour where smaller ones cannot (Godfray, 1994; Bell, 2012). Observations on searching behaviour will improve our understanding on host stage choice strategies of parasitoids.

Effects of experiences on the parasitism efficiency of parasitoids

The parasitism efficiency of parasitoids can be improved from experience (Outreman, 2005). Experienced parasitoids can be more efficient in finding and handling hosts. In observations, effects of experiences on the parasitism efficiency of parasitoids can be investigated through the comparisons between the first parasitism (naive) and the rest (experienced).

8. Research questions

This thesis is concerned with six main research questions.

- How does temperature affect the development and oviposition of *E. warrae*?
- Which species of parasitoid has greater development and survival at extreme temperatures, *E. warrae* or *En. formosa*?
- How does immature developmental temperature and adult temperature influence the body-size, life-time oviposition and longevity of *E. warrae*?

- Which instar of greenhouse whitefly is preferred by *E. warrae*?
- Is the development and searching behaviour of *E. warrae* influenced by the stage of hosts?
- Are host stage choices and searching behaviour affected by the relative body-sizes of *E. warrae* and its host?

9. Aims and significance of the project

This research project aimed to study effects of temperature on the biology of *E. warrae*. I studied effects of temperature on development, body-size, oviposition and longevity of *E. warrae* at different temperatures and compared the performance of *E. warrae* and *En. formosa* to find out if *E. warrae* can perform better at higher temperatures. I also investigated the effects of relative body-size on the host stage choice and searching behaviour of *E. warrae*.

This research will elucidate the temperature-dependent biology of *E. warrae* and suggest its potential in biological control of greenhouse whitefly. The results should assist in the development of more effective methods for the mass rearing and releasing this parasitoid. Effects of temperature on immature development and adult biology will provide an enhanced knowledge of the temperature-dependent biology of parasitoids. Moreover, the results will also enhance understanding of the host stage choice strategy and searching behaviour of parasitoids.

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CHAPTER TWO

The effects of temperature on the development, fecundity and mortality of *Eretmocerus warrae*: Is *E. warrae* better adapted to high temperatures than *Encarsia formosa*?

Statement of Authorship

The effects of temperature on the development, fecundity and mortality of *Eretmocerus warrae*: Is *E. warrae* better adapted to high temperatures than *Encarsia formosa*?

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Submitted to Biological Control

TW conceived and designed the experiments, carried out the experiments, generated and analysed the data and wrote the manuscript. MK conceived and designed the experiments, analysed the data, reviewed the manuscript. KH provided conceptual and editorial advice throughout the project.

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Name of Principal Author (Candidate)	Tao Wang		
Contribution to the Paper	Conceived, designed and carried out the experiments, generated and analysed the data, and wrote the manuscript.		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	23 December 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Michael A. Keller		
Contribution to the Paper	Reviewed and provided advice on the design of experiments, assisted data analysis, and reviewed and suggested significant changes to the manuscript.		
Signature		Date	23 December 2016

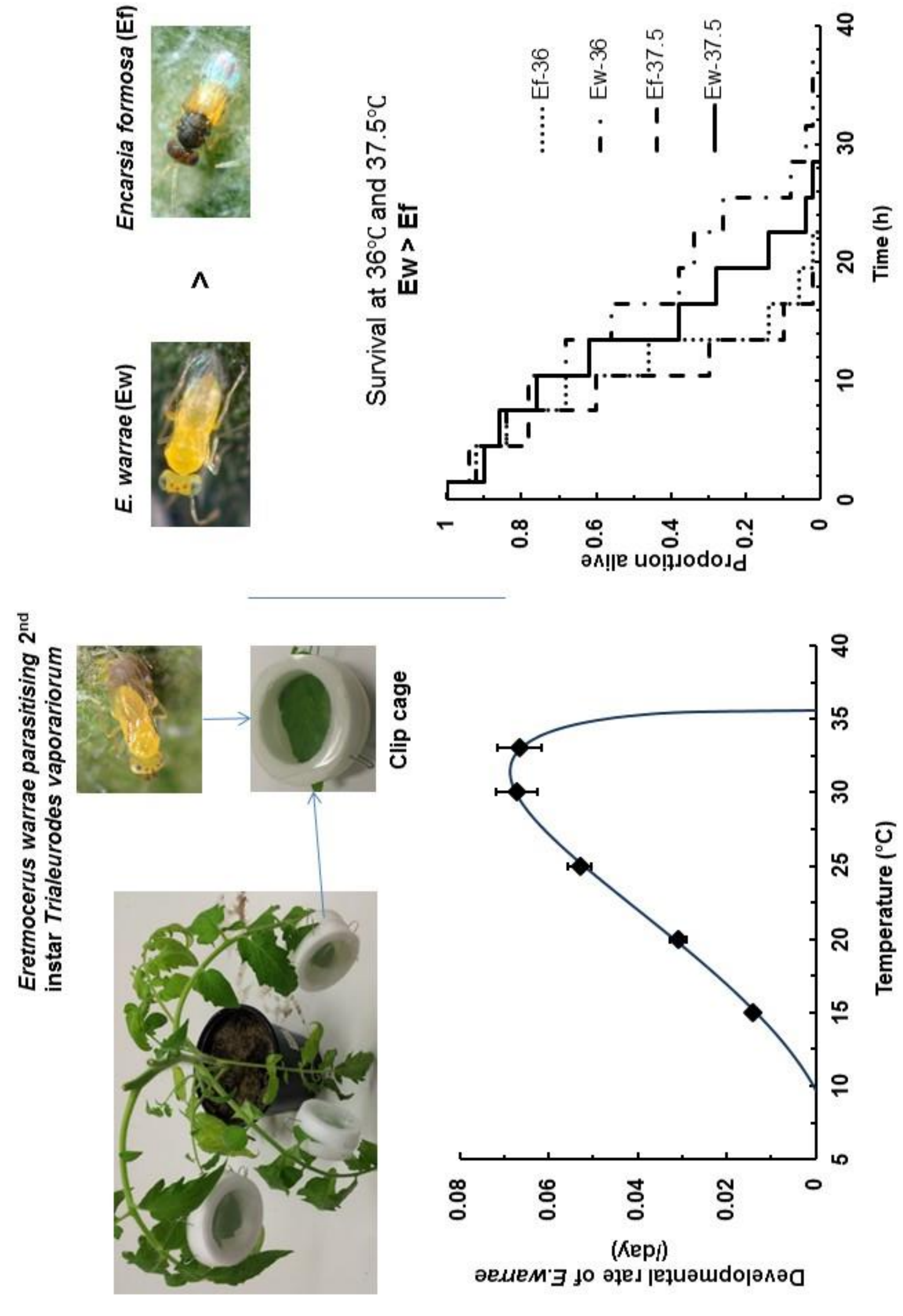
Name of Co-Author	Katja Hogendoorn		
Contribution to the Paper	Provided conceptual and editorial advice throughout the project.		
Signature		Date	23 December 2016

Please cut and paste additional co-author panels here as required.

Highlights

- *Eretmocerus warrae* shows a non-linear developmental response to temperature
- Reproduction is predicted to occur over a more limited range of temperatures
- Development in fluctuating temperatures was accurately predicted
- *E. warrae* cannot develop above 34.5 °C; *En. formosa* cannot develop above 33.0 °C
- Critical thermal maximum of *E. warrae* is 42.7°C, 0.6°C above that of *En. formosa*

Graphical Abstract



Abstract:

Eretmocerus warrae (Hymenoptera: Aphelinidae) is a parasitoid of the greenhouse whitefly，*Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). It is suspected to be a better biological control agent at high temperatures than *Encarsia formosa* (Hymenoptera: Aphelinidae), a wasp which is widely sold for control of *T. vaporariorum*. Experiments were conducted to investigate how temperature affects the development and behaviour of *E. warrae.* It attained the highest estimated developmental rate at 31.5 °C and the optimum temperature for oviposition was 30.5 °C. Developmental times of *E. warrae* at fluctuating temperatures that simulate night-day patterns were similar to those predicted based on constant temperatures. Above the optimum temperature, *E. warrae* tolerated higher temperatures than *En. formosa* during development and as adults. Using a ramping temperature approach, the critical thermal maxima of *E. warrae* was 42.7 ± 0.5 °C, 0.6 °C higher than that of *En. formosa*. Thus, *E. warrae* is better adapted to high temperatures than *En. formosa*, and it could be a complementary or superior biological control agent during summer months in hot regions.

Key Words:

Trialeurodes vaporariorum, critical thermal maximum, ramping temperature, survival, fluctuating temperature, aphelinid

1. Introduction

Greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) attacks an extensive range of ornamental plants and vegetables, and causes severe damage to greenhouse crops when present at high densities (Byrne and Bellows, 1991). Although insecticides can suppress this pest, resistance of greenhouse whitefly to insecticides has been demonstrated and biological control is widely used for suppression. *Eretmocerus warrae* (Nauman & Schmidt) (Hymenoptera: Aphelinidae) is a parasitoid of the greenhouse whitefly that is suspected to be effective as a biological agent (De Barro et al., 2000a). Observations suggest that *E. warrae* actively parasitizes greenhouse whitefly at higher temperatures than *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) (James Altmann, personal communication). If this proves to be true, then *E. warrae* should be a complementary biological control agent to *En. formosa*, and might be a better control agent when the temperature is high during summer. This study was carried out to study the effects of temperature on the biology of *E. warrae* and determine whether it is able to tolerate higher temperatures than *En. formosa*.

Among species of biological control agents, the release of *En. formosa* has received much attention in the biological control of greenhouse whitefly (Hoddle et al., 1998). It has been one of the most widely used and very effective parasitoids in control of whiteflies in greenhouses in many parts of the world since the 1920s (Waage and Hassell, 1982). However, there are some weaknesses that can reduce the efficacy of *En. formosa* in biological control. For instance, greenhouse whitefly colonies still grow in the hot summer when *En. formosa*'s activity and population growth are decreased by extreme temperatures, and thus it cannot control this pest effectively at high temperatures (Gerling, 1990). Species that have a broader tolerance for extreme temperatures are needed to replace or complement *En. formosa.*

The solitary parasitoid *E. warrae* was found in New Zealand in 1997 by Workman et al. (2008). It was first studied in Australia by De Barro et al. (2000b), who presented the morphological and molecular characteristics of this wasp. It has been suspected that *E. warrae* is a better biological control agent at high temperatures than *En. formosa*. Because of its potential in suppressing greenhouse whitefly, *E. warrae* is being reared for release in commercial greenhouses in Australia (Biological Services, 2015).

Temperature is a key environmental factor that affects all aspects of arthropod life, from physiology to behavioural patterns (Ludwig, 1928). Therefore, how development, longevity and behaviour of *E. warrae* are affected by temperature is significant in utilizing it in greenhouses. The development of insects shows a non-linear response to temperature (Fig. 1), with the highest developmental rate achieved at an intermediate optimum temperature. Basic parameters, such as lower developmental threshold temperature, developmental rates under different temperature conditions and critical lethal maxima are needed to predict the generation time, which affects the effectiveness of parasitoid populations. The determination of these parameters should enable an advanced release strategy to be formulated in consideration of expected temperature conditions in greenhouses. The effects of fluctuating temperatures on *E. warrae* need to be studied because daily fluctuations in temperature are inherent in the operation of greenhouses.

For integrated pest management (IPM), the upper threshold temperatures may be more critical than the optimum temperature (Briere et al., 1999). When the temperature exceeds the optimum, it can cause death or irreversible injury (Fig. 1), or severely limit development and behaviour (Briere et al., 1999; Tang et al., 2000). Insects may also become dormant or quiescent when exposed to extreme temperatures. Therefore methods to estimate the upper lethal threshold are valuable in understanding a species' response to temperature. The dynamic method for estimating upper lethal temperatures uses ramping temperatures to assess the critical upper limit (Terblanche et al., 2007). It is increasingly used and thought to be more ecologically-relevant than the static method, which uses a range of fixed constant temperatures to estimate the upper lethal temperature.

To better understand the effects of temperature on the development and mortality of *E. warrae,* we assessed the influence of temperature on its development, mortality and oviposition. The effects of fluctuating temperatures on *E. warrae* were also investigated. Furthermore, to investigate the potential of *E. warrae* as a complementary biological control agent of *En. formosa* in greenhouses at high temperatures, we compared the developmental rate and survival of *E. warrae* and *En. formosa* at a range of high temperatures. Both constant temperature and ramping temperatures were used. These results will facilitate rearing and effective deployment of *E. warrae* in greenhouses.

2. Materials and Methods

2.1. Rearing parasitoids and host

Greenhouse whiteflies were collected from eggplant, *Solanum melongena,* in the greenhouses at the Waite Campus of The University of Adelaide and used to initiate a culture. Tobacco, *Nicotiana tabacum,* plants with at least five fully expanded leaves and approximately 30 cm high were used to rear the whitefly culture because they can support high densities of this insects. A greenhouse whitefly culture was kept at 26.0 °C, and a photoperiod 14 L: 10D.

Pupae of *E. warrae* and *En. formosa* were provided by Biological Services, Loxton, South Australia. A breeding culture of the parasitoids was maintained on greenhouse whiteflies feeding on tomato plants, *Solanum lycopersicum,* at 26 °C. The cultivar ‘*Improved Appolo*’ was used in moderate temperature conditions (15.0 – 33.0 °C) whereas ‘*Summerstar*’ was used at higher temperatures (30.0 - 37.5 °C) because it can better withstand temperatures up to 37.5 °C. Six to seven week old tomato plants were used in experiments as a host plant for greenhouse whitefly. The tomato plants had six fully expanded leaves and were approximately 50 cm high.

The pupae of the two species of parasitoids were kept in two separate incubators at 8 °C to arrest development. For each species, when adult parasitoids were needed, pupae were moved from 8°C at 20:00 h to another incubator which was set at 26 °C to allow them to emerge. Most adults emerged during the morning (< 24 h) when experiments commenced. Honey drops were provided as food.

2.2. Experimental materials

Temperature experiments were conducted in five incubators. The rearing temperatures were set according to the experiments and the photoperiod was 14L : 10D. Clip cages were used to confine insects that had inside and outside dimensions of 40 mm and 55 mm, respectively. They were made of 12 mm thickness of polyethylene foam. There was a transparent cellulose acetate sheet on bottom of each cage which allowed wasps to be observed and fine organza on top for aeration. An aspirator made of plastic tubing was used to handle wasps. Honey drops were placed on the organza of clip cages as food for *E. warrae*.

2.3. Effects of temperature on the developmental rate of *E. warrae*

An experiment was conducted to investigate the effects of temperature on the development of *E. warrae*. Five incubators were set at 15, 20, 25, 30 and 33 °C, respectively. One tomato plant infested with 2nd instar greenhouse whiteflies was put in each incubator. A pure cohort of 2nd instar nymphs was obtained by separating the adults from tomato plants after six hours of exposure to a greenhouse whitefly culture and rearing these cohorts in incubators at 26 °C. Three clip cages were attached to each plant on selected leaves which were in good condition with a minimum of 100 2nd instar greenhouse whiteflies.

In the temperature range15 - 33 °C, the wasp numbers were 13, 4, 3, 2 and 2, respectively. The plants were transferred to the experimental incubators where nymphs were exposed to varying numbers of newly-emerged adult *E. warrae* for six hours, after which the adults were removed from the clip cages using an aspirator. Greater numbers of wasps were used at lower temperatures, to compensate for their lower activity levels. The parasitised greenhouse whitefly nymphs were kept in clip cages until the next generation of adult *E. warrae* emerged. Development of *E. warrae* was monitored using a hand lens. Every week, a new plant infested with greenhouse whitefly nymphs was put in each incubator and the nymphs were exposed to *E. warrae* as described. There were four replicate plants at each temperature.

The equation of the Briere model was used to analyse the developmental rate of *E. warrae* (Briere et al., 1999). It is described as

$$R(T) = \begin{cases} 0 \; T \leq T_0 \\ aT(T - T_0)(T_L - T)^{\frac{1}{m}} \quad T_0 \leq T \leq T_L \\ 0 \; T \geq T_L \end{cases} \tag{1}$$

where R is the rate of development; T is the temperature; T_L is the upper threshold temperature and T_0 is the lower threshold temperature; a and m are empirical constants.

This model has advantages compared to other non-linear models (Logan, 1988; Schoolfield et al., 1981; Sharpe et al., 1981). It has few parameters, is biologically descriptive and incorporates both high and low threshold temperatures. Unlike a degree-day model which does not include the nonlinear relationship between developmental rate and temperature at extreme low and high temperatures, this model fits the broad non-linear relationship across all temperatures (Briere et al., 1999). The model has three or four parameters. The lower (T_0) and upper (T_L) temperature threshold parameters have biological meaning. The model of Briere et al. (1999) has a form that can potentially fit the relationship between temperature and other biological rates. It was also used to evaluate the relationship between temperature and oviposition rate.

The data of developmental rate of *E. warrae* were analysed using non-linear regression in R version 3.2.0 (2015-04-16) to estimate the parameters of the model of Briere et al. (1999).

2.4. Effects of temperature on the oviposition activity of *E. warrae*

The oviposition avitivity of *E. warrae* was assessed at 15.0, 20.0, 25.0, 30.0 and 33.0 °C to determine how temperature influences rates of oviposition. Before an experiment, wasps were kept at 25.0 °C. Each adult *E. warrae* was exposed to 2nd instar greenhouse whitefly for two hours to become experienced in host searching. The wasps were then separated from hosts for one day. This procedure ensured that *E. warrae* would lay eggs quickly when hosts were available. Tomato leaves infested with 2nd instar greenhouse whitefly were placed into the incubators one hour before the experiment. The greenhouse whitefly infested leaves were covered by clip cages. Four experienced adult *E. warrae* were released into each clip cage at the experimental temperature. The wasps were removed from the cages after three hours. Because *E. warrae* lays eggs under the ventral part of nymphs, all the nymphs were turned over using a dissecting needle and the number of eggs laid was recorded. Observations at each temperature were replicated four times. The analysis of the effect of temperature on oviposition rate of *E. warrae* were the same as that of experiment 2.3.

2.5. The effects of fluctuating temperature on the developmental times of *E. warrae*

An experiment was conducted to investigate the development of *E. warrae* under fluctuating temperature conditions to determine whether these varied substantially from development at constant temperatures. The methods were similar to experiment 2.3. Two fluctuating temperature regimes were chosen. The temperatures were 33.0 °C in light and 26.0 °C in dark in the high fluctuating temperature regime, and 25.0 °C in light and15.0 °C in dark in the low fluctuating temperature regime. The photoperiod was 14L: 10D. 2nd instar of parasitised greenhouse whiteflies developed in clip cages until adults emerged.

The numbers of *E. warrae* were two and four in high and low fluctuating temperature regimes, according to the activity of the wasps. The developmental times of the parasitoids from egg to adult were recorded and compared to development that was predicted based on development at constant temperatures. This experiment had four replicates. The results of this experiment were compared to that of experiment 2.3 to check if the constant model can be used to predict the development of *E. warrae* at fluctuating temperatures.

2.6. The effects of high temperature on emergence and development of greenhouse whiteflies

The development and survival of greenhouse whitefly under the high temperature conditions were investigated at 30.0, 33.0, 34.5, 36.0 and 37.5 °C, respectively. The cultivar ‘*Summerstar*’ was used. Greenhouse whiteflies were reared on the plants until the 2nd instar. The number of 2nd instar nymphs in each replicate at each temperature was 100. Excessive numbers of nymphs were removed from the leaf with a pin. The developmental time and the number of greenhouse whitefly adults that emerged were recorded. This experiment has four replications.

2.7. The effects of high temperatures on emergence and development of *E. warrae* and *En. formosa*

An experiment was conducted to assess how high temperature affect the development and survival of *E. warrae* and *En. formosa*. The temperatures were the same as the aforementioned high temperature experiment (see 2.6). The development of *E. warrae* was compared with *En. formosa* under a set of higher temperatures and the procedure was the same as experiment 2.3. The numbers of parasitoids used for each species were 6, 6, 9, 18 and 36 at temperatures from 30.0 °C to 37.5 °C, respectively. Ten parasitoid pupae from each temperature replicate were selected randomly. The developmental time and emergence of both species were recorded for each species in four replications.

2.8. The mortality of adult *E. warrae* and *En. formosa* at high temperatures

The effects of high temperature on the mortality of adult *E. warrae* and *En. formosa* were investigated. Adult *E. warrae* and *En. formosa* were put into vials (5 ml) that rested in close-fitting semi-circular grooves in a dense wooden block (12.5 × 10 × 2.5 cm^3) that had been heated to 36.0 °C or 37.5 °C, which, according to the results of experiment 2.7, were stressful temperatures for both wasp species. The wooden block was painted white and served as a thermal ballast to maintain a constant temperature inside the vials during brief periods of observation when the vials were removed from incubators. Honey and water were provided on a dental cotton wick to ensure that the wasps did not die from starvation or dehydration. There were ten wasps in each vial and five replications at each temperature. The number of dead wasps was recorded every three hours until all wasps died. The proportional hazards regression function (Statistix version 10.0, Analytical Software, Tallahassee, Florida, USA) was used to analyse of the survival rate of the parasitoids.

2.9. The critical thermal maxima of adult *E. warrae* and *En. formosa* under ramping temperature conditions

The critical thermal maxima of adult *E. warrae* and *En. formosa* were assessed using the ramping temperature method. A water bath was used for this test and a calibrated thermometer was used to measure the temperature. Starting at 26.0 °C, temperatures were increased by 1°C every two minutes in which the temperature increased gradually in the first minute and kept constantly in the second minute. Temperatures were controlled at ±0.1 °C. *E. warrae* and *En. formosa* were put into two separate small glass vials with lids. A shelf was made of iron wire to fix the vial in the water bath. The shelf and vials were totally submerged into the water bath during the experiment. The observation took less than ten seconds to ensure that the temperature in the vials did not change. 10 % honey solution was provided on cotton wicks as the water and food source of the parasitic wasps. Ten wasps were put into each vial and this experiment has eight replications. The number of dead wasps was recorded. Logistic regression (Statistix 10.0) was used to estimate critical thermal maxima of *E. warrae* and *En. formosa*. In all cases, parameter estimates are given as mean ± standard error.

3. Results

3.1. Effects of temperature on the developmental rate of *E. warrae*

The developmental rate of *E. warrae* increased as the temperature rose from 15.0 °C to 30.0 °C (Fig. 2). The estimated optimum temperature for the development of *E. warrae* was 31.5 °C and the upper threshold was predicted at 35.6 ± 0.4 °C. The lower threshold temperature of *E. warrae* was 9.6 ± 0.3 °C; and the parameter “a” in Briere model was $6.3e^{-5} \pm 8.9e^{-6}$ and “m” was 3.07 ± 0.30 ($n = 550$, $r^2 = 0.98$). At 15.0 °C, it took more than two months for *E. warrae* to develop from egg to adult, which is around four times of the developmental time at 30.0 °C.

3.2. Effects of temperature on oviposition of *E. warrae*

The relationship between oviposition and temperature had the same form as the relationship between developmental rate and temperature (Fig. 3). *E. warrae* produced more eggs in three hours at 30.0 °C than at 33.0 °C. At temperatures around 30.0 °C, the adults laid on average 36 eggs in three hours. The empirical model parameters “a” and “m” in the model were estimated to be 0.04 ± 0.01 and 2.43 ± 0.95 ($n = 459$, $r^2 = 0.97$). The estimated low temperature threshold for oviposition by *E. warrae* was 13.7 ± 0.8 °C and the upper lethal threshold was estimated at 34.9 ± 1.4 °C.

3.3. The effects of fluctuating temperatures on developmental rate of *E. warrae*

The observed developmental rates were predicted accurately based on the calculated mean temperatures. The average temperatures for two fluctuating temperatures were 29.7 °C for the 33.0 / 26.0 °C regime and 20.8 °C for the 25.0 / 15.0 °C regime. The developmental rate of *E. warrae* was 0.067 ± 0.001 and 0.036 ± 0.001 (1/D) at high and low fluctuating temperatures and it took roughly 15 and 28 days (*n* = 194), for *E. warrae* to develop from egg to adult, which were statistically not different from the predicted times (Table 1).

3.4. The effects of high temperatures on emergence and development of greenhouse whitefly

There was a strong inverse relationship between high temperatures and the emergence and developmental rate of greenhouse whiteflies. The number of emerged greenhouse whiteflies decreased markedly when the temperature increased from 30.0 °C to 34.5 °C (Table 2). The percentage of adults emerged dropped from 44.3 ± 5.6 at 30.0 °C to zero at 36.0 °C and 37.5 °C (*n* = 2000). Only less than 5 % of adults on average emerged at 34.5 °C, which is roughly 10 times fewer than at 30.0 °C. It took roughly one more day for greenhouse whiteflies to grow from the 2nd instar to adult at 34.5 °C than at 30.0 °C, while the duration of development was similar at 30.0 and 33.0 °C (*n* = 264).

3.5. The effects of high temperatures on emergence and development of *E. warrae* and *En. formosa*

While adults of *E. warrae* emerged at 34.5 °C, *En. formosa* did not, and the developmental rate of both decreased as the temperature increased within the experimental temperature range (Table 3). Both *E. warrae* and *En. formosa* had reduced adult emergence numbers at 33.0 °C compared to 30.0 °C. Only less than five adults of *E. warrae* emerged at 34.5 °C whereas no *En. formosa* emerged. The developmental rate of *E. warrae* (n = 170) decreased with increasing temperature, from 0.069 ± 0.006 (1/D) at 30.0 °C to zero at 36 °C. For *En. formosa*, developmental rate at 33.0 °C was lower than that of 30.0 °C (*n* = 136). No development was recorded at 34.5, 36.0 and 37.5 °C because no *En. formosa* emerged at those temperatures.

3.6. The mortality of adult *E. warrae* and *En. formosa* at high temperatures

Adult *E. warrae* tolerated both 36.0 °C and 37.5 °C for longer periods of time than *En. formosa* ($\chi^2 = 28.78$, $df = 2$, $P < 0.001$). There was no difference in the mortality rates of *E. warrae* and *En. formosa* in the first nine hours of the experiment (Fig. 4). However, after 15 hours the survival rate of *E. warrae* was much higher than *En. formosa*. The majority of *En. formosa* perished within 15 hours and none of them survived longer than 18 hours at 37.5 °C. In contrast, almost half of *E. warrae* survived in 15 hours at both 36.0 °C and 37.5 °C and some of them lived up to 36 hours when the temperature was 36.0 °C ($n = 200$).

3.7. The critical thermal maxima of adult *E. warrae* and *En. formosa* under ramping temperature conditions

The 50 % death temperature for *E. warrae* was at 42.7 ± 0.5 °C and for *En. formosa* was at 42.1 ± 0.2 °C (*E. warrae*: Deviance = 188.52, $df = 158$, $P = 0.049$; *En. formosa*: Deviance = 143.99, $df = 154$, $P = 0.71$). The critical thermal maxima of *E. warrae* is 0.6 °C higher than *En. formosa*, and no *En. formosa* could survive above 33.0 °C whereas 38 % of *E. warrae* survived at 33.0 °C. One *E. warrae* in replicate three died when the temperature was 35.0 °C; the data of this single wasp was discarded because it was a statistical outlier.

4. Discussion

The developmental rate of *E. warrae* in range of 15.0 to 25.0 °C is broadly similar to *En. formosa*, *E. mundus* and *E. eremicus*, which are widely used in biological control (Qiu et al., 2004). *E. warrae* completes its life cycle in about two weeks at 30 °C, which is similar to *En. formosa*, *E. eremicus* and *E. mundus* (Qiu et al., 2004). However, the predicted developmental rate of *E. warrae* is still increasing at 30.0 °C (Fig. 2) while that of *En. formosa* begins to decrease at this temperature (van Roermund, 1995). The optimum temperature for the development of *E. warrae* is predicted at 31.5 °C, which is commonly observed during the summer months. *Eretmocerus* spp. are easy to establish in greenhouses (Stouthamer, 1993), which is convenient for farmers and researchers, and our research reflected this. The higer developmental rate of *E. warrae* at high tempatures indicates the potential of using *E. warrae* and *En. formosa* in combination, as *E. warrae* should suppress greenhouse whitefly more effectively during the hot summer while *En. formosa* should contribute to control at lower temperatures. This is analogous to the complementary relationship between *En. formosa* and *E. eremicus*, which are released for control of *T. vaporariorum* and *Bemisia tabaci* in European greenhouses (Qiu et al 2004). The results of the temperature experiments will be useful for optimising methods for the mass rearing and use of this parasitoid. Temperature also influences many other aspects of its fitness, such as the body-size, oviposition and foraging behaviour and these aspects of fitness warrant further investigation. Such information is essential to determine the most appropriate role of this parasitic wasp in biological control.

E. warrae had a relatively high oviposition rate even when the temperature was 33.0 °C (Fig. 3), while the oviposition behaviour of *En. formosa* is known to be adversely affected by temperatures higher than 31.0 °C (van Roermund, 1995). In this research, we did not compare the oviposition of *E. warrae* under different temperature conditions with the fecundity of *En. formosa*. However, *En. formosa* is said to mature only eight to ten eggs per day (Kajita and van Lenteren, 1982), which is less than 1 / 3 that of *E. warrae* in just three hours. Although the conditions of experiments are somewhat different, the results reveal the effectiveness of *E. warrae* in oviposition and its potential of supressing greenhouse whitefly within a short time period. The application of *E. warrae* in inundative control of greenhouse whitefly and the co-existence of *E. warrae* and *En. formosa* warrant further investigation.

When *E. warrae* was reared in fluctuating temperatures, its developmental was virtually the same as that predicted based on rearing at the same mean constant temperatures (Table 1). This suggests that the model of the developmental rate based on development at constant temperatures can be adequately applied to predict the approximate timing of developmental under moderate fluctuating temperatures. There is limited research on the effects fluctuating temperatue on parasitoids, but fluctuating temperatures are normal in production systems. Insects have the chance to recover at night during the hot summer when the daily maximum temperatures causes stress. The developmental rate of *En. formosa* under fluctuating temperatues was found to be faster than that at constant temperatures (Stenseth, 1975), which was not found with *E. warrae*.

The emgence rate of greenhouse whitefly decreased as the temperature increased within the experimental range of temperatures where the duration of development from 2nd instar to adult increased (Cui et al., 2008) (Table 2). No adults of greenhouse whitefly emerged at both 36.0 °C and 37.5 °C, and the emergence rate dropped almost ten folds. High temperature strongly affected the development and emergence of greenhouse whitefly. The effect of heat shock on the survival of greenhouse whitefly has been investigated (Cui et al., 2008), who showed most greenhouse whiteflies perish after an hour of exposure at 43.0 °C. No parasitoid could complete its development above 36.0 °C, since host adaptation to high temperature constrains the development of parasitoid. The result also indicate that high temperature could be used to effectively control greenhouse whiteflies provided the development and quality of the plants are not affected.

E. warrae completed development and emerged at 34.5 °C, whereas all *En. formosa* died at this temperature (Table 3). The results imply that the mortality of *E. warrae* at 36.0 °C and 37.5 °C might be caused by host mortality rather than an intrinsic metabolic limitation (Table 2). *E. warrae* may be able to withstand higher temperatures than its host. In contrast, *En.*

formosa did not complete development at 34.5 °C while its host is able to survive. Thus means *En. formosa* cannot suppress greenhouse whitefly effectively at high temperatures around 34.5 °C. Previous research (Qiu et al., 2004) showed that the developmental rates of *Eretmocerus* spp. were higher than that of *En. formosa* at 32.0 °C, and our results reflect this. During the hot summer, the peak temperature could reach 34.5 °C easily in some regions. Notably in greenhouses, the temperature can be higher inside than outside a greenhouse and reach particularly high levels (Qian et al., 2010), thus the application of *E. warrae* at high temperatures is more appropriate than *En. formosa*. Therefore releasing *E. warrae* during hot summer months could deliver better biological control than *En. formosa*.

When exposed to static high temperatures, most *E. warrae* could survive longer than *En. formosa* when the temperature was higher than 36 °C (Fig. 4). *E. warrae* displayed better survival at extreme high temperatures. When the temperature increased to 36.0 °C or above, its longer survival time makes *E. warrae* superior to *En. formosa*. Extreme high temperatures typically persist for only a few hours during the afternoon, so it is possible that parasitoids that do survive extreme temperatures can recover when the temperature drops in the evening. The performance of another species of *Eretmocerus, E. eremicus* was reported to be more effective at high temperatures than *En. formosa,* which is consistent with our results (Greenberg et al., 2000).

The critical thermal maximum of *E. warrae* was 0.6 °C higher than that of *En. formosa*. It can withstand 43.0 °C for more than two minutes whereas *En. formosa* cannot (Fig. 5). Although the 0.6 °C higher critical thermal maxima gap is not substantial, it matters in the commercial application of parasitoids because short periods of extreme temperature around 42.0 °C occur in some regions. Most insects will perish under extreme temperature conditions, thus it is essential to know the critical thermal maximum of a parasitic wasp when developing a biological control program against a pest. This is even more critical in a global warming environment. On the one hand, the use of the ramping temperature method to estimate the critical thermal maximum has proved to be closely related to organismic ecology (Overgaard et al., 2012; Terblanche et al., 2007). On the other hand, the static temperature method demonstrated a longer period of survival at high temperature, that was more than 34 hours for *E. warrae* at 36.0 °C, while the ramping temperature approach did not show this difference. Both these methods have their advantages, and thus the combination of both static and ramping temperature methods can reveal different aspects of the effects of high temperature on insects like *E. warrae*.

Our results suggest that *E. warrae* should be a complementary biological control agent to *En. formosa* when the temperature is high. The superior oviposition ability within a short time of *E. warrae* compared to *En. formosa* indicates its potential for control. *E. warrae* should be used alone or in combination with *En. formosa*, notably in hot regions. This research should enable farmers to use *E. warrae* together with *En. formosa* to achieve more effective biological control. The effectiveness of *E. warrae* needs validation in greenhouses in summer.

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Figure legends

Figure 1. Temperature-dependent development of insects. T_0 refers to the lower threshold temperature for insects, T_{OPT} is the optimum temperature and T_L is the upper threshold temperature. The dashed lines indicate that the mortality of insects increased because of stressful high temperature.

Figure 2. Temperature-dependent developmental rate (mean ± SD) of *Eretmocerus warrae*. In the Briere model, $a = 0.00006$, $m = 3.07$, $T_0 = 9.62$, $T_L = 35.63$. Where no error bar is visible, the standard deviations were ≤ 0.001 /day. 550 observations in total.

Figure 3. Temperature-dependent fecundity (mean ± SD) of *Eretmocerus warrae*. In the Briere model, $a = 0.004$, $m = 2.43$, $T_0 = 13.70$, $T_L = 34.94$. The standard deviation was 0.96 at 15 °C. 459 observations in total.

Figure 4. Survival analysis of *Eretmocerus warrae* and *Encarsia formosa* at 36 and 37.5 °C. Dotted line is the survival rate of *En. formosa* at 36 °C, dash- and dotted line is that of *E. warrae* at 36 °C, dashed line is *En. formosa* at 37.5 °C and solid line is *E. warrae* at 37.5 °C. ($\chi^2=28.78$, $df = 2$, $P < 0.001$)

Figure 5. Survival of *Eretmocerus warrae* and *Encarsia formosa* using ramping temperature. Curves fitted by logistic regression: a) Survival rate of *E. warrae,* the constant is 115.76, deviance 188.52, $P = 0.049$, $df = 158$; b) Survival rate of *E. formosa,* the constant is 155.98, deviance 143.99, $P = 0.71$, $df = 154$.

Figure 1.

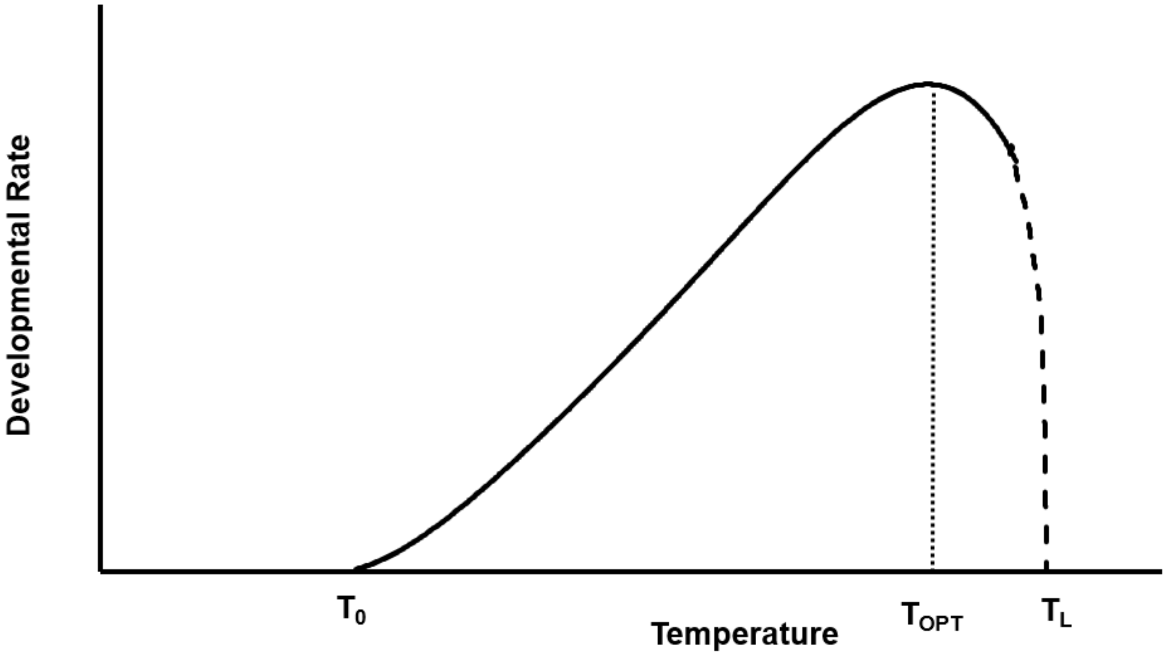


Figure 2.

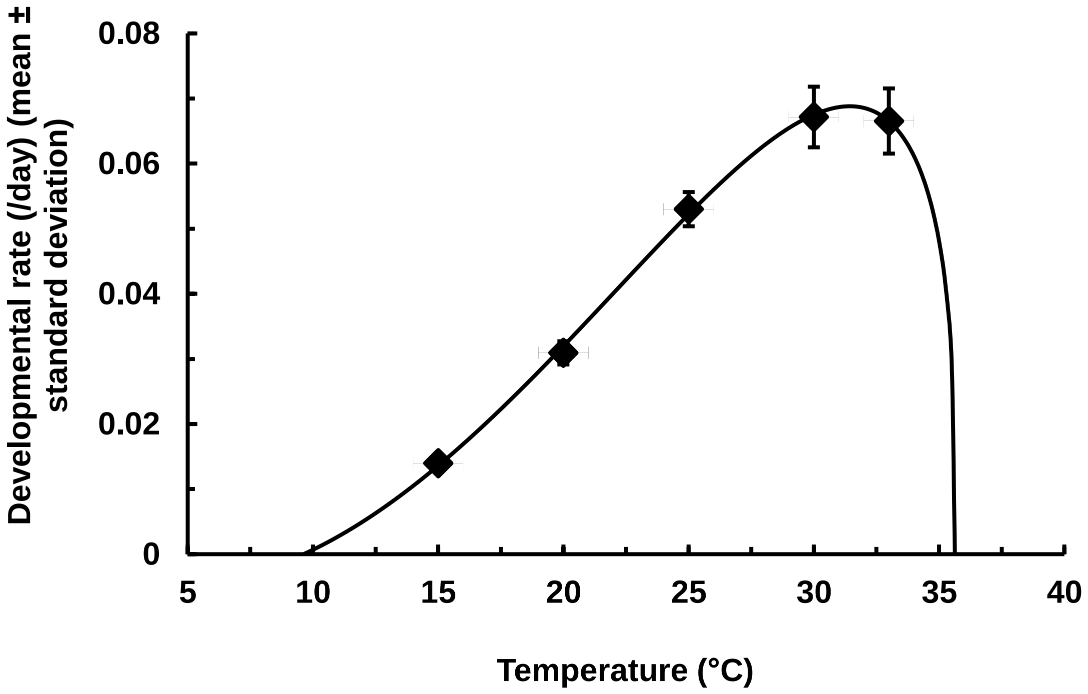

0.08
0.06
0.04
0.02
0
Developmental rate (/day) (mean ± standard deviation)
5
10
15
20
25
30
35
40
Temperature (°C)

Figure 3.

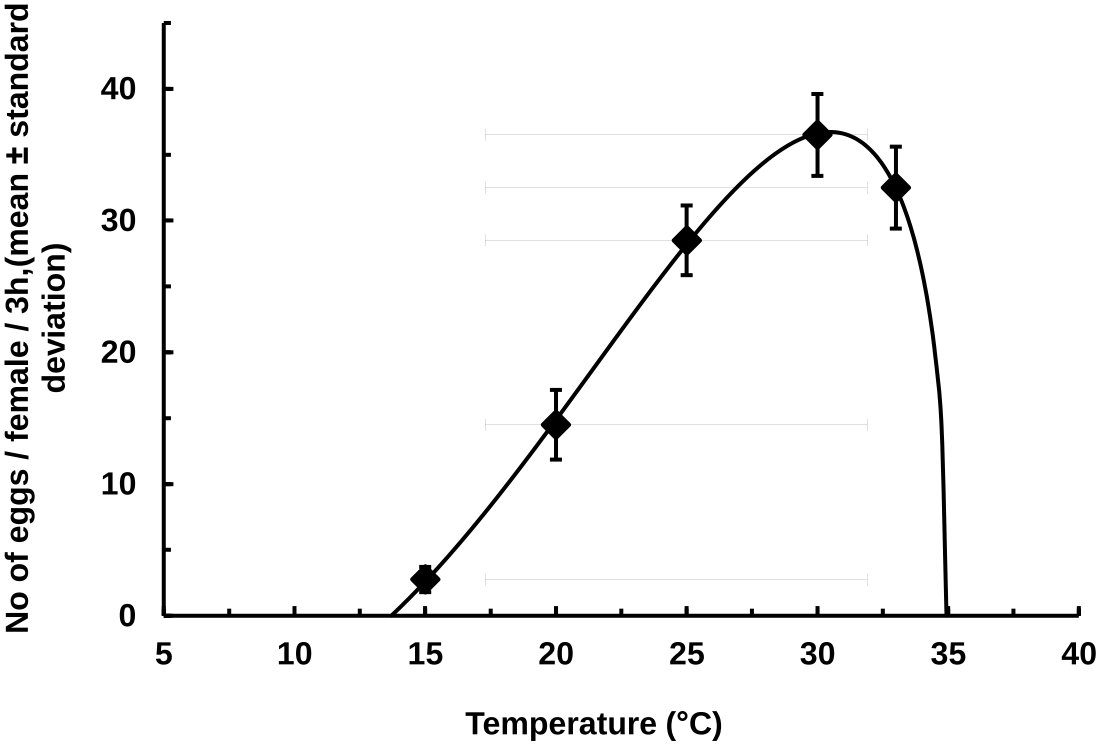

No of eggs / female / 3h,(mean ± standard deviation)
40
30
20
10
0
5
10
15
20
25
30
35
40
Temperature (°C)

Figure 4.

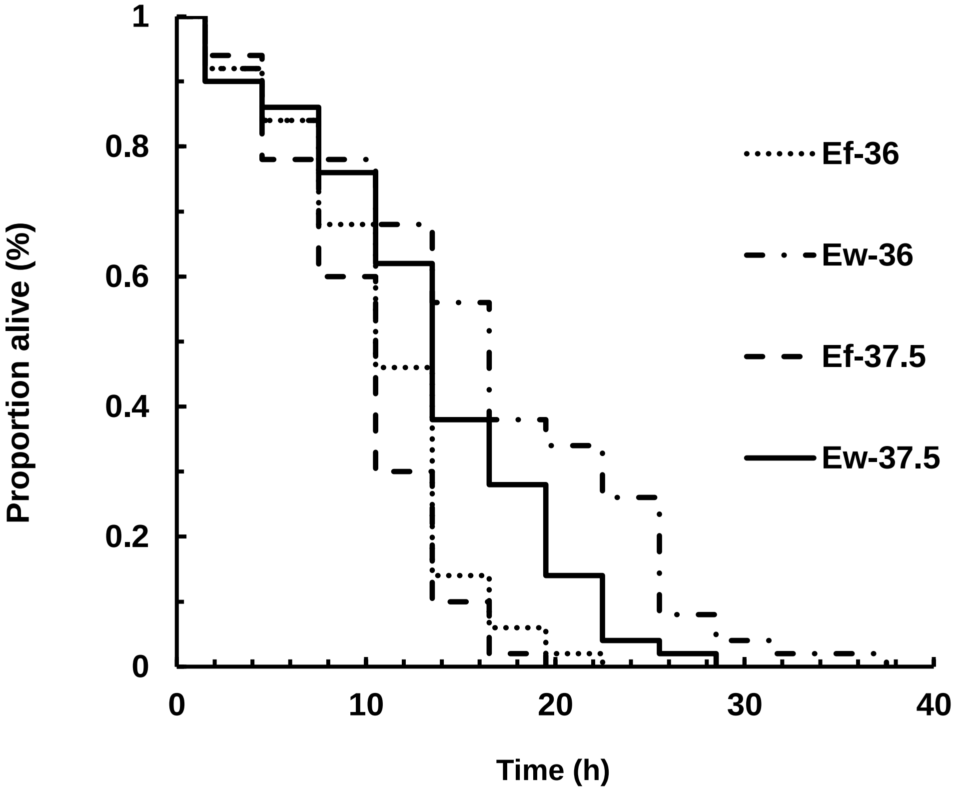
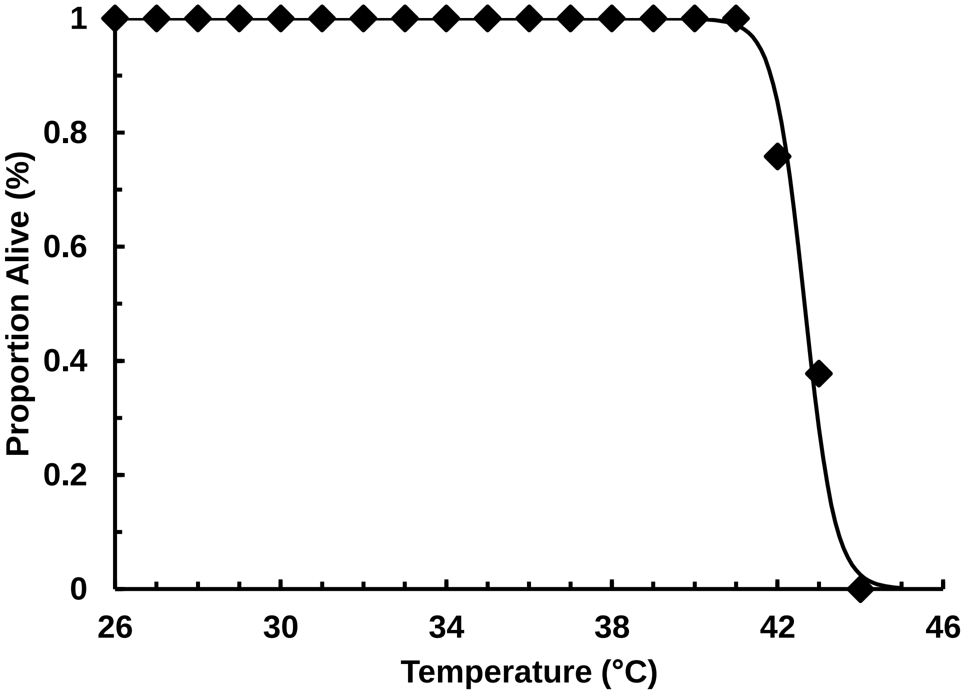

1
0.8
0.6
0.4
0.2
0
Proportion alive (%)
0
10
20
30
40
Time (h)
Ef-36
Ew-36
Ef-37.5
Ew-37.5

Figure 5.

a. E. warrae



b. En. formosa

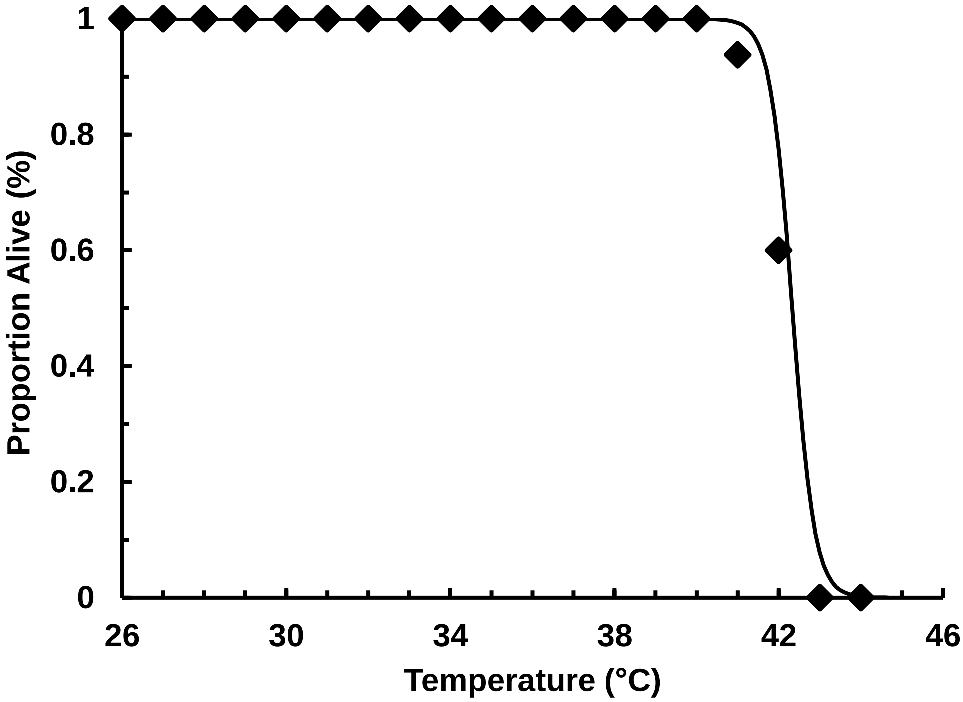


Table 1. The effects of fluctuating temperature on developmental rate (mean ± SD) of *Eretmocerus warrae* fit in the model of constant temperature. The average temperature for 33 / 26 °C and 25 / 15 °C Light / Dark is 29.67 °C and 20.83 °C. The standard deviations were ≤ 0.001 / day, thus no error bars are visible for both sets. The predicted developmental time was obtained from the constant temperature experiment using Briere model, where a = 0.00006, m = 3.07, T_0 = 9.62 and T_L = 35.63, see experiment 2.3.1. (Fig. 2).

Temperature design (°C)		**Developmental time (Days)**	
14 h Light	**10 h Dark**	**Observed**	**Predicted**
33.0	**26.0**	**14.98 ± 0.23 (88)**	**14.94**
25.0	**15.0**	**27.93 ± 0.90 (106)**	**28.25**

Table 2. The effects of high temperature on emergence and development of greenhouse whitefly. Values are mean ± standard deviation (sample size).

Temperature (°C)	**Mean number of adult greenhouse whiteflies emerged (max = 100)**	**Duration from 2nd instar to adult (Days)**
30.0	**44.25 ±5.56 (400)**	**5.63 ± 0.52 (177)**
33.0	**17.25 ±3.10 (400)**	**5.86 ± 0.89 (69)**
34.5	**4.5 ±1.91 (400)**	**6.31 ± 1.41 (18)**
36.0	**0 (400)**	**0**
37.5	**0 (400)**	**0**

Table 3. The effects of high temperature on emergence and development of *Eretmocerus warrae* and *Encarsia formosa*. Values are mean ± standard deviation.

Temperature (°C)	**No. of *E. warrae* emerged**	**Developmental Time of *E. warrae* (day^{-1})**	**No. of *En. formosa* emerged**	**Developmental Time of *En. formosa* (day^{-1})**
30.0	25.8 ± 3.8	14.46 ± 1.27 (103)	25.5 ± 4.2	14.31 ± 1.28 (102)
33.0	12.0 ± 3.1	14.34 ± 1.23 (48)	8.5 ± 2.5	15.79 ± 1.44 (34)
34.5	4.8 ± 1.6	15.58 ± 2.13 (19)	0	0
36.0	0	0	0	0
37.5	0	0	0	0

CHAPTER THREE

The effects of temperature on body-size, longevity and life-time oviposition of *Eretmocerus warrae*

Statement of Authorship

The effects of temperature on body-size, longevity and life-time oviposition of *Eretmocerus warrae*

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For submission to Bulletin of Entomological Research

TW conceived, designed and carried out the experiments, generated and analysed the data, and wrote the manuscript. MK reviewed and provided advice on the design of experiments, assisted data analysis, and reviewed and suggested significant changes to the manuscript.

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Statement of Authorship

Title of Paper	The effects of temperature on body-size, longevity and life-time oviposition of *Eretmocerus warrae*
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	For submission to *Bulletin of Entomological Research*

Principal Author

Name of Principal Author (Candidate)	Tao Wang		
Contribution to the Paper	Conceived, designed and carried out the experiments, generated and analysed the data, and wrote the manuscript.		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	23 December 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Michael A. Keller		
Contribution to the Paper	Reviewed and provided advice on the design of experiments, assisted data analysis, and reviewed and suggested significant changes to the manuscript.		
Signature		Date	23 December 2016

Abstract:

Eretmocerus warrae (Hymenoptera: Aphelinidae), is a specialist parasitoid that is used for the control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). The body-size of both this parasitoid and its host are negatively influenced by temperature. Body volume indices that reflect body sizes roe by 274.1 % in *E. warrae,* compared to 91.1 % in *T. vaporariorum* when temperature rose from 32 to 20 °C. The life-time oviposition of female adult of *E. warrae* that grew at immature developmental temperature of 20 °C was 86 ± 22 eggs, more than that 66 ± 11 eggs at 26 °C and 65 ± 23 eggs at 32 °C. Besides the influence on fecundity, temperature also influences the oviposition behaviour at the adult stage. More eggs were oviposited at 20 and 26 °C than at 32 °C. Life-time oviposition was shown to be influenced by the temperatures experienced by immature and adult wasps. Higher temperature reduced survival in the immature developmental stages and longevity in adults. Adult females lived a maximum of 8.9 ± 1.8 days at 20 °C and laid a maximum of 97.4 ± 23.2 eggs when reared at 20 °C and held at 26 °C as adults. Adult body-size is positively correlated with life-time oviposition but not adult longevity.

Key Words:

Trialeurodes vaporariorum, fitness, fecundity, immature developmental temperature, aphelinid

Introduction:

Temperature is an ecological factor that moderates many aspects of insect fitness, including body-size, longevity and oviposition. Insects are poikilothermic, so temperature influences physiology and development. Immature development in relatively cooler conditions often leads to a larger body-size (Nunney and Cheung, 1997, Angilletta et al., 2004). This change in body-size affects fecundity in a more complicated manner. Angilletta et al. (2004) found that individuals grow slower in colder ambient environments, and argued that there should be no substantial increase in fecundity in such conditions. However, adult insects are usually more active in warmer conditions, so higher temperatures generally lead to a higher oviposition rate (Bale et al., 2002). Warmer environmental conditions result in higher levels of metabolism and higher developmental rates. Thus the longevity of insects normally has an inverse relationship with the ambient temperature (Smith, 1958). Therefore temperature can affect the number of eggs that are laid during an individual’s lifetime.

The body-size, fecundity and longevity of insects are interrelated. Generally, a larger body-size means more ovarioles (Honěk, 1993) and a higher fecundity (Roff, 2002). Being larger can also result in greater longevity (Miller and Thomas, 1958). Moreover, there are potential trade-offs between fecundity and longevity. Higher fecundity might result in a higher oviposition rate and cause higher energy consumption, and thus reduce longevity. Longer lifespans in insects gives them more chances to oviposit and usually lead to a higher life-time oviposition (Blackburn, 1991). Kingsolver and Huey (2008) concisely summarised these aspects of the biology of ectotherms in three “rules”: bigger is better, hotter is smaller, and hotter is better.

Temperature affects parasitoids directly and also indirectly through the responses of host insects and plants. Plants can display physical differences and chemical changes in response to temperature (Berry and Bjorkman, 1980). These changing plant characteristics directly influence the development and behaviour of host insects, and thus influence the parasitoids directly or indirectly. The size and quality of host insects will also vary under different temperature conditions. Hosts that are larger and offer better quality resources to parasitoids generally deliver better fitness gains to parasitoids that develop on them (Godfray, 1994). Host size is particularly key to solitary species of parasitoids, since parasitoids that emerge from larger hosts tend to be larger. However, the level of host acceptance might be lower since there might be higher levels of defence of larger hosts that are associated with higher fitness gain.

The research reported here investigated how temperature affects the body-size, life-time oviposition and longevity of an idiobiont parasitoid and its host. Of particular interest is oviposition over the life-time of parasitoids, which is directly is related to life-time parasitism of hosts, and therefore contributes to the level of biological control of host insects. It is also valuable to understand how temperature affects the biology of parasitoids, to assist with developing optimal rearing procedures for commercial production.

The model parasitoid used in this research was *Eretmocerus warrae* (Hymenoptera: Aphelinidae) (Nauman & Schmidt). Its host insect is greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). Greenhouse whitefly attacks a wide variety of agricultural and horticultural plants from 249 genera and 84 angiosperm plant families (Russell, 1977). Tomato, *Solanum lycopersicum* L, was the host plant used in this investigation. *E. warrae* has been developed as a commercial product by Biological Services (Loxton, South Australia). There has been limited research on *E. warrae* and its role in the biological control of whiteflies. Notably there has been no investigation of the influence of temperature on its body-size, longevity and life-time fecundity. This research will help with understanding the effects of temperature on different aspects of the fitness of *E. warrae* and its host. It will also be useful in rearing and release of this parasitoid in commercial greenhouses.

2. Methods

2.1. Rearing insects

The leaves of tomato plants (‘Improved Appolo’) around 50 cm tall with five fully expanded leaves were used in experiments.

A culture of greenhouse whitefly was established from insects that were collected from eggplant, *Solanum melongena*, in greenhouses at the Waite Campus of The University of Adelaide. The whitefly culture was maintained on tobacco, *Nicotiana tabacum*, at 26 °C and a 14 L: 10 D photoperiod.

E. warrae prefers to parasitise the 2nd instar nymphs of greenhouse whitefly. To obtain cohorts of 2nd instar nymphs, tomato plants were exposed to ovipositing adult whiteflies for six hours. Then the adults were blown away with a hair dryer. These plants were grown at selected experimental temperatures until the whitefly nymphs reached the appropriate stage.

A breeding culture of *E. warrae* was initiated with pupae provided by Biological Services (Loxton, South Australia). It was maintained at 26 °C. When necessary, the pupae of *E. warrae* were kept in an incubator at 8 °C to arrest development. When parasitoids were needed for experiments, pupae were moved out of the 8 °C incubator at 20:00 h to another incubator which was set at 26 °C to stimulate them to emerge. Most *E. warrae* emerge during the morning. Honey drops were provided as food for adults.

Circular clip cages were used to confine experimental insects that had inside and outside dimensions of 40 mm and 55 mm, respectively. They were made of 12 mm polyethylene foam. There was a transparent cellulose acetate sheet on bottom of each cage which allowed wasps to be observed and fine organza on top for aeration. An aspirator made of plastic tubing was used to handle wasps. Honey drops were placed on the organza of clip cages as food for *E. warrae*.

2.2. Experiments

A two-stage experiment was conducted to determine the effects of temperature on both the immature and adult stages of *E. warrae*. The experiment was conducted using three constant temperatures, 20, 26 and 32 °C.

To determine the effects of temperature on the growth of 2nd instar greenhouse whitefly, 2nd instars, which were not exposed to parasitoids, were chosen randomly for body-size measurements. The length and width of each nymph was measured using an ocular micrometer on a dissecting microscope at a magnification of 80 *, and an index of body volume was calculated as (length * width) $^{3/2}$. The body volume index should be proportional to the actual body volume. Variations in body size measurements of greenhouse whitefly among temperatures were subjected to regression analyses with linear and quadratic terms using R version 3.1.1 (2014-07-10). In all cases, parameter estimates are given as mean ± standard deviation since the aims were to estimate life history characteristics.

The effects of temperature on the development, fecundity and longevity of *E. warrae* were evaluated as follows. 2nd instar host nymphs were exposed to adult *E. warrae* for six hours in clip cages. The wasp numbers in each clip cage were 4, 2, and 2 at 20, 26 and 32 °C, respectively, since the rate of oviposition was lower at 20 °C. A new replicate was initiated once each week, for five weeks.

Five adult wasps each were chosen randomly when they emerged and placed in individual clip cages at 20, 26 and 32 °C. One tomato leaf infested with 2nd instar greenhouse whitefly nymphs was provided each day until the wasp died. After exposure to wasps, whitefly nymphs were turned over using an insect-mounting pin to count the number of eggs laid.

The head width and hind-tibia length of each dead wasp were measured using an ocular micrometer as previously described. The longevity of these wasps was also recorded. Data were analysed using regression analysis on linear, quadratic and interaction terams of temperature during immature development and adult life using R version 3.1.1 (2014-07-10).

3. Results

3.1. Effect of temperature on body-size of greenhouse whitefly and *Eretmocerus warrae*

The body-sizes of nymphs of greenhouse whiteflies and adult *E. warrae* were affected by temperature. Regression analysis indicated statistically significant ($P < 0.001$) linear and quadratic responses in body sizes to varying temperature, which indicates overall nonlinear responses to temperature. Observed body lengths and widths of greenhouse whiteflies were smallest at 32 °C (Fig. 2a). Likewise, observed head widths (Fig. 2b) and hind tibia lengths (Fig. 2c) of *E. warrae* were smallest at 32 °C. Body volume indices derived from linear body measurements also varied in a nonlinear manner. The greatest decline in the body size of greenhouse whitefly occurred between 26 and 32 °C, whereas the sharpest decline for *E. warrae* occurred between 20 and 26 °C (Fig. 3).

3.2. The effects of temperature on life-time oviposition and longevity of *Eretmocerus warrae*

Life-time oviposition

The temperature during immature development and the adult stage interacted to influence both the life-time oviposition and adult longevity of *E. warrae*. The maximum observed oviposition was 97.36 ± 23.20 (mean ± SD) eggs / female when the immature stages were reared at 20 °C and adults were held at 26 °C ($P < 0.05$, Fig. 4). Oviposition was greatest in adults that were reared at 20 °C and declined as temperature increased up to 32 °C, while it was similar when the temperature adults were held at was 20 and 26 °C and then lower when adults were exposed to 32 °C.

Adult longevity

Adult longevity displayed a more complex pattern of response to temperature. Longevity decreased as the temperature adults were held at increased. However, this pattern was not consistent across the range of temperatures at which immatures were reared. This is reflected in the greater longevity of adults when they were reared at 32 °C and held at 26 and 32 °C, than adults that were reared at 26 °C and held at the same temperatures.

3.3. The effects of temperature on the number of eggs laid each day by *Eretmocerus warrae*

There was a consistent complex pattern of daily oviposition by *E. warrae* after emergence. Regardless of temperature conditions, the largest number of eggs was laid on the first day after emergence and numbers declined thereafter. Females laid more eggs at 26 °C than at 20 and 32 °C on the first and second days (Fig. 5). By the third day, females that were held at 20 °C laid the most eggs and numbers were progressively lower at 26 and 32 °C.

3.4. The effects of body-size on the life-time oviposition and longevity of adult *Eretmocerus warrae*

The body-size of *E. warrae* was positively correlated with the total number of eggs laid, but the pattern depended on the temperature of immature development (Fig. 6). No such correlations were found when longevity was analysed against head width and hind-tibia length.

4. Discussion

Temperature influences the body-sizes of the host and parasitoid in different ways. In both cases, body size was largest when development occurred at 20 °C, and declined as temperature increased (Fig. 2 and 3). Larger body size is generally associated with greater fitness (Kingsolver and Huey, 2008). Body-size is an essential indicator of the quality of a parasitoid (Sagarra and Stewart, 2001). *E. warrae* grew to a relatively larger size as temperature decreased from 26 to 20 °C, which could be useful in practice. If the relative size of the parasitoids compared to the host is larger, then this could lead to more effective biological control. However, there are many other factors are involved in practice, and the costs and benefits of producing wasps of different sizes should be analysed systematically to determine whether it is profitable in a commercial environment.

Besides body-size, the life-time oviposition and longevity of *E. warrae* were also highest at 20 °C. Temperature during both immature development and the adult stage affected oviposition (Fig. 4 and 5). The pattern of oviposition over the lifetime of suggests that there is an interaction between the fecundity of females, which is largely established during immature development, and the effects of temperature on adult behaviour and activity levels during the adult stage (Fig. 6). The increased longevity of wasps that were reared and held at 32 °C as adults compared to those that were reared at 26 °C and held at 32 °C suggests that higher levels of oviposition reduce longevity, perhaps as a result of energetic or nutrient costs. The life-time oviposition of *E. warrae* is closely related to fecundity and parasitism when hosts are present. The life-time oviposition of 93.16 eggs of *E. warrae* of at 20 °C is greater than the congeneric species *E. eremicus* and *E. mundus*, which are only 13 and 43 at 20 °C, respectively (Qiu et al., 2004). The latter two *Eretmocerus* species are effectively used in commercial greenhouses in Europe, which suggests that *E. warrae* has promise in Australia.

E. warrae lays more eggs per day in the first two days of adult life than *Encarsia formosa*, which only produces eight eggs each day (Kajita and van Lenteren, 1982). The results indicate the potential of using these two parasitoid together to control greenhouse whitefly in greenhouses, where *E. warrae* could be more effective when the pest density is high. The complementarity of *En. formosa* and *E. eremicus* in European greenhouses (Qiu et al, 2004), suggests that similar complementarity should be achieved with *En. formosa* and *E. warrae* in Australia.

Being large is generally superior in many respects, including greater fecundity and longevity (Kingsolver and Huey, 2008). This is the case with *E. warrae*, which grew larger at low temperature during the immature stages, and generally have a higher life-time oviposition and longevity. Adult size positively influences fitness of parasitoid females (King, 1987; Visser, 1994). Besides fecundity and longevity, the effects of body-size on searching behaviour in this parasitoid warrant further investigation.

Acknowledgments

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Figure legends

Figure 1. Experimental design: different stage, temperature and elements measured.

Figure 2. Effects of immature developmental temperature on body-size of greenhouse whitefly and *Eretmocerus warrae* (mean ± SD). (a). Body length and width of greenhouse whitefly; (b). Head width of *E. warrae*; (c). Hind-tibia length width of *E. warrae*.

Figure 3. Body volume indices of *Eretmocerus warrae* and greenhouse whitefly. Values followed by different letters are significantly different ($P < 0.001$).

Figure 4. Effects of immature developmental temperature on life-time oviposition of *Eretmocerus warrae* (mean ± SD). Values followed by different letters are significantly different ($P < 0.01$).

Figure 5. Effects of adult temperature on life-time oviposition and longevity of *Eretmocerus warrae*. (a). Life-time oviposition (mean ± SD); (b). Longevity (mean ± SE). Values followed by different letters are significantly different ($P < 0.01$).

Figure 6. The number of eggs laid each day (± SD). (a). Immature development at 20 °C; (b). Immature development at 26 °C; (c). Immature development at 32 °C. For all three figures, to prevent overlaps, $(x + 0.2)$ for adults exposed at 20 °C and $(x – 0.2)$ for adults exposed at 32 °C.

Figure 7. Effects of body-size on life-time oviposition of *Eretmocerus warrae.* (a). Head width on life-time oviposition; (b). Hind-tibia length on life-time oviposition.

Figure 1. Experimental design

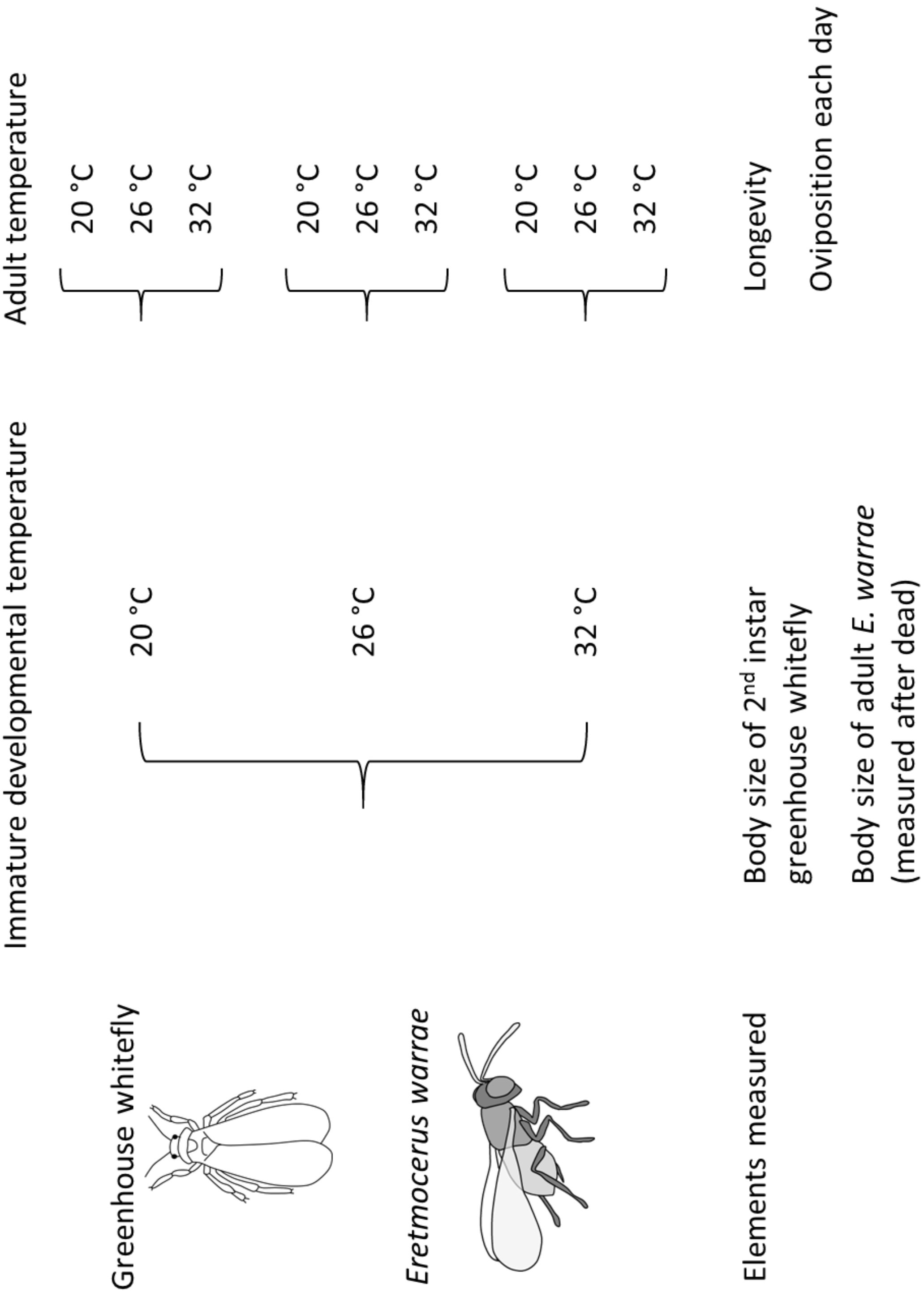
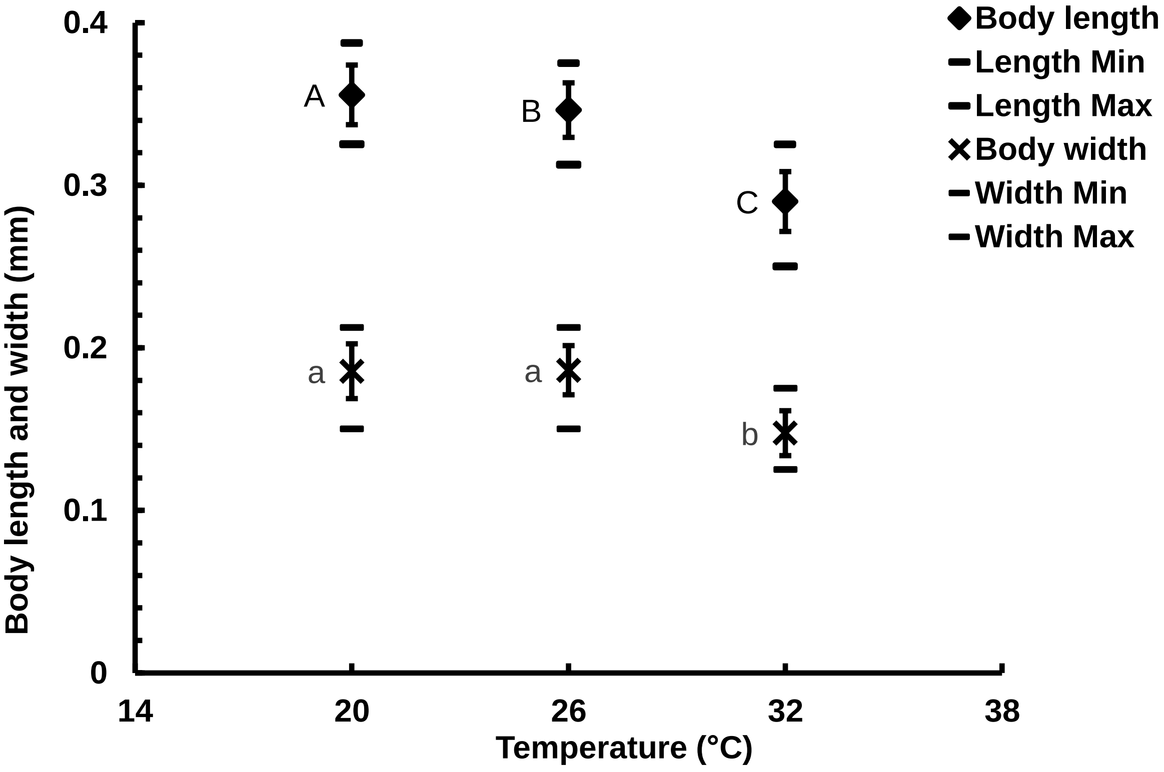
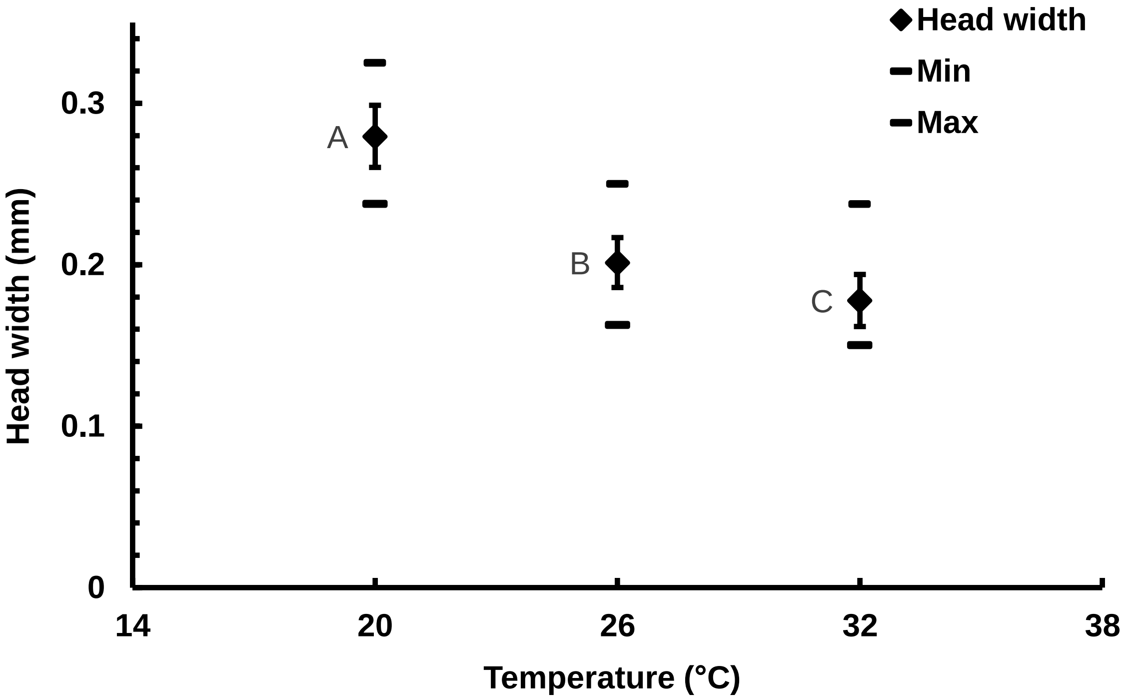


Figure 2. Effects of immature developmental temperature on body-size of greenhouse whitefly and *Eretmocerus warrae* .Values followed by different letters are significantly different (*P* < 0.001).

(a). Body length and width of 2nd instar greenhouse whitefly (mean ± SD). Values followed by the same letter did not differ statistically (*P* ≥ 0.05).



(b). Head width of *E. warrae* (mean ± SD).



(c). Hind-tibia length width of *E. warrae* (mean ± SD).

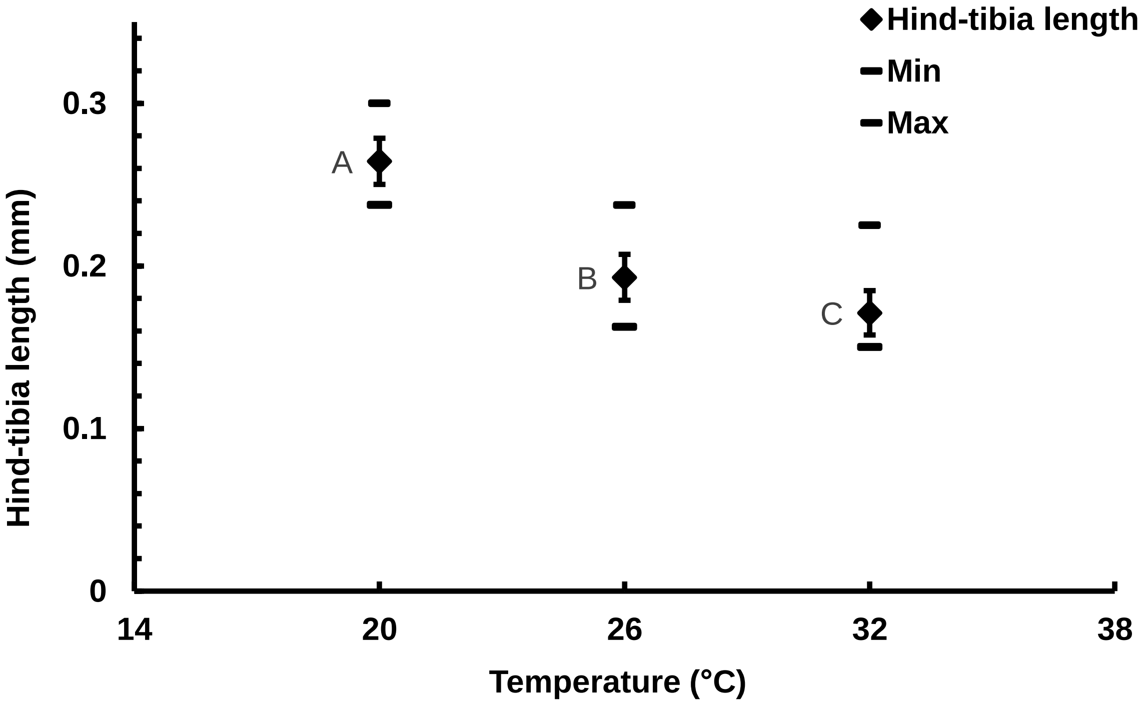


Figure 3. Body volume indices of *Eretmocerus warrae* VS greenhouse whitefly (mean ± SD). The body volume index was calculated as (body length * body width) ^ (3/2) for greenhouse whitefly and (head width * hind-tibia length) ^ (3/2) for *E. warrae*.

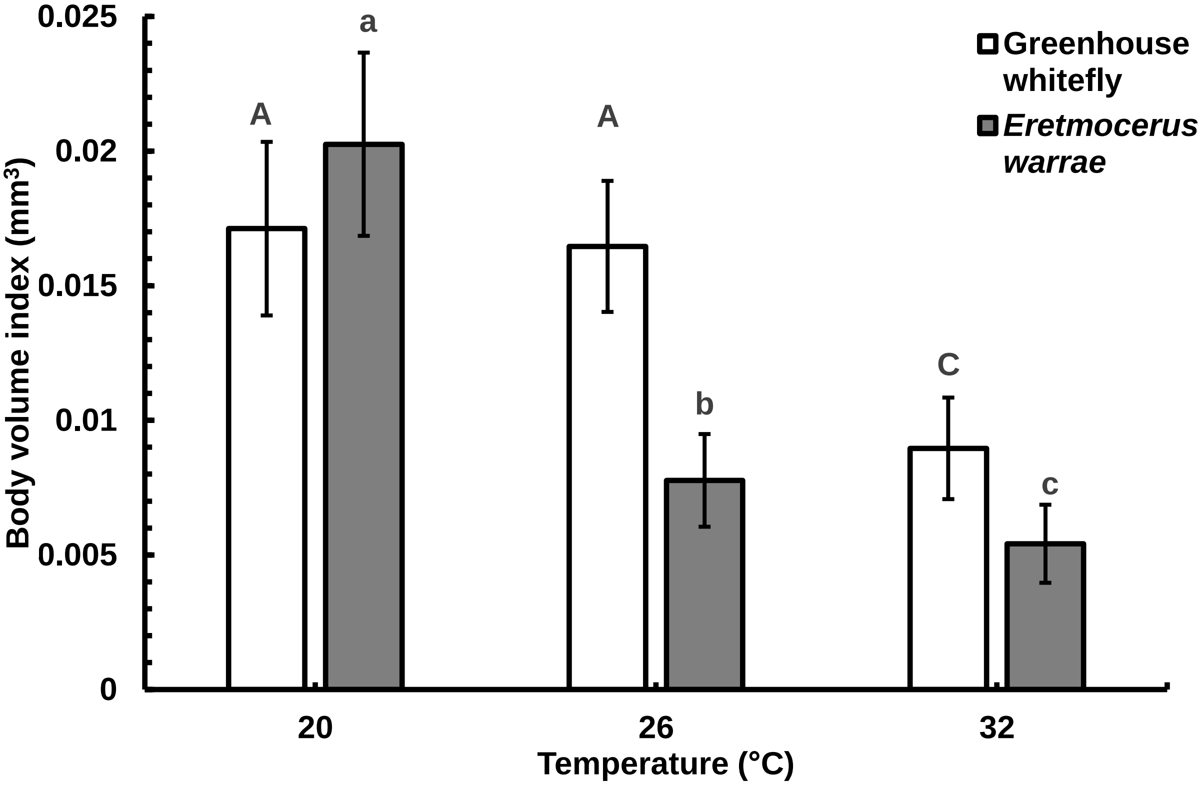


Figure 4. Effects of immature developmental temperature on life-time oviposition of *Eretmocerus warrae* (mean ± SD). Values followed by different letters are significantly different (P < 0.01).

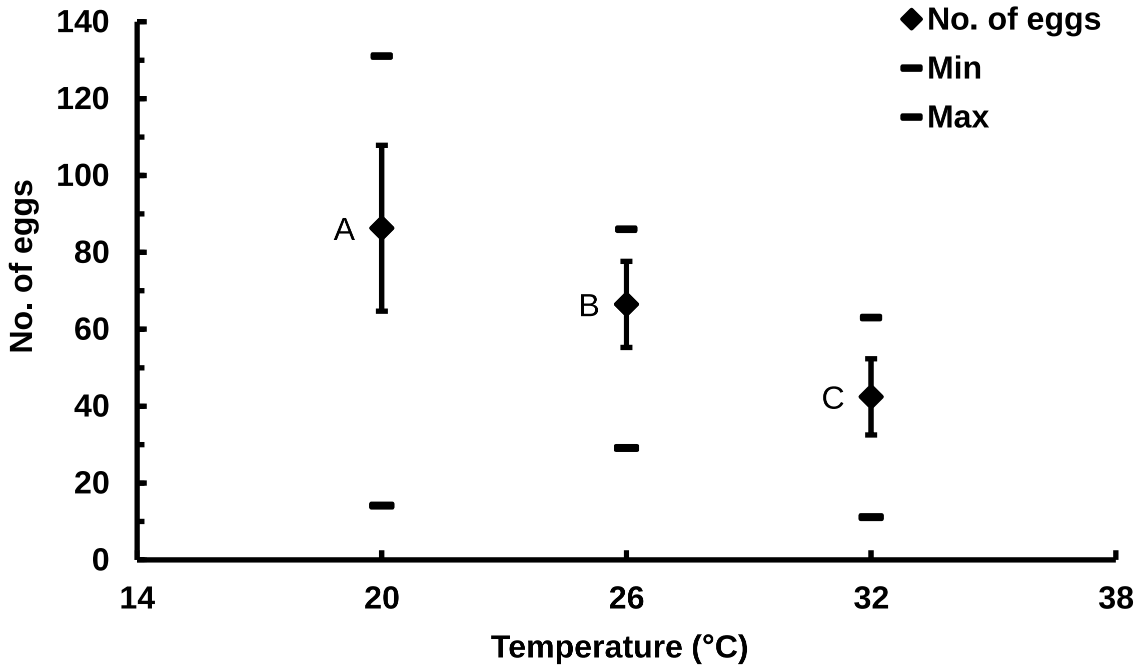
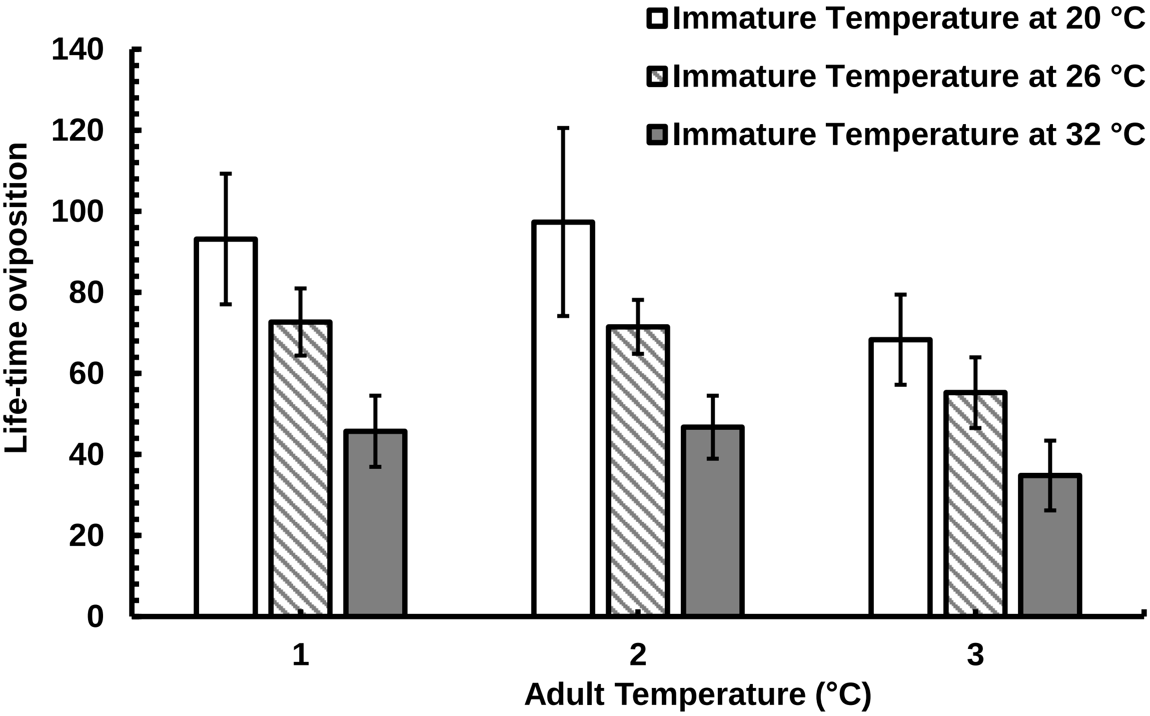


Figure 5. Effects of adult temperature on life-time oviposition and longevity of *Eretmocerus warrae.* Values followed by different letters are significantly different compared with the same immature developmental temperature group (P < 0.01).

(a). Life-time oviposition (mean ± SD)



(b). Longevity (mean ± SE)

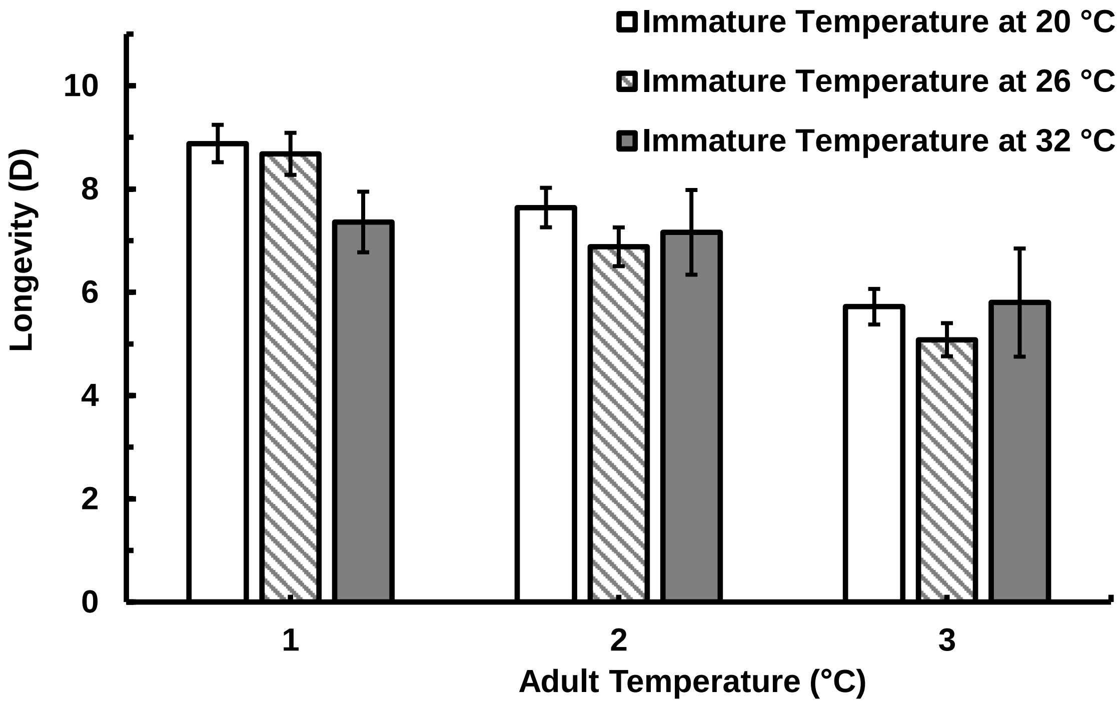
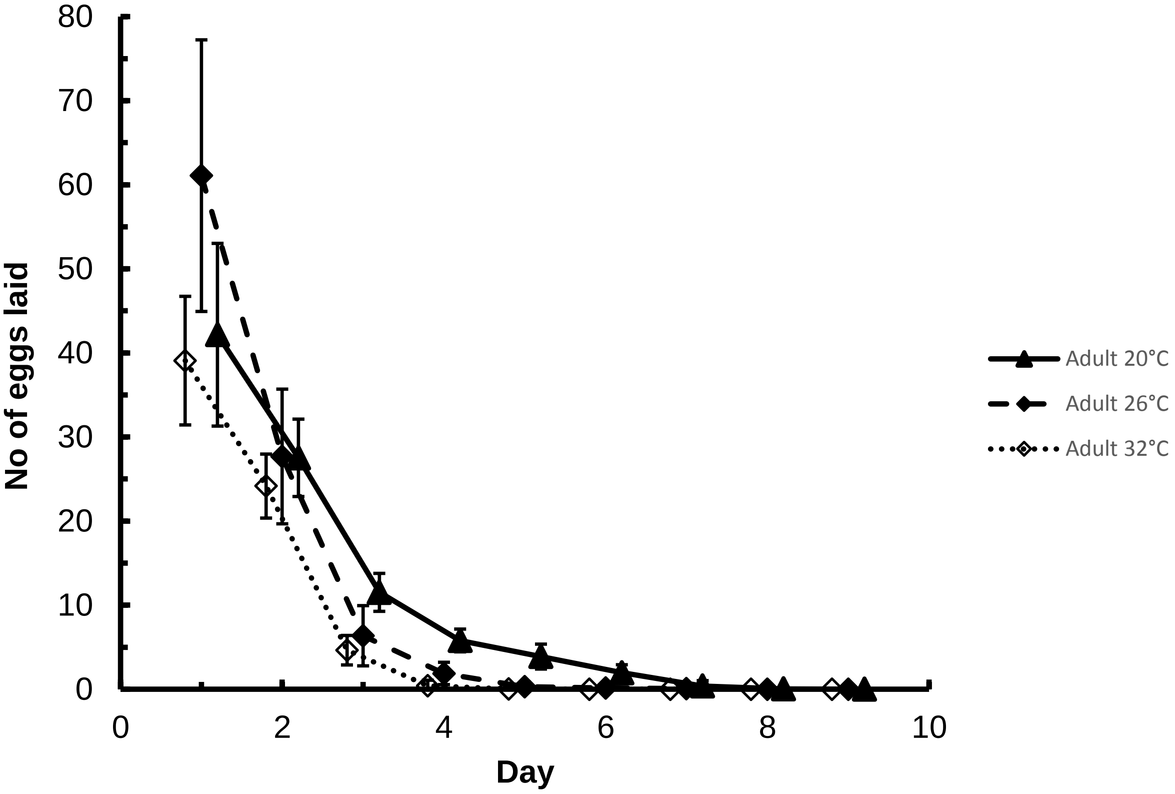
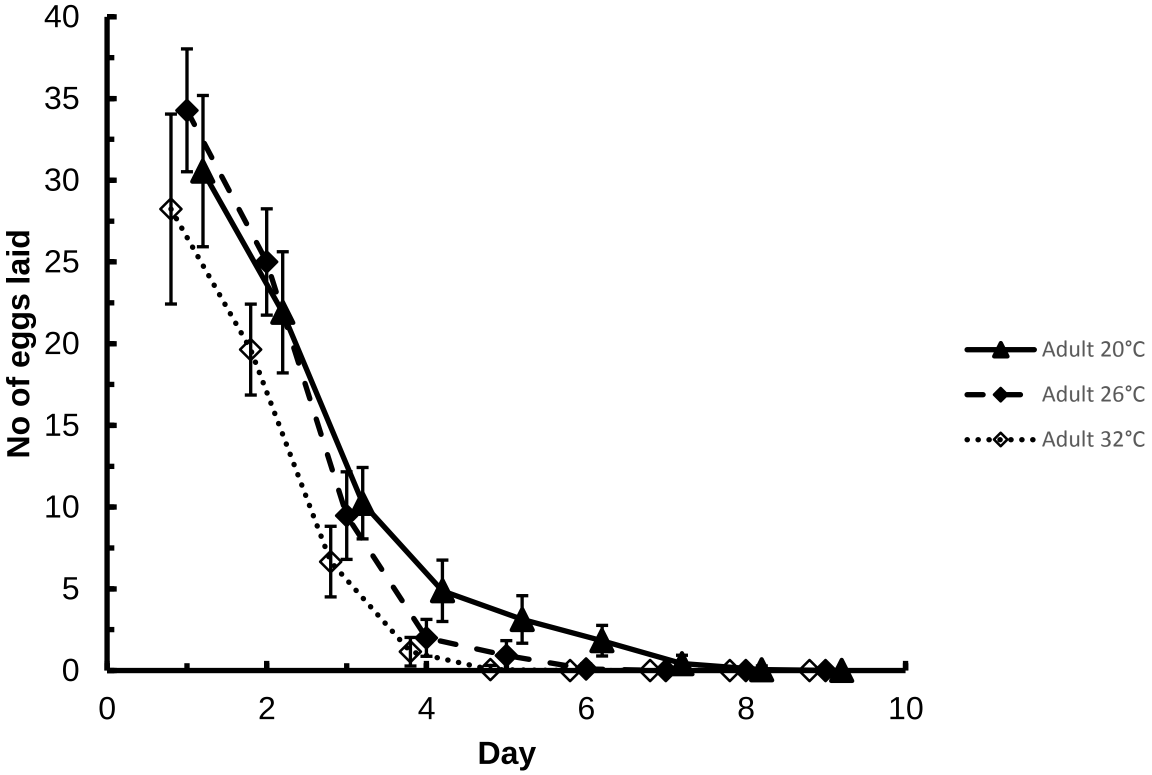


Figure 5. The number of eggs laid each day (mean ± SD). Some values are offset to enhance clarity, (+ 0.2 days at 20 °C and – 0.2days at 32 °C).

(a). Immature development at 20 °C



(b). Immature development at 26 °C



(c). Immature development at 32 °C

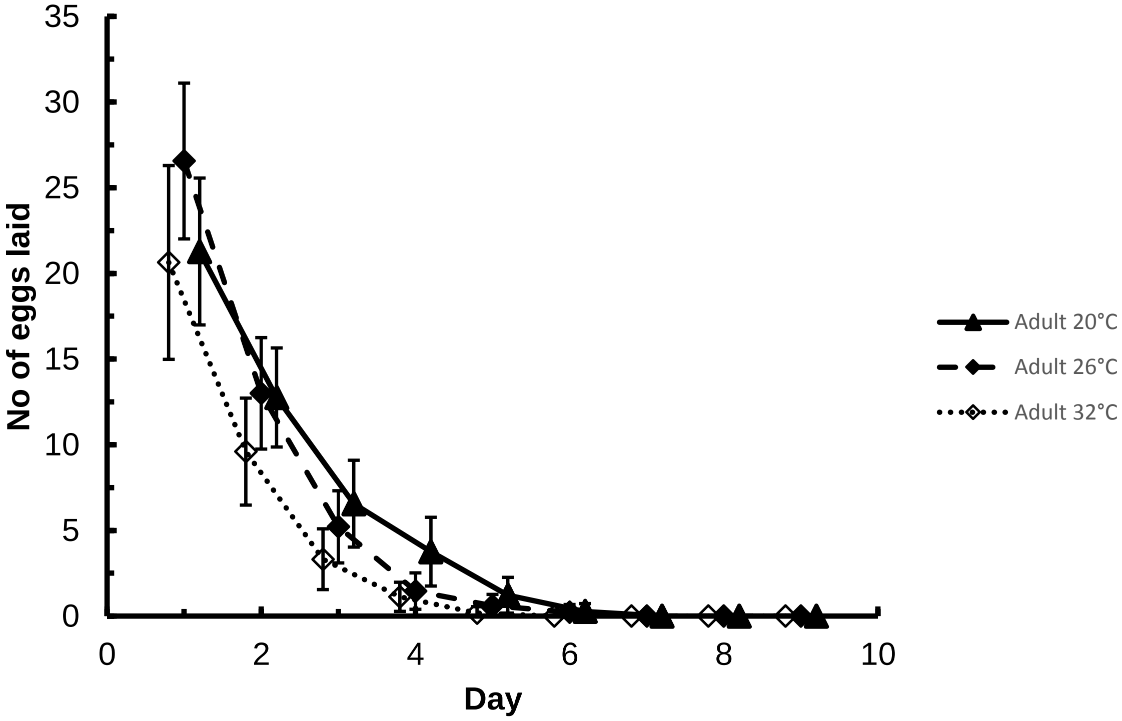
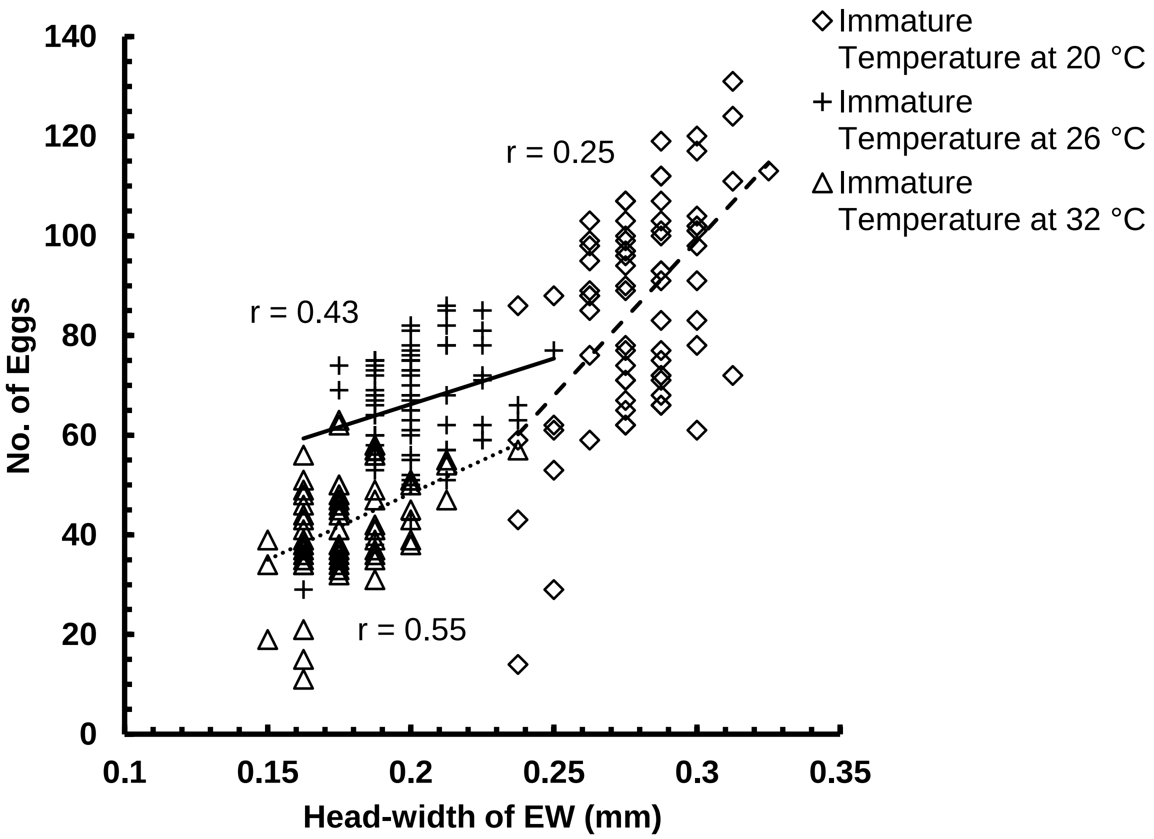
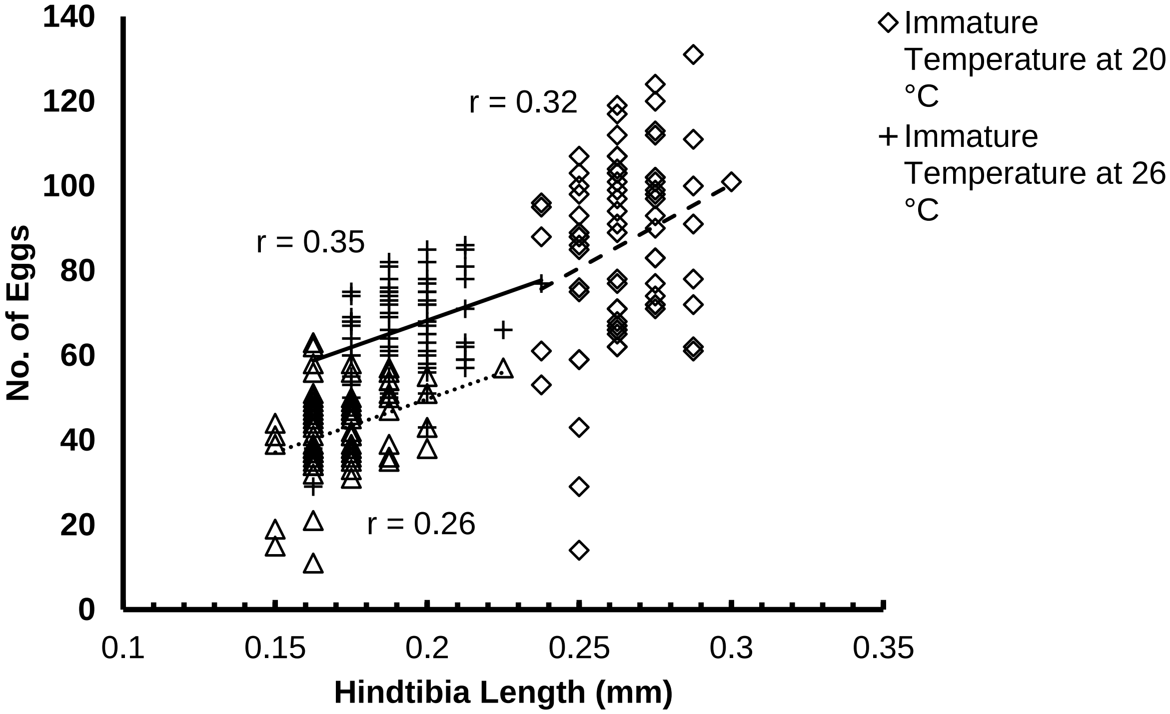


Figure 6. Effects of body-size on life-time oviposition of *Eretmocerus warrae*.

(a). Head width on life-time oviposition;



(b). Hind-tibia length on life-time oviposition



CHAPTER FOUR

Effects of relative body-size on the host stage choices of parasitoids: Developmental temperature affects interactions between *Eretmocerus warrae* and its host

Statement of Authorship

Effects of relative body-size on the host stage choices of parasitoids: Developmental temperature affects interactions between *Eretmocerus warrae* and its host

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For submission to Journal of Pest Science

TW conceived, designed and carried out the experiments, generated and analysed the data, and wrote the manuscript. MK reviewed and provided advice on the design of experiments, assisted data analysis, and reviewed and suggested significant changes to the manuscript.

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Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Tao Wang		
Contribution to the Paper	Conceived, designed and carried out the experiments, generated and analysed the data, and wrote the manuscript.		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	23 December 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Michael A. Keller		
Contribution to the Paper	MK reviewed and provided advice on the design of experiments, assisted data analysis, and reviewed and suggested significant changes to the manuscript.		
Signature		Date	23 December 2016

Abstract:

Experiments were conducted to investigate the effects of (1) host stage on the behaviour and development of *Eretmocerus warrae* (Hymenoptera: Aphelinidae), and (2) the body-size of adult *E. warrae* relative to its host on the behaviour and fitness of this parasitoid. In both no choice and choice tests, *E. warrae* preferred the 2nd instar of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) for parasitism. The behavioural patterns of the parasitoid were observed when handling different instars of hosts. The significantly largest proportion of behaviour was devoted to stinging (40.7 %) when the second instar hosts were provided to parasitoids. The host acceptance (84.1 ± 8.9 %) and emergence rates (83.2 ± 7.8 %) from second instar hosts were also the highest, which indicated that second instars are the preferred stage to attack.

The body size of *E. warrae* is affected by temperature during immature development. Wasps that develop at 20 ^{o}C are larger than those that develop at 32 ^{o}C. The relative body-size of the parasitoid compared to its host was shown to affect the host stage choice of the parasitoids. Smaller wasps preferred smaller host instars while larger wasps preferred larger hosts. However, both small and large wasps parasitised 2nd instar hosts most frequently. The behavioural patterns of small and large wasps handling second instar hosts were compared. Smaller wasps spent longer periods on antennating hosts than larger ones. Despite displaying similar proportions of the sting behaviour, the frequencies of stinging behaviour of larger parasitoids (35.4 ± 4.1 acts / h) were much higher than those of smaller ones (17.4 ± 6.0 acts / h), which reflected the greater fecundity and oviposition activity of larger wasps. In addition, experience was shown to reduce the duration of stinging behaviour of *E. warrae*.

Key Words:

Host stage, body-size, *Trialeurodes vaporariorum*, aphelinid, behaviour pattern, flow diagram

Introduction:

The relationship between size and fitness in parasitic wasps (Visser, 1994) and more generally ectotherms (Kingslover and Huey 2008) is well established. Larger parasitoids typically live longer and have higher fecundity. Within a species, body size, in addition to the rate of development, can be influenced by temperature during development (Angilletta et al, 2004). Thus there can be a range of effects of temperature on the fitness of parasitic wasps.

Eretmocerus warrae (Hymenoptera: Aphelinidae) (Nauman & Schmidt) is a parasitoid of greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), which is one of the most damaging pest in greenhouses (Russell, 1977). It is suspected to be a better biological control agent at high temperatures than *Encarsia formosa* (Hymenoptera: Aphelinidae), a wasp which is widely sold for control of greenhouse whitefly. *E. warrae* has been commercialised in Australia because it is suspected to be a better biological control agent than *Encarsia formosa* (Hemiptera: Aleyrodidae) at high temperatures (Biological Services, 2015). Experiments have elucidated how temperature affects the development, survival and reproduction of *E. warrae* (Chapters 2 and 3). Adult wasps are larger when they develop at 20 ^{o}C than at higher temperatures. Larger *E. warrae* tend to live longer and have higher rates of oviposition. The observation that daily oviposition varies according to adult size suggests that other aspects of the behaviour of *E. warrae*, including the host stage choice, are likely to vary with adult size.

When confronted different stages of hosts, parasitoids need to make choices among them. Theoretically, parasitoids should select the stages that can lead to higher levels of reproductive success (Godfray, 1994). Typically larger body-sized hosts serve as a better source of food (Godfray, 1994; Da Rocha, 2007). Parasitoids depend on various cues to find hosts, and different stages of hosts may produce different amounts or qualities of these cues (Mattiacci and Dicke, 1995). Some stages of hosts may be easier to find due to their associated cues, e.g., those with a larger body size should be easier to see. However, older stages of the host tend to have higher levels of host defence against parasitoids and the level of host acceptance can also vary in response to different stages (Godfray, 1994). Thus parasitoids do not always choose older and larger stages of hosts to parasitise.

To understand host stage choice and searching behaviour of parasitoids, it is essential to observe the mode of searching behaviour. Parasitoids display different types of behaviours when searching and handling different stages of hosts because they are different physically and possibly chemically. Searching behaviours of parasitoids typically involve still, walk, fly, groom, antennate and sting, and these behaviours differs between different species (Wang and Keller 2002, Yazdani et al., 2015). Recording and analysing different types and patterns of behaviour, including the duration and frequency of each type of behaviour are essential to understanding the searching strategies of parasitoids. Searching behaviour is influenced by a broad range of factors and operates at a range of spatial scales (Bell, 2012).

Parasitoids should adopt different behaviour patterns according to host stage (Bell, 2012). The older stages of hosts are generally larger in size, produces more odours and causes worse damage to plants (Thompson, 1999), and thus should be easier to detect by parasitoids. Moreover, the behaviour of parasitoids also depends on the levels of host defence, which can vary with stage (Godfray, 1994).

Host stage choice influences the fitness of parasitoids, particularly for solitary species (Visser, 1994; Harvey, 2004). The different host nutritional levels of different stages of hosts affect the fecundity, longevity and behaviour of parasitoids (Jervis, 2008). For solitary parasitoids like *Eretmocerus* and *Encarsia* species, their development totally depends on the quality of hosts. To understand host stage choices of a parasitoid, effects of different stages of hosts on the development of parasitoids need to be investigated.

The body-size difference of parasitoids might lead to different host stage choice strategies. Parasitoids of different body-size usually have different levels of fecundity and longevity (Honěk, 1993). The host choice of parasitoids can be affected by the body-size of the parasitoids in that larger parasitoids generally tend to parasitise older stages of the hosts (Müller et al., 1996). For parasitoids, being larger also means hosts are relatively smaller. Should they adjust their host stage choices according to their relative size? This question has not yet been answered and warrants investigation.

For parasitoids and hosts of different relative body-sizes, there should be differences in searching behaviours of parasitoids that relate to their host stage preferences. Larger parasitoids normally have a higher levels of searching ability and can handle hosts that have higher levels of defence behaviour (Godfray, 1994; Bell, 2012).

Parasitism experience influences the parasitism efficiency of parasitoids to a large extent. Parasitoids can improve their searching and singing efficiency through learning (Outreman, 2005). For naïve parasitoids, their lack of parasitism experience can affect the efficiency of parasitism. In comparison, experienced parasitoids can search and handle hosts easier than newly emerged individuals that have not been exposed to host cues (Bell, 1990; Outreman, 2005).

This research aimed to determine if there is a relationship between the body-size of *E. warrae* and its host stage choice, and to evaluate the searching behaviour when its body size varies.

This research will lead to refined understanding of host stage choices of *E. warrae* and the effects of developmental temperature on interactions between this parasitoid and its host. It will elucidate the effects of relative body-size on interactions between *E. warrae* and its host. This research could also be useful in informing how *E. warrae* can be used in augmentative biological control, in which farmers can release parasitoids with an optimum body-size.

2. Methods

2.1. Plants and insects

Tomato plants, greenhouse whiteflies and *E. warrae* were grown using the same methods as described in Chapter 2. The pure greenhouse whitefly culture was kept at 26 ± 2 °C, in a 14 L: 10 D photoperiod in insectary rooms.

2.2. The effects of host instar on choice and searching behaviour of *Eretmocerus warrae*

2.2.1. Host choice of *Eretmocerus warrae*

Tomato plants were exposed to greenhouse whiteflies on different days to obtain each of the stages of greenhouse whitefly at the start of an experiment. Tomato plants were put into the incubator set at 26 °C after exposure. There were 15 plants for no choice tests and five plants for choice tests.

Before each experiment, leaves with the appropriate size and density of nymphs f were chosen and marked. Excessive nymphs were flipped off the experimental leaves using a dissecting needle until the selected number of nymphs of different instars remained. In no choice tests, there were 90 nymphs of a single instar in the clip cage arena and six clip cages on each plant. Four of these clip cages were sampled to determine parasitism rates. In choice tests, there were 30 nymphs of each instar and four clip cages on each plant. The numbers of nymphs were selected based on results of a pilot experiment. Newly emerged adult *E. warrae* was kept away from hosts at 26 °C for one day before experiment (See Chapter 2). At 9:00 am next day, the wasps were put into clip cages. After one hour, the wasps were removed. The parasitism of nymphs was assessed within 24 hours after exposure to parasitoids. The parasitism were checked by counting the number of eggs laid under each instar nymphs of hosts. The no-choice and choice experiments were replicated five times (Fig. 1a).

2.2.2. Effects of host stage on fitness of *Eretmocerus warrae*

For every group of plants in no choice tests, two clip cages were left on the plants and kept until the new generation of adults emerged. The developmental times were recorded. And then three wasps were chosen randomly from each clip cage. If a clip cage did not have three wasps, a wasp from the other clip cage on the same plant was used to reach the desired total. The head width and hind tibia each adult was measured as an indicator of body size (See Chapter 3). The F test in IBM SPSS Statistics 24 was used for data analysis of this experiment.

2.2.3. The searching behaviour of *Eretmocerus warrae* related to different instars of hosts

The newly emerged *E. warrae* were used in this experiment. Parasitoids were kept away from hosts 24 hours. The different instars of greenhouse whiteflies are achieved by exposing new plants to greenhouse whiteflies at different times.

One 24 h old wasp was released into a clip cage with 60 nymphs of greenhouse whitefly. Females were observed under a dissecting microscope and their behaviour was recorded with

The Observer XT 11 continuously for 20 mins. If a wasp left the leaf or did not handle any nymphs for five mins, it was eliminated from the experiment.

A behavioural catalogue for *E. warrae* was established based on preliminary observations (Table 1). For simplicity, the act of inserting the ovipositor into a nymph and feeding on host body fluid was pooled into the category of host-feeding; behaviours not described were pooled into an ‘other’ rare category. The Kruskal Wallis test in IBM SPSS Statistics 24 was used to test for behavioural differences among the instars that were attacked.

Patterns of searching behaviour were examined by constructing behavioural flow diagrams (Field and Keller, 1993). This analysis aimed to elucidate how parasitoids organise their behaviour. First-order behavioural transition matrices were constructed for each individual, with rows corresponding to preceding behaviours and columns to following behaviours. Data from all individual parasitoids for each host instar were pooled in the analysis. By pooling, it was assumed that there were no significant differences among individuals. Some further pooling of behaviour in the composite matrices was necessary so that no expected values were less than one and no more than 20% of the expected values were less than five. In our caseanalysis, the behaviours *antennate, host-feed* and *other* were pooled together in the analysis. The principal diagonal elements of these matrices were logical zeros since behaviours could not follow themselves in our records. The expected values of the matrix cells were determined using the iterative proportional fitting method of Goodman (1968). The significance of this analysis was adjusted to a table-wide level of 0.05 using the sequential Bonferoni Method (Rice 1989). The results of analysis of transition matrices are presented graphically in kinetograms (Field and Keller, 1993; Wang and Keller 2002).

2.3. The effects of relative body-size on host stage preference and searching behaviour of *Eretmocerus warrae*

2.3.1. The host stage preference of smaller and larger parasitoids

An experiment was conducted to find out if the host stage preference of parasitoids are affected by their body-size relative to the size of their host (Figure 1b). Based on previous research (Chapter 3), *E. warrae* reared at 20 °C were considered to be larger parasitoids, those reared at 26 °C to be medium-sized, and those at 32 °C to be smaller.

The experimental procedure for determination of host stage preference was described above (2.2.1). Parasitism rates in each trial were determined. The size of the hosts and the adult wasps were measured at the end of the experiment. The length and width of host nymphs were measured. The head width and hind-tibia length of samples of *E. warrae* were measured

2.3.2. The effects of body-size on searching behaviour of *Eretmocerus warrae*

An experiment was conducted to determine if the searching behaviour of large and small *E. warrae* differ. Naïve *E. warrae* of different body-sizes were deprived of hosts for 24 hours before the start of observations. Medium-sized 2nd instar nymphs of greenhouse whitefly that developed at 26 °C were used.

The procedure of this experiment was described above (2.2.3) and the same behavioural catalogue was used. For both large and small wasps, there were 20 observations. The sizes of the parasitoids were measured after each observation.

3. Results

3.1. The effects of host instar on choice and searching behaviour of *Eretmocerus warrae*

3.1.1. Host choice of *Eretmocerus warrae*

E. warrae preferred the 2nd instar of greenhouse whitefly for parasitism in both choice and no choice tests ($P < 0.0001$; Fig. 2). Host preferences were the same in both no-choice and choice tests.

3.1.2. Effects of host stage on fitness of *Eretmocerus warrae*

E. warrae with the exception of emergence rate, fitness indicators for *E. warrae* were largest when the 3rd instars were parasitised (Table 2). The duration of development was shortest at 16.6 ± 1.5 days and body size was largest when wasps developed on large 3rd instar hosts. But the highest emergence rate of *E. warrae* at 83.2 ± 7.8 % was observed when the 2nd instar was parasitised. Although progeny that emerged from the 3rd instar hosts had the longest observed longevity, there were no significant differences in wasp longevity among different host stages.

3.1.3. The effects of host stages on searching behaviour and host acceptance of *Eretmocerus warrae*

The searching behaviour of *E. warrae* varied in the presence of different host instars (Table 3 and 4, Figure 2-4). Females parasitised the 2nd instar most frequently in no-choice and choice tests (Figure 2). The highest rate of parasitism was observed when females searched for 2nd instar hosts in the no-choice test at 19.65 ± 3.75 hosts in one hour. The overall rate of parasitism in the choice test was 17.05 hosts in one hour. The proportion of time that was devoted to different types of behaviour varied among instars. Antennate took *E. warrae* the largest proportion of time when the first and third instar hosts were present, while the most time was were spent on stinging when the second instar was provided (Fig. 2).

Among all host instars, antennate and sting constituted more than half of all the observation time, compared to still, walk, groom and ‘other’, which in aggregate took up less than 25 % of the whole time. This indicates that searching for and handling hosts dominates behaviour when young adult *E. warrae* encounters high-density patches of hosts. Host-feeding was least common when the first instar hosts were provided, compared to when the parasitoid was searching for second and third instar hosts.

Several aspects of the duration and frequency of each type of behaviour in the presence of different host instars are noteworthy. The frequency of antennating, probing and stinging was statistically greatest in the presence of 1st instars, while the duration of each of these acts was longest in the presence of 3rd instars. Host-feeding took the longest time in the presence of 3rd instars, but was most frequent in the presence of 2nd instars.

The pattern of behaviour exhibited by *E. warrae* varied when host stages were different (Fig. 4). In all cases there were statistically more frequent than random transitions from antennate to probe to sting. Sting was non-randomly followed by antennate in the presence of 2nd and 3rd instars hosts. Non-random transitions among groom, walk and still were common.

The preference of *E. warrae* for 2nd instar hosts was clearly indicated in its behaviour (Table 4). It displayed the highest rate host acceptance (sting / probe) and parasitism (hosts parasitised / sting) in the presence of 2nd instar hosts.

The duration of stinging by *E. warrae* was affected by experience (Table 4). While each act of stinging took longer as host instar increased, the first act of stinging hosts took significantly longer than the mean of subsequent acts of stinging on all host instars.

3.2. The effects of relative body-size on choice and searching behaviour of *Eretmocerus warrae* handling the second instar nymphs of greenhouse whitefly

3.2.1. The host choice of small and large parasitoids

Host stage preference was affected by the size of adult *E. warrae*. In both no choice and choice tests, 2nd instar hosts were preferred by smaller *E. warrae*, followed by the first instar (Fig. 3.a). In contrast, large *E. warrae* obviously tended to parasitise 2nd and 3rd instar hosts in no-choice and choice tests (Fig. 3.b). In other words, small wasps more frequently parasitised smaller hosts and large wasps more frequently parasitised larger hosts.

3.2.2. The effects of body-size on searching behaviour of *Eretmocerus warrae*

The searching behaviour of small and large *E. warrae* varied in the presence of 2nd instar hosts (Table 5 and 6, Figure 6 and 7). Although large *E. warrae* sting more hosts than small ones, the proportion of total time spent on sting for large and small wasps was highest and similar for both (Fig. 5). The combined behaviours associated with searching for and handling hosts (antennate, probe, sting, host feed) dominated the time of both wasp sizes.

Several aspects of the duration and frequency of each type of behaviour expressed by different sizes of *E. warrae* are noteworthy. All individual categories of behavioural acts were shorter when expressed by small wasps, except antennate. The shorter duration of antennate expressed by large wasps reflected their quicker arrival at the next host when searching. The frequency of probe, sting and host feed was greater in large wasps, while the frequency of sting was greater in small wasps.

The greater efficiency of large *E. warrae* when attacking 2nd instar hosts was clearly indicated in its behaviour (Table 6). Large wasps displayed a higher rate host acceptance (sting / probe) and parasitism (hosts parasitised / sting) than small wasps.

The duration of stinging by large and small *E. warrae* was affected by their size experience (Table 6). Each act of stinging took longer for small wasps, while the first act of stinging hosts took significantly longer than the mean of subsequent acts of stinging for both small and large wasps.

The differing behavioural patterns of large and small *E. warrae* are reflected in behavioural flow diagrams (Fig. 7). In all cases there were transitions that were statistically more frequent than random from antennate to probe to sting, which again highlights the core of searching and host acceptance behaviour in this species. Sting was non-randomly followed by antennate in large wasps, but by groom in small wasps. Non-random transitions among groom, walk and still were common.

4. Discussion

The 2nd instar nymphs of greenhouse whitefly were more frequently parasitised by *E. warrae* in both no choice and choice tests (Fig. 2). This is different as *En. formosa*, which prefers the older stages of hosts (Qiu et al., 2004). Host stage choices like this typically reflect the fitness gained when a parasitoids searches for hosts (Godfray, 1994). Theoretically, the 3rd instar hosts should produce better fitness gains due to their larger size. However, the highest emergence rate for the parasitoids occurred when they parasitised 2nd instar hosts, and the duration of development was longer (Table 2). Host acceptance and parasitism rates were highest when 2nd instars were attacked (Table 4). But the emergence rate of *E. warrae* from the 3rd instar host was much lower than that from the second instar. Parasitoid preference for the middle stage of host development rather than older stages was also found in *E. eremicus* (Qiu et al., 2004). For parasitoids, host handling efficiency is another factor, beside nutrient levels, that can affect host preference.

When *E. warrae* is searching in the presence of high host densities like those used in these experiments, most of their time is devoted to behaviour that involves searching for and handling hosts (Fig. 3). Female *E. warrae* tend to antennate (search) less frequently (Table 2) and devote less time to antennate (Fig. 3) when the second instar hosts were provided and probe and sting more frequently (Table 3), which suggests that 2nd instar hosts might be relatively easier to find and attack. Although relatively long periods were spent on acts of host-feeding (Table 3), the low frequency of this behaviour and small proportion of time devoted to it (Fig. 3) suggests that *E. warrae* does not need to feed often, and the amount of food obtained from 1st instar hosts is small.

The varying pattern of behaviour exhibited by *E. warrae* when offered different host stages gave insights into its interactions with hosts. The statistically frequent transitions from antennate to probe to sting highlight the core of searching and host acceptance behaviour in this species. The observation that sting was non-randomly followed by antennate in the presence of 2nd and 3rd instars hosts may reflect the greater amount of chemical cues associated with these instars or simply their larger size. The frequency of non-random transitions among groom, walk and still suggests that these are behaviours are expressed when females are not actively searching for hosts.

The host stage preference of *E. warrae* is clearly influenced by the body-size of the adult parasitoid relative to its host. Small wasps parasitised mostly first and second instar hosts while large wasps parasitised mostly 2nd and 3rd instar hosts. Yet as the quality of the egg is not expected to differ among wasp sizes, the outcome of parasitism is expected to be the same for both large and small wasps (Table 2). That is, the emergence rate is expected to be highest when 2nd instars are attacked, but the resulting size of adult progeny is expected to be larger. It is possible that manner in which large wasps attack 3rd instar hosts increases the emergence rate of their offspring which, when combined with the larger size of adult offspring, should deliver greater fitness than when attacking 2nd instars. The effects of adult wasp size on the fitness of their offspring should be investigated.

Large *E. warrae* were more efficient in searching for and parasitising the second instar of greenhouse whiteflies (Table 6). Larger size played a role in changing the pattern of behaviour in ways that must have influenced this efficiency (Table 5-6, Fig. 6-7). Larger wasps can sting more frequently than those smaller ones. As was theoretically expected (Kingsolver and Huey, 2008), larger wasps have greater indicators of fitness that are underpinned by behavioural differences.

In the two sets of behavioural observations, experience played a role in the duration of the sting behaviour of *E. warrae*. Learning like this is common among parasitoids wasps, as it improves their efficiency (Turlings et al, 1993).

In summary, the host stage preference and searching behaviour of *E. warrae* are affected by the relative body-sizes of parasitoids and hosts. This conclusion could be applicable to other species of parasitoids, especially those that attack whiteflies. However, relative body-size between parasitoids and hosts is not the only elements that decides the host-stage choice of parasitoids. There are many other factors that might influence the host-choice stage of parasitoids as well (Godfray, 1994).

This research has some implications in commercial rearing and releasing of *E. warrae*. First, temperature can be used in modifying the body-size in rearing parasitoids, which will influence the fecundity, longevity and host preferences of *E. warrae*. For example, if there are more hosts of older stages in greenhouse, releasing smaller parasitoids should not be the best choice. Second, larger parasitoids can be used to more quickly suppress pest hosts because they are more efficient in parasitising them. Finally, when the hosts are at low density, parasitoids experienced with handling hosts could be able to suppress hosts more effectively, so releasing them at hot spots should be best practice.

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Figure legends:

Figure 1. Experimental design: host stage choice and behaviour observation of different-sized *Eretmocerus warrae*. (a). Medium-sized *E. warrae*; (b). Larger and Smaller *E. warrae*.

Figure 2. Host stage choice of *Eretmocerus warrae* (mean ± SD). White bars for no choice test and grey bars for choice test. Values followed by different letters are significantly different ($P < 0.05$).

Figure 3. Proportion of total time of each behaviour that *Eretmocerus warrae* spent when searching for different stages of greenhouse whitefly. (a). First instar host, (b). Second instar host, (c). Third instar host.

Figure 4. Flow diagrams showing the sequence of searching behaviour of *Eretmocerus warrae* handling different stages of hosts. See Table1 for a description of behavioural categories. The areas of circles are proportional to the overall frequency of each type of behaviour. The arrows represent behavioural flows that are significantly greater than expected ($P < 0.05$) and the widths of arrows are proportional to the standardised residuals of positive deviations from expected values. (a). First instar host; (b). Second instar host; (c). Third instar host.

Figure 5. Host stage choice of smaller and larger *Eretmocerus warrae* (mean ± SD). White bars for no choice test and grey bars for choice test. Values followed by different letters are significantly different ($P < 0.05$). (a). Smaller wasps, (b) Larger wasps, the number of parasitism at the third instar was 0.2 ± 0.4.

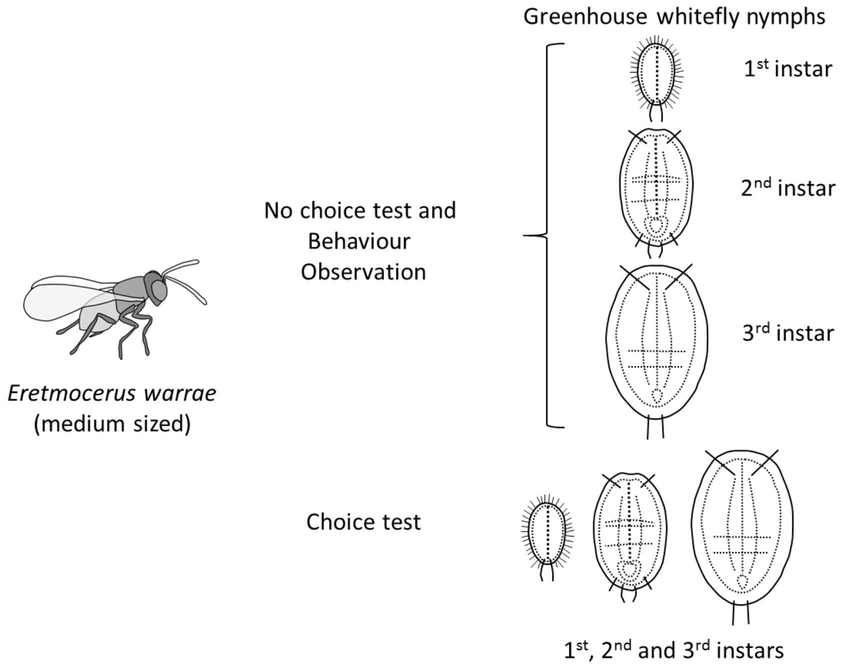
Figure 6. Proportion of total time of each behaviour that small and large *Eretmocerus warrae* spent on the second instar nymphs of greenhouse whitefly. (a). Smaller wasps, (b) Larger wasps.

Figure 7. Flow diagrams showing the sequence of searching behaviour of smaller and larger *Eretmocerus warrae* handling different stages of hosts. See Table1 for a description of

behavioural categories. The areas of circles are proportional to the overall frequency of each behavioural event. The arrows represent the significant behavioural flows ($P < 0.05$) and the widths of arrows are proportional to the standardised residuals of positive deviations from expected values. (a). Small wasps, (b) Large wasps.

Figure 1.

(a). Medium-sized *Eretmocerus warrae*



(b). Larger and smaller *Eretmocerus warrae*

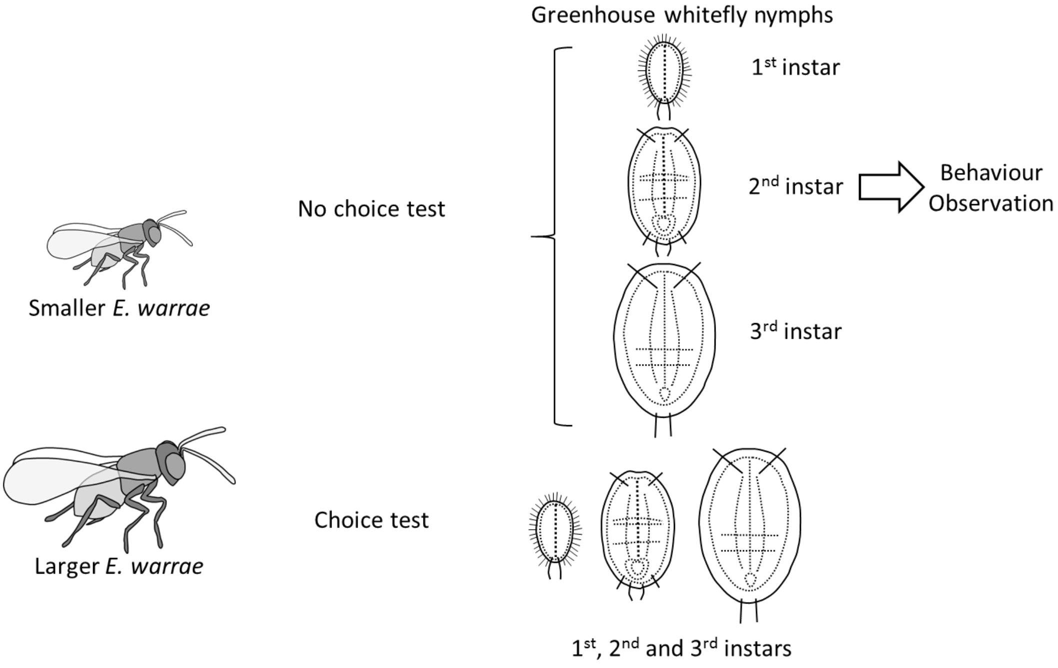


Figure 2.

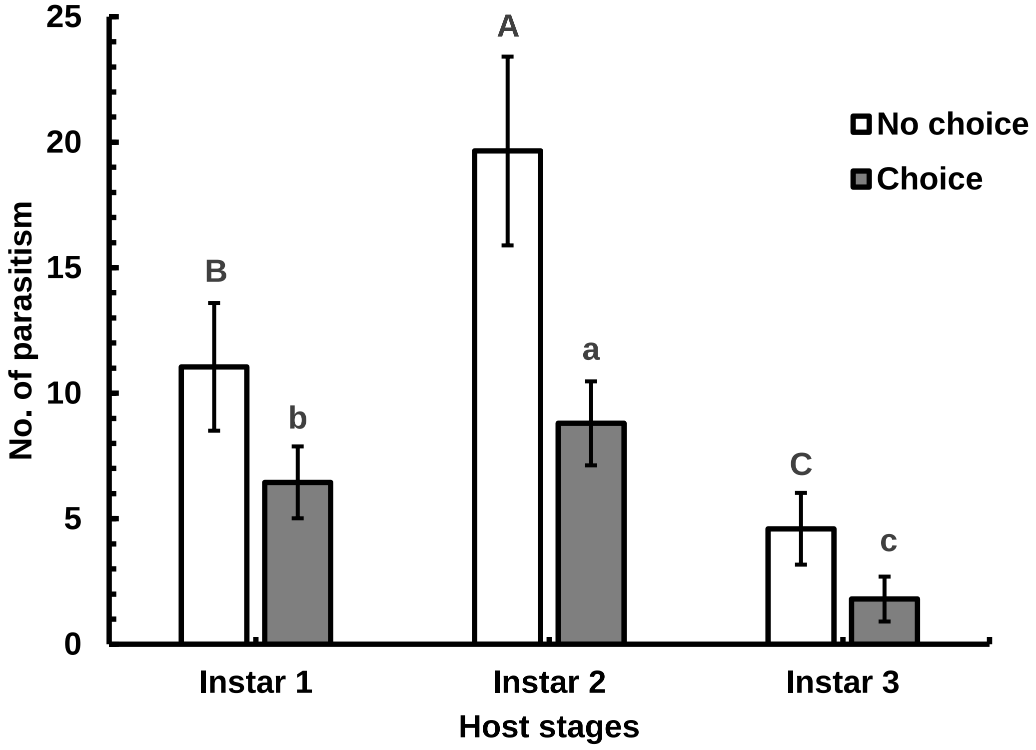
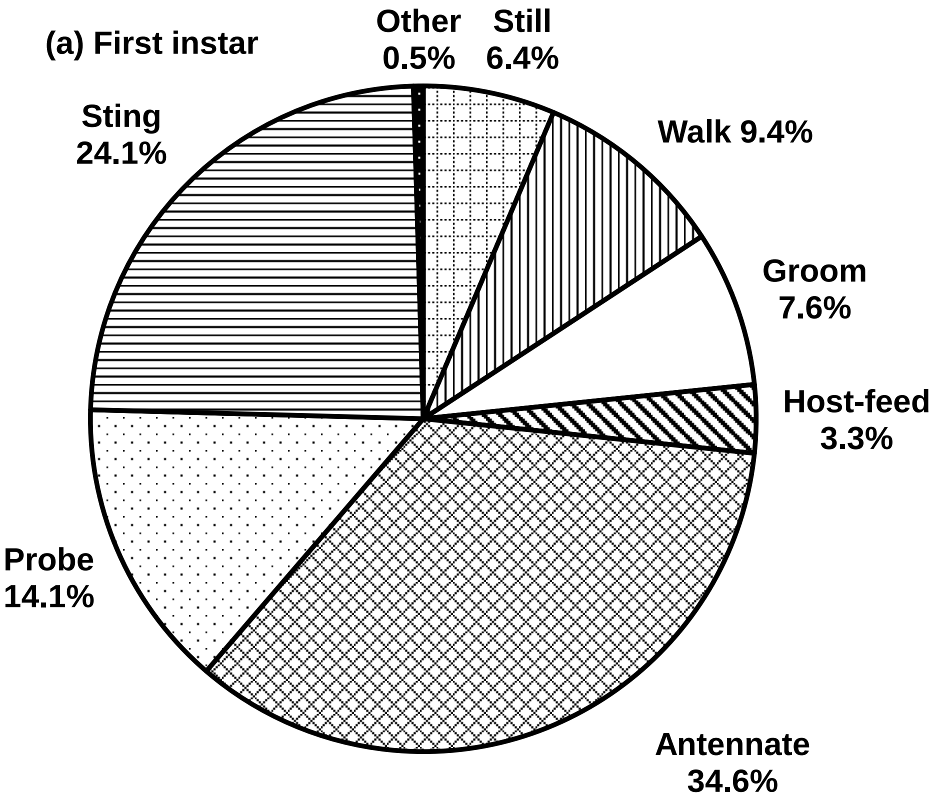
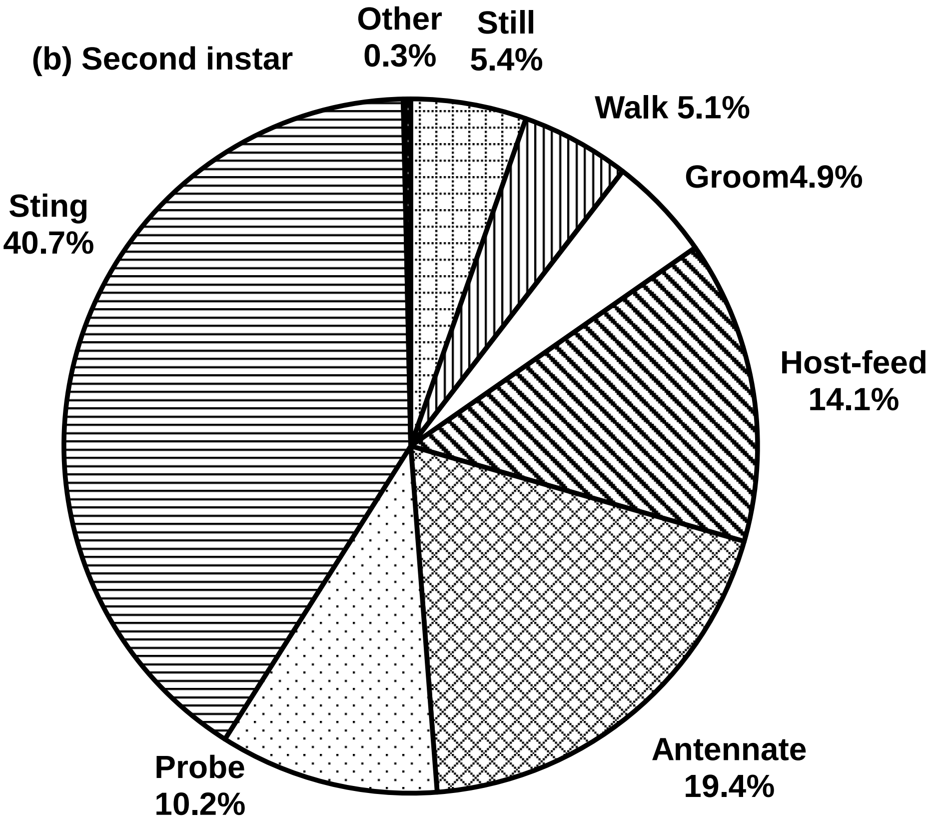

25
20
15
10
5
0
No. of parasitism
A
B
a
b
C
c
No choice
Choice
Instar 1
Instar 2
Instar 3
Host stages

Figure 3.


(a) First instar


(b) Second instar

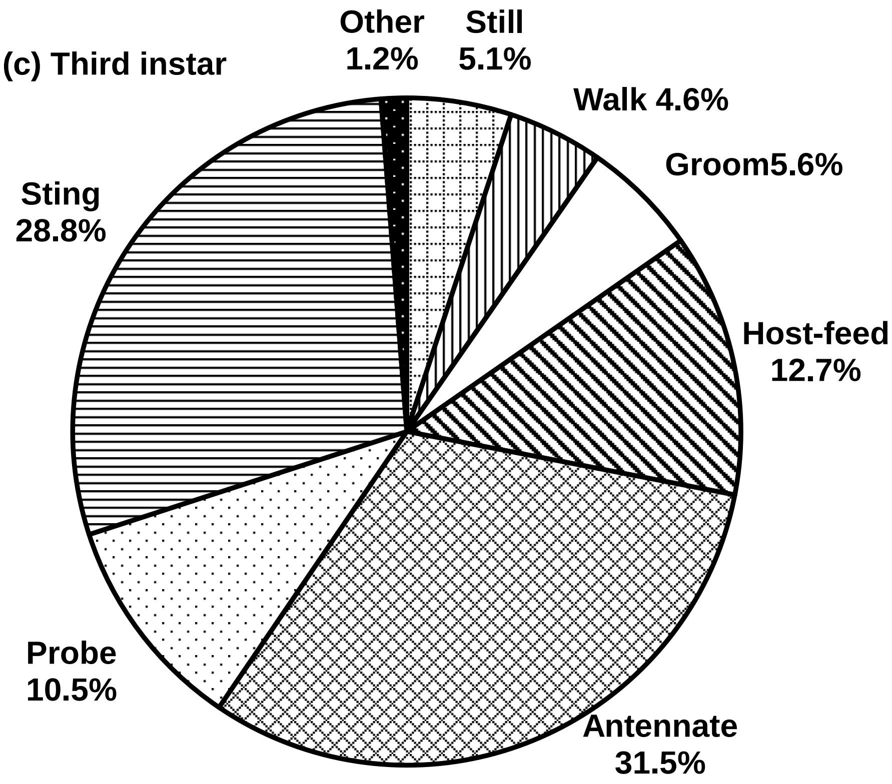
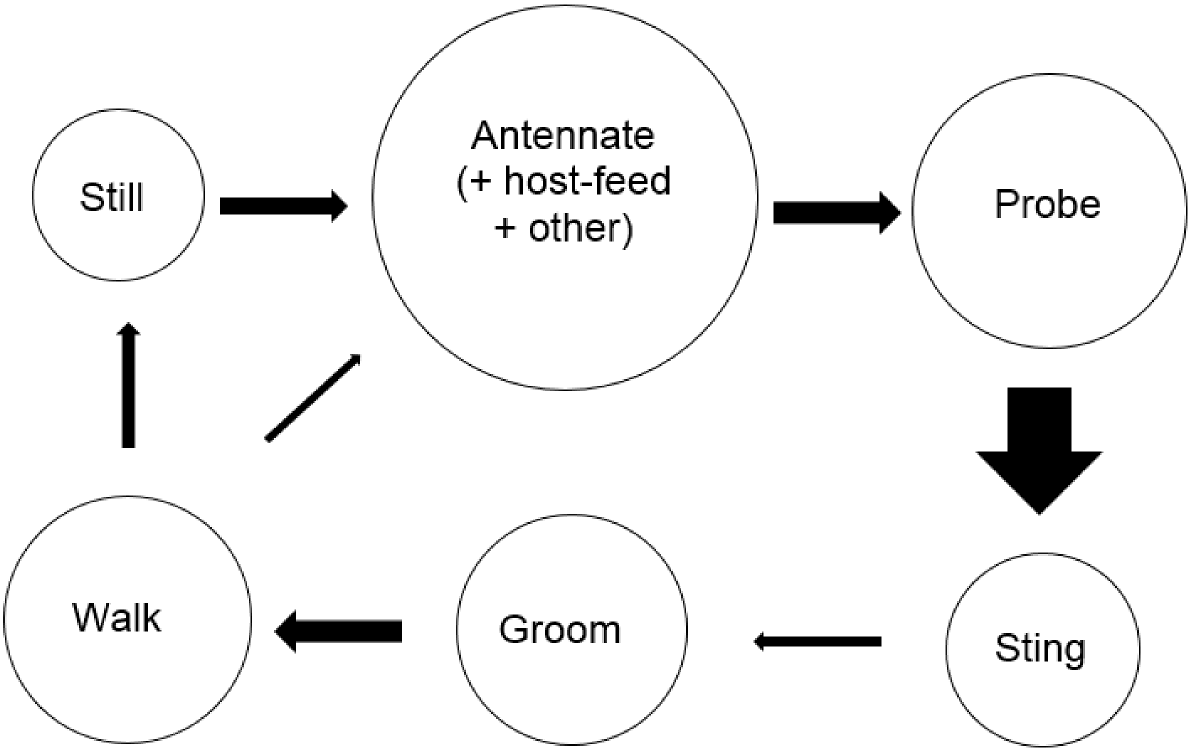
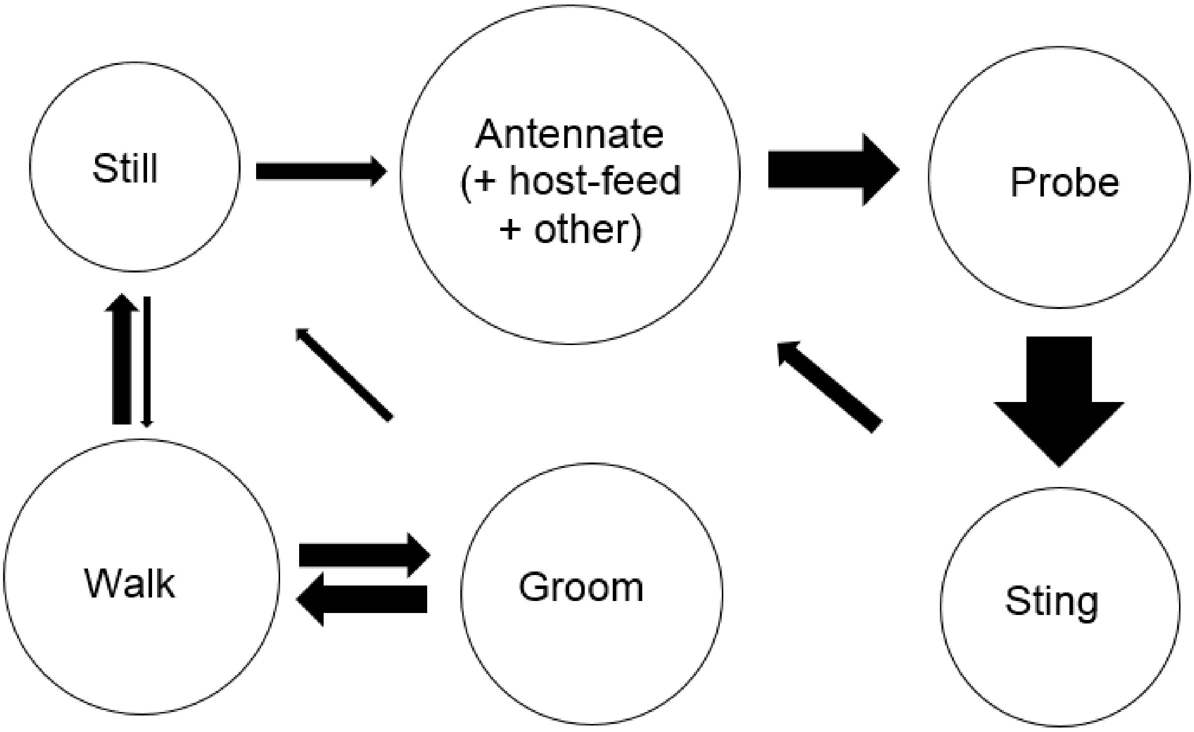

(c) Third instar
Other 1.2%
Still 5.1%
Walk 4.6%
Groom5.6%
Host-feed 12.7%
Antennate 31.5%
Probe 10.5%
Sting 28.8%

Figure 4.

(a). First instar host



(b). Second instar host



(c). Third instar host

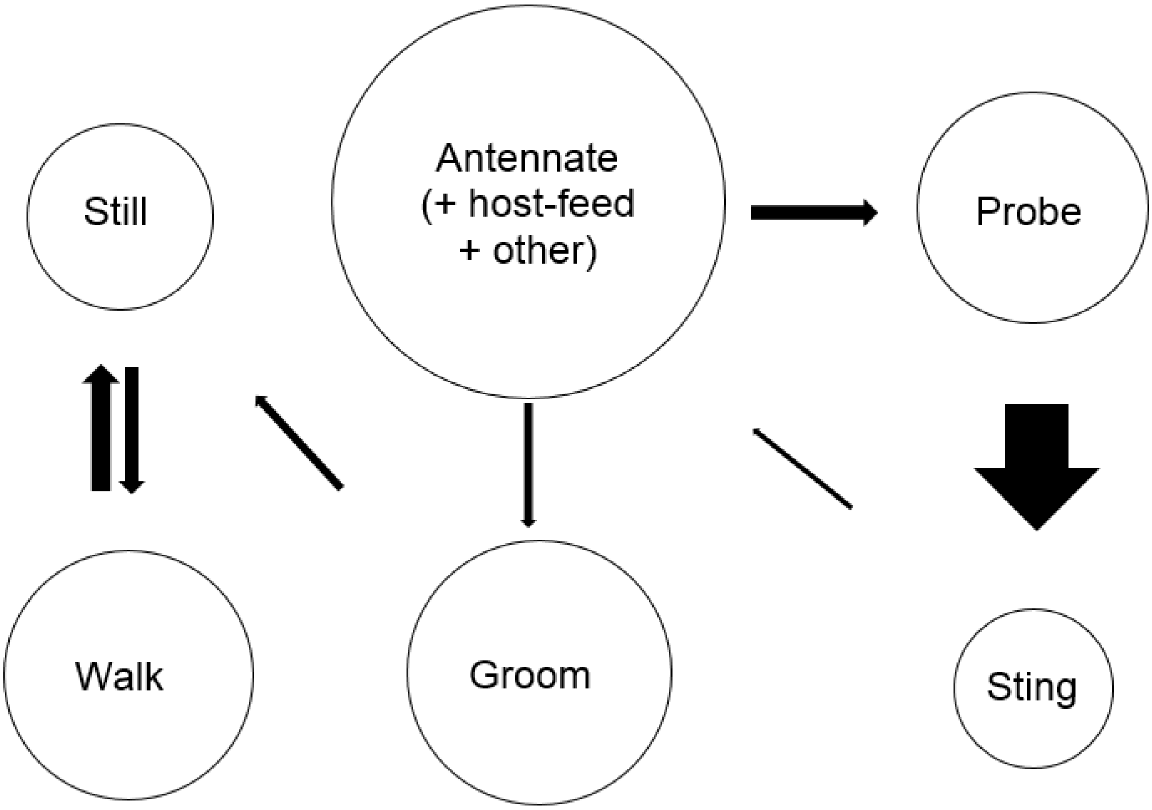
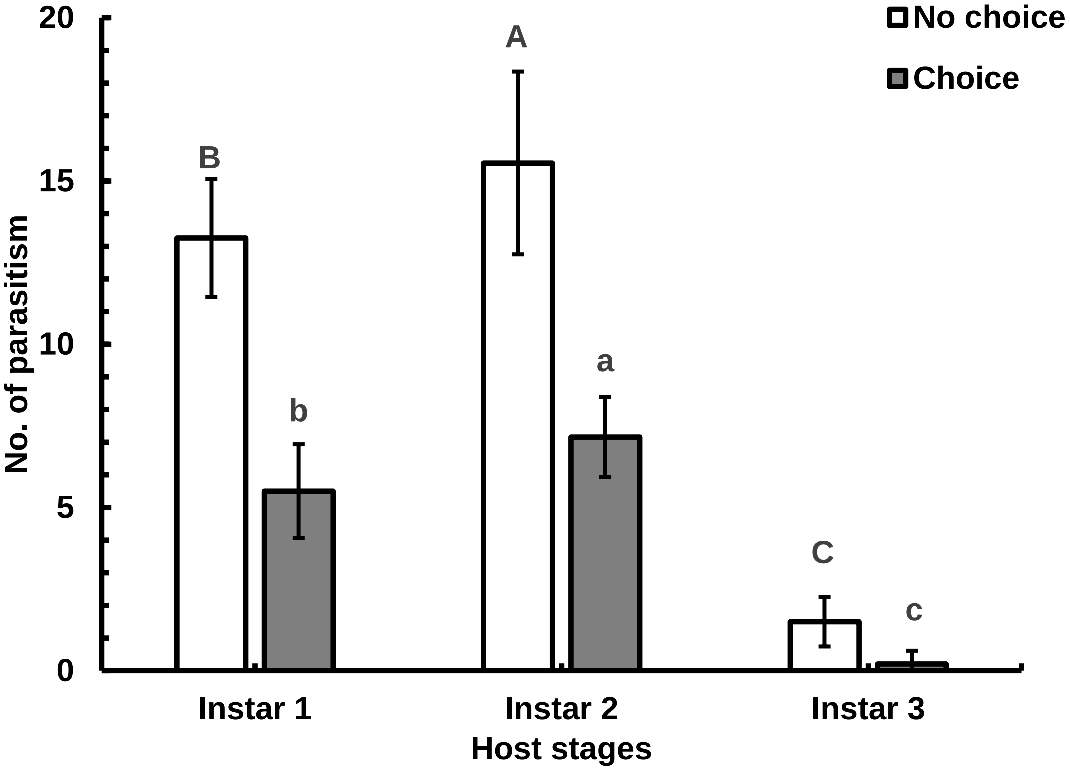


Figure 5.

(a). Small wasp



(b). Large wasp

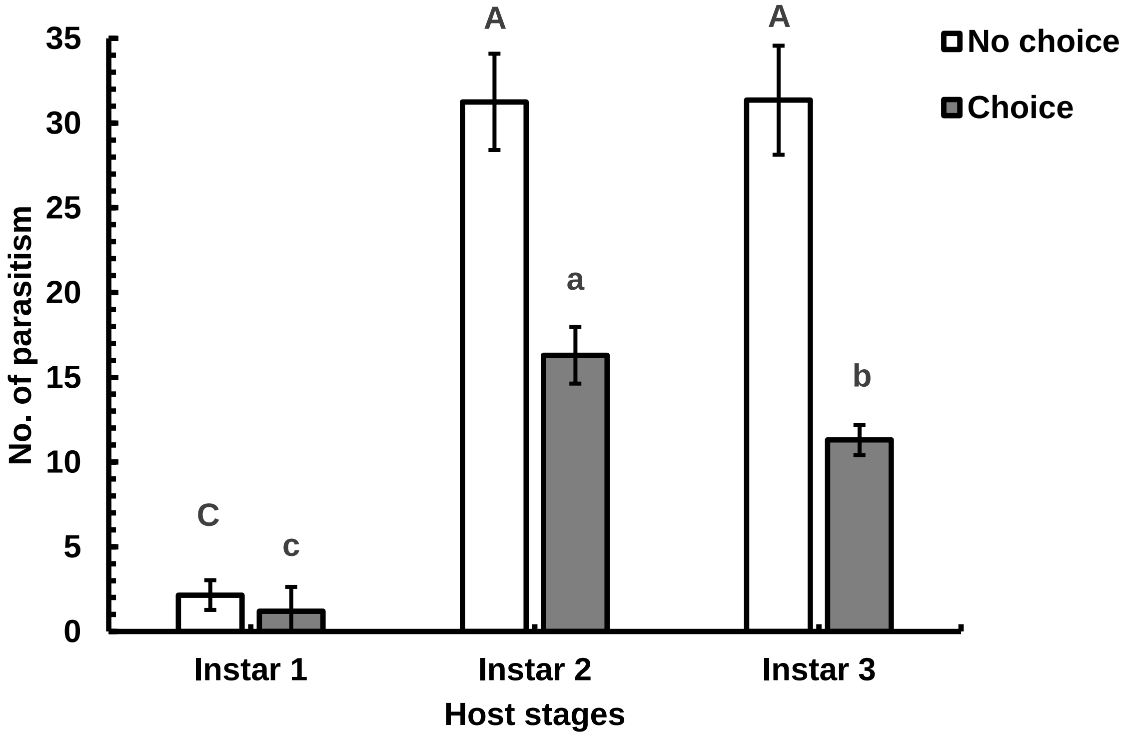
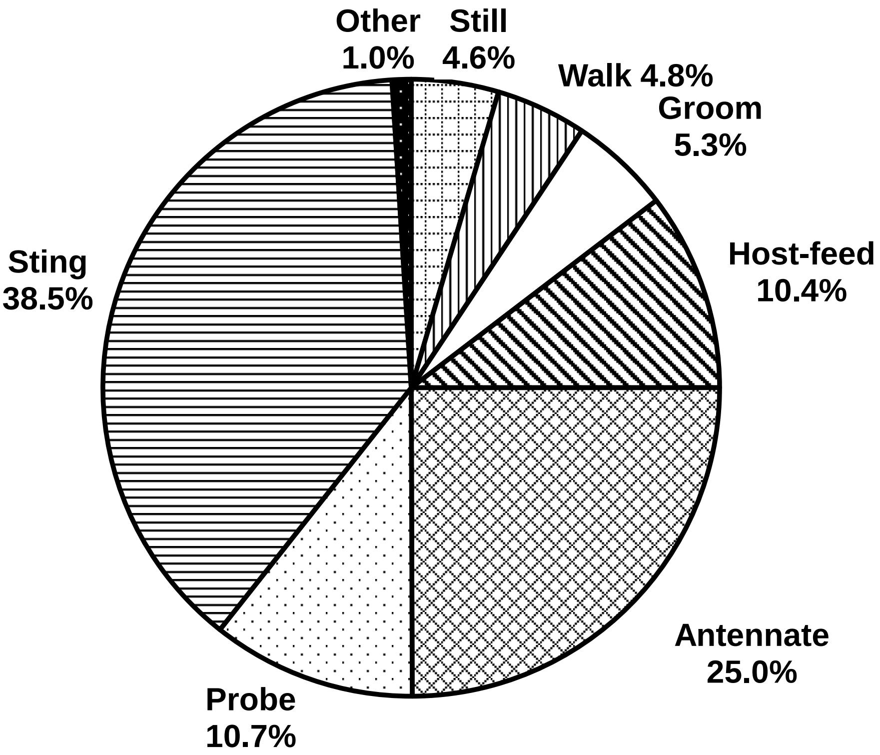


Figure 6.

(a). Small wasp



(b). Large wasp

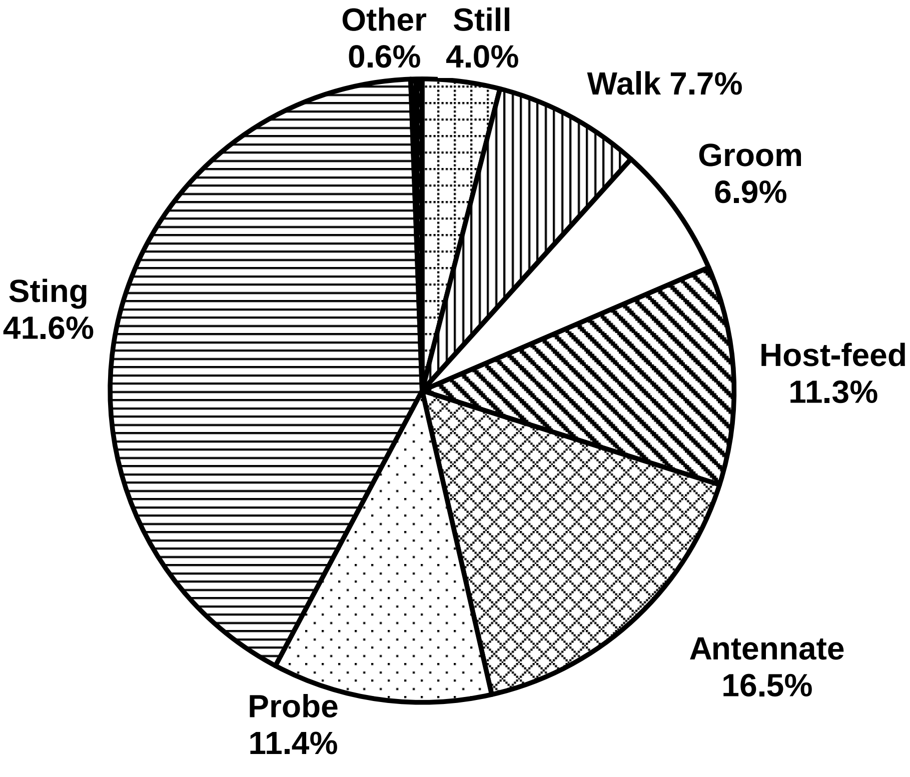
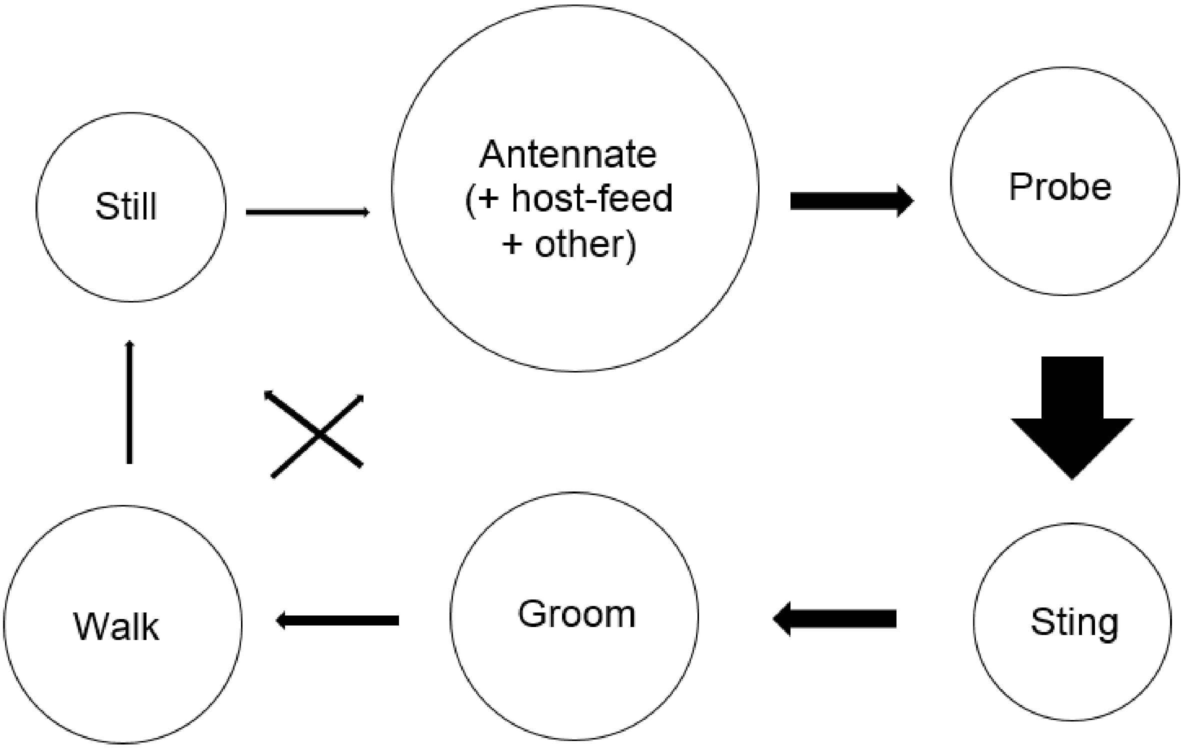


Figure 7.

(a). Small wasps



(b). Large wasps

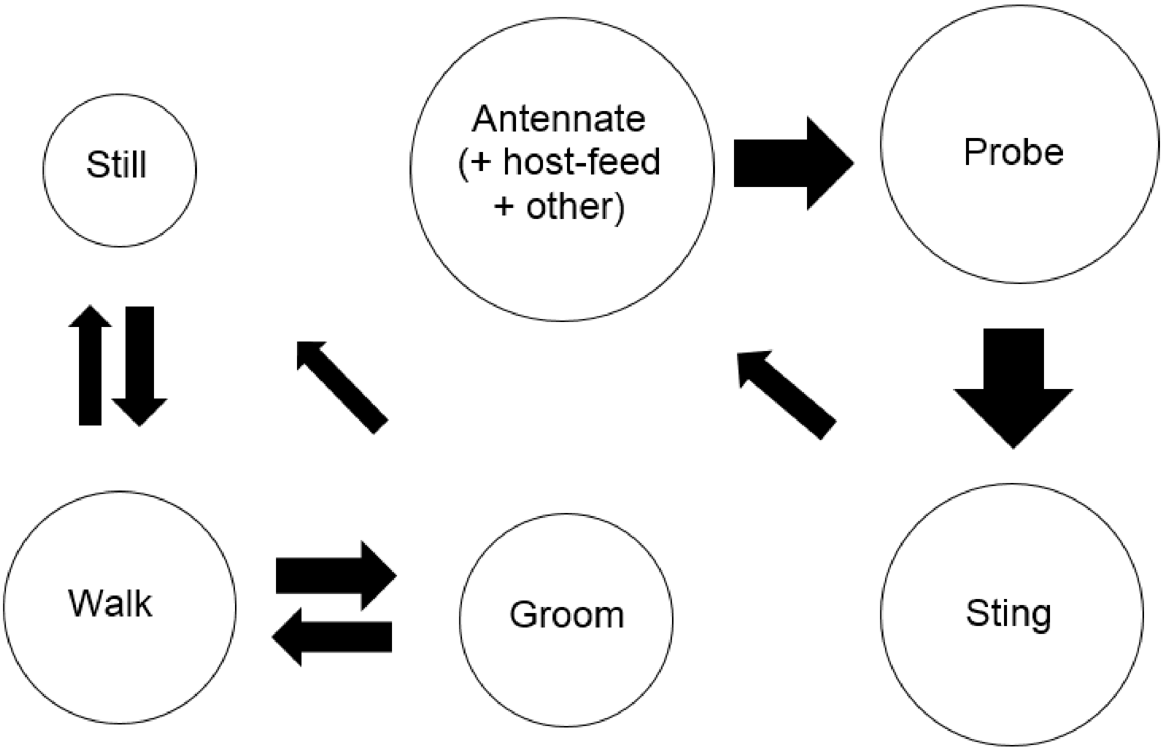


Table 1. A catalogue of behavioural acts of *Eretmocerus warrae* searching for greenhouse whitefly

Event	**Description**
Walk	Parasitoid walks on the surface of a leaf;
Antennate	Parasitoid taps the substrate with the tips of its antennae;
Still	Parasitoid motionless;
Groom	Parasitoid brushing parts of its body with legs;
Probe	Parasitoid touching a host with the tips of its antennae and circling around the body of the nymph;
Sting	Parasitoid inserting its ovipositor between the nymph and leaf surface;
Host-feed	Parasitoid inserting its ovipositor into the nymph and feeding on the wound on the host’s body;
Other	Any behaviour not described above.

Table 2.The effects of host stage on fitness indicators of *Eretmocerus warrae* (mean ± SD).

Host instar	**No. of adults emerged / parasitism (%)**	**Duration of development (day)**	**Head width (μm)**	**Hind-tibia length (μm)**	**Longevity (day)**
1st	59.7 ± 9.5 c	19.4 ± 1.3 a	17.9 ± 1.3 c	16.9 ± 0.8 c	6.9 ± 1.7 a
2nd	83.2 ± 7.8 a	17.9 ± 1.3 b	20.3 ± 1.5 b	19.3 ± 1.4 b	7.2 ± 1.9 a
3rd	72.4 ± 14.3 b	16.6 ± 1.5 c	21.6 ± 0.9 a	21.0 ± 1.5 a	7.5 ± 1.6 a

F test for data analysis. Mean followed by the same letters are not significantly different ($P > 0.05$).

Table 3. Durations and frequencies of each type of behaviour of *Eretmocerus warrae* handling different stages of greenhouse whiteflies (mean ± SD).

Host stage	**First instar**	**Second instar**	**Third instar**
Duration (s)			
Still	15.7 ± 8.7 a	11.6 ± 9.7 b	12.4 ± 10.7 c
Walk	10.9 ± 6.3 a	6.2 ± 4.1 b	6.2 ± 4.3 b
Groom	10.0 ± 5.9 a	6.5 ± 4.2 b	7.0 ± 4.3 b
Host-feed	198.1 ± 12.2 c	282.3 ± 29.9 b	382.1 ± 28.7 a
Antennate	16.2 ± 5.0 c	17.1 ± 4.7 b	19.0 ± 6.4 a
Probe	13.5 ± 3.0 b	14.1 ± 2.5 b	16.8 ± 3.7 a
Sting	45.9 ± 18.5 c	67.8 ± 13.7 b	98.2 ± 18.0 a
Other	11.3 ± 3.8 c	13.9 ± 3.9 b	24.7 ± 17.7 a
Frequency (number/h)			
Still	14.1 ± 3.8 b	16.3 ± 3.6 a	14.4 ± 3.1 b
Walk	29.6 ± 7.4 a	28.6 ± 6.5 a	25.9 ± 6.0 b
Groom	26.1 ± 6.2 b	25.7 ± 5.5 b	28.7 ± 5.0 a
Host-feed	0.7 ±1.3 b	2.0 ± 1.7 a	1.6 ± 2.2 a
Antennate	73.6 ± 16.5 a	44.3 ± 11.7 c	65.9 ± 16.7 b
Probe	36.1 ± 9.9 a	25.1 ± 7.0 b	21.9 ± 7.8 c
Sting	18.1 ± 5.3 a	20.9 ± 5.4 a	10.4 ± 3.4 b
Other	1.6 ± 1.5 a	1.1 ± 1.8 a	1.6 ± 2.2 a

Different letters in the same row indicate significant differences between host stages (Kruskal Wallis tests, $P < 0.05$).

Table 4. Host acceptance rate, parasitism rate, and duration of first- and subsequent stinging behaviours of *Eretmocerus warrae* handling different stages of greenhouse whiteflies (mean ± SD).

Host stage	**First instar**	**Second instar**	**Third instar**
Host acceptance rate (Sting / Probe) (%)	53.5 ± 15.6 b	84.1 ± 8.9 a	49.5 ± 13.0 b
Parasitism rate (No. of host parasitised / sting) (%)	79.3 ± 9.5 c	93.6 ± 8.1 a	86.5 ± 13.9 b
Duration of first-sting (s)	85.4 ± 8.1 c	97.4 ± 7.1 b	123.9 ± 10.6 a
Duration of subsequent stings (s)	38.4 ± 6.4 c	63.0 ± 6.6 b	88.0 ± 5.8 a

Different letters in the same row indicate significant differences between host stages (Kruskal Wallis tests, $P < 0.05$).

Table 5. Durations and frequencies of each type of behaviour of larger and smaller *Eretmocerus warrae* handling the second instar of greenhouse whiteflies (mean ± SD).

Host stage	**Small parasitoids**	**Large parasitoids**
Duration (s)		
Still	10.0 ± 6.6 b	12.0 ± 10.2 a
Walk	6.6 ± 4.1 b	10.0 ± 4.8 a
Groom	6.7 ± 4.1 b	10.4 ± 5.0 a
Host-feed	312.4 ± 15.6 c	226.8 ± 10.2 a
Antennate	16.2 ± 5.0 a	11.9 ± 4.3 b
Probe	15.9 ± 3.6 a	10.5 ± 3.1 b
Sting	79.6 ± 16.3 a	42.7 ± 14.0 b
Other	24.1 ± 5.8 a	17.6 ± 9.8 b
Frequency (number / h)		
Still	16.2 ± 2.8 a	12.0 ± 1.4 b
Walk	26.1 ± 5.7 a	27.6 ± 4.7 a
Groom	28.5 ± 4.0 a	23.7 ± 4.4 b
Host-feed	1.2 ± 1.5 b	1.8 ± 1.5 a
Antennate	61.5 ± 4.8 a	49.8 ± 3.7 b
Probe	23.4 ± 6.0 b	39.3 ± 2.9 a
Sting	17.4 ± 5.5 b	35.4 ± 4.1 a
Other	1.5 ± 1.5 a	1.2 ± 1.5 b

Different letters in the same row indicate significant differences between host stages (Kruskal Wallis tests, $P < 0.05$).

Table 6. Host acceptance rate, parasitism rate, duration of first- and subsequent stinging behaviours, and sizes of larger and smaller *Eretmocerus warrae* handling the second instar of greenhouse whitefly (mean ± SD).

Host stage	**Small parasitoids**	**Large parasitoids**
Host acceptance rate (Sting / Probe) (%)	75.9 ± 18.3 b	90.1 ± 9.0 a
Parasitism rate (No. of host parasitised / sting) (%)	79.6 ± 6.5 b	92.5 ± 2.6 a
Duration of first-sting (s)	109.6 ± 8.6 a	86.0 ± 4.9 b
Duration of subsequent stings (s)	73.4 ± 8.7 a	38.6 ± 4.5 b
Head widths of parasitoids (µm)	17.6 ± 1.4 b	27.6 ± 2.2 a
Hind-tibia lengths of parasitoids (µm)	16.9 ± 1.2 b	26.0 ± 1.6 a

Different letters in the same row indicate significant differences between host stages (Kruskal Wallis tests, $P < 0.05$).

CHAPTER FIVE

General Discussion

My research has contributed to an understanding of many of the effects of temperature on the biology of *E. warrae*. The effects of temperature on its rate of development, longevity, oviposition and survival at high temperatures were quantified (Chapter 2). It is noteworthy that *E. warrae* has slightly greater tolerance of high temperatures than the widely used alternative biological control agent, *En. formosa*. Selected temperatures were also shown to affect the body size, longevity and realized fecundity of *E. warrae* (Chapter 3). The effects of temperature on the body sizes of *E. warrae* and its host *T. vaporariorum* were found to influence the behaviour of the parasitoid in predictable ways (Chapter 4). Thus, all of the investigated characteristics of *E. warrae* are affected by temperature, which is typical of invertebrates. These findings have important implications for the use of *E. warrae* and similar species in the biological control of insect pests. It is important to understand the limitations of this research when inferring such broader implications. Further research should be useful in making practical use of *E. warrae* in the control of greenhouse whitefly.

Several findings suggest that *E. warrae* should be a complementary biological control agent to *En. formosa*. First, *E. warrae* was shown to develop and survive at marginally higher temperatures than *En. formosa* (Chapter 2), which validates the empirical observations of James Altmann (Biological Services, personal communication, 2015). This could make it a better control agent when temperatures are high in the summer. Second, *E. warrae* can lay more eggs each day than *En. formosa* (Chapter 3). Thus it could more quickly and efficiently suppress its host if numbers released are equal, or it may be possible to release fewer *E. warrae* to achieve effective control. *En. formosa* is a well-established commercial biological control agent. Further research is necessary to clearly show that *E. warrae* can play a complementary or alternative role as a control agent to *En. formosa*.

The behaviour of *E. warrae* and *En. formosa* at high temperatures should be investigated. In my investigation of the effects of extreme high temperatures on these parasitoids, the insects were confined, which prevented them from displaying any behavioural responses to temperature. For example, the desert darkling beetle, *Pimelia senegalensis* (Coleoptera: Tenebrionidae), seeks refuge when daytime temperatures are extremely hot (Maeno et al., 2014), and microhabitat preferences of the groundhopper, *Tetrix tenuicornis* (Orthoptera: Tetrigidae), are influenced by temperature (Musiolek and Kočárek, 2016). Such behavioural responses to temperature have not been extensively investigated. Preventing adult *E. warrae* from exhibiting behavioural responses to temperature may be a limitation of my work. It is possible that there are differences in the behaviour of these species at high temperatures that could further distinguish them and influence their ability to control whiteflies. For instance, the endomychid beetle *Stenotarsus rotundus* will diapause in adapt to high temperatures (Wolda and Denlinger, 1984). If this is so, then the marginally higher tolerance of *E warrae* to extreme high temperatures could be more biologically and commercially significant.

It is noteworthy that *En. formosa* and *Eretmocerus eremicus* are used in combination to control *Bemisia tabaci* in Europe (Qiu et al 2004). In that case, *En. formosa* is predicted to provide better control when temperatures are lower than 20 ºC and *E. eremicus* to provide better control at higher temperatures. Also, *E. eremicus* lays most of its eggs in the first few days of adult life, while *En. formosa* parasitises host over a longer period of time. Thus the former species is thought to suppress the pest while the latter is better suited to maintaining it at acceptably low densities. The results of my thesis suggest that similar complementarity exists between *E. warrae* and *En. formosa*.

The results of my study provide insights into the way that *E. warrae* is reared and used in augmentative biological control of the greenhouse whitefly. Ideally biological control agents should be reared in a manner that optimises their capacity to control the target pest. On the one hands, my results show how controlling temperature can affect the production of *E. warrae*. As temperature is raised, the developmental time decreases which makes production of cohorts of adults faster. On the other hand, wasps that develop faster are smaller, which causes them to have lower fecundity and they are less likely to parasitise larger whitefly nymphs. Controlling temperature involves costs, so the trade-offs between cost and the efficiency of production and adult wasp quality should be investigated. On the other hand, my results give insights into how the development and quality of *E. warrae* will vary during different seasonal conditions if temperature is not controlled (Fig. 1.). Biological control agents are typically produced in greenhouses. If temperatures are not controlled, then relatively large size and quality variations in adult *E. warrae* are likely to occur. But greenhouses are usually heated in winter and cooled in summer, which moderates temperature extremes and promotes the growth of host plants, host insects and biological control agents. In such a situation, the size and quality of *E. warrae* will vary. Producers of this insect could measure samples of wasps in order to monitor quality changes throughout the year. It may be appropriate to vary the numbers of wasps that are released in biological control programs at different times of the year since their size and quality are now known to vary seasonally.

Varying release rates of biological control agents has not generally improved the level of biological control in augmentative programs (Crowder 2007). Releasing more agents than is optimal increases cost without delivering better control in most cases. Factors such as synchrony with pest populations and integration with other control practices, especially insecticide use, have been shown to be more important than release rates in many cases (Crowder 2007).

This research did not include field experiments, which are crucial to validation of experimental results that relate to the release of *E. warrae* in commercial greenhouses. Future research should be conducted in commercial greenhouses to confirm the practical value of the proposed applications of my experimental findings.

E. warrae is already being produced and sold as a biological control agent (Biological Services, 2015). The research in my thesis adds to the understanding of this wasp's biology and ecology. It should contribute to the effective deployment of *E. warrae* and related species from this genus in the biological control of whiteflies in future.

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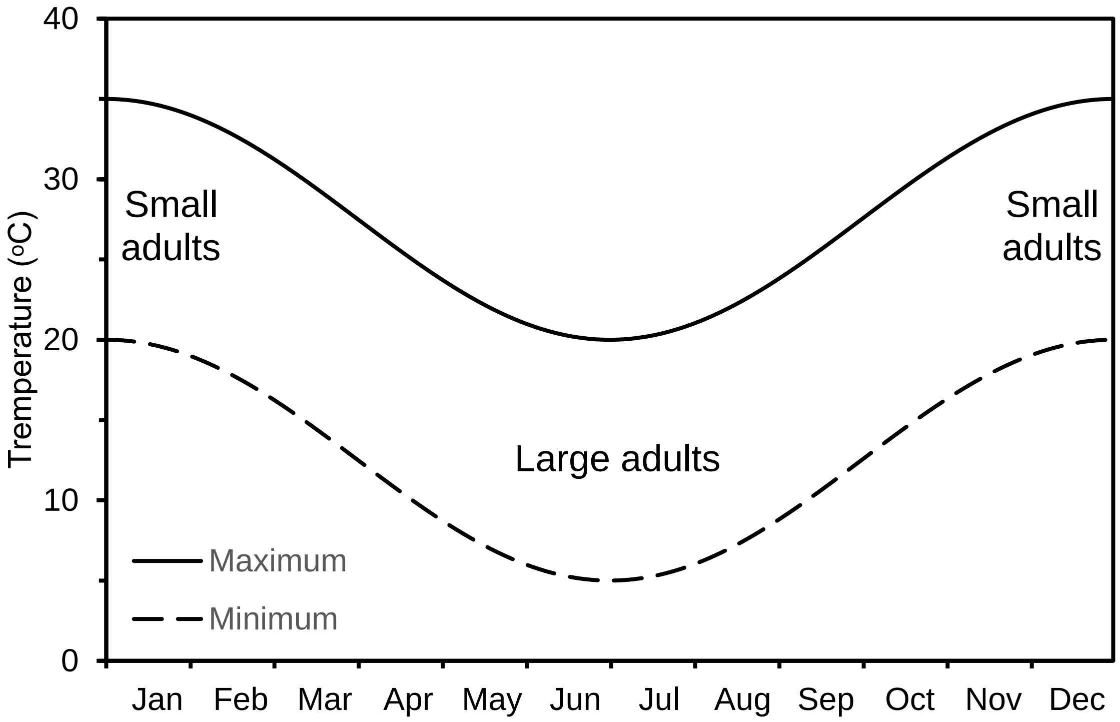
Figure 1. Pattern of seasonal temperature variation in greenhouses and predicted pattern of size variation in adult *E. warrae*. (a) Unmoderated temperatures. (b) Temperature variations when heating and cooling are installed.

(a).



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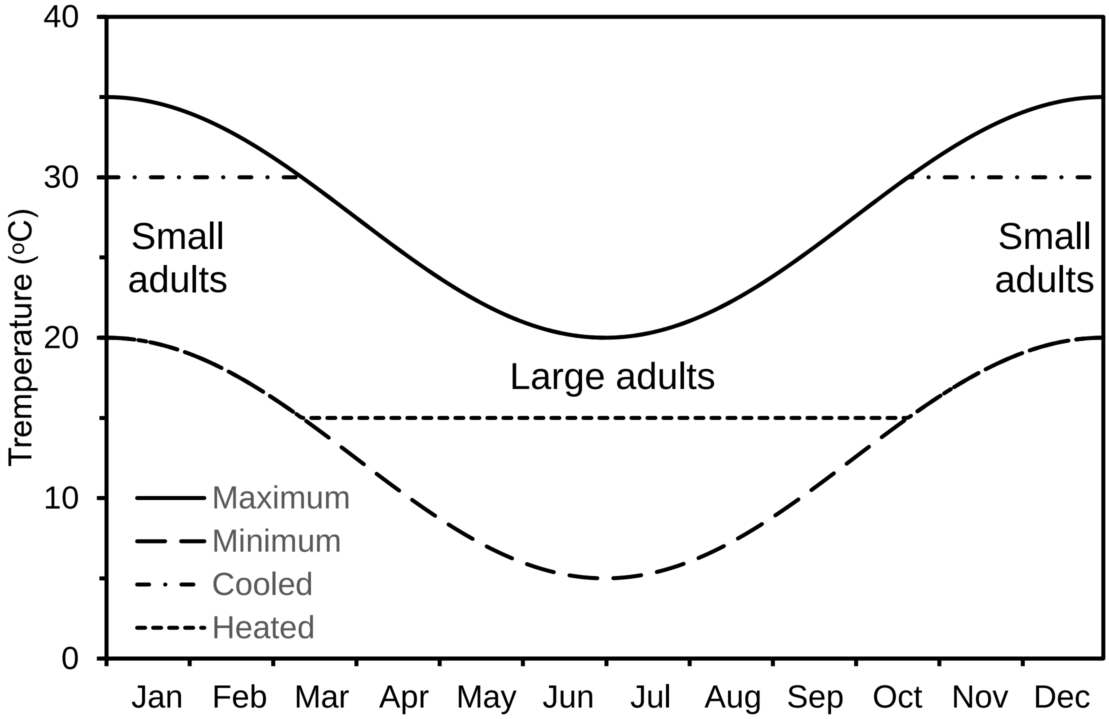
40
30
20
10
0
Trempeature (oC)
Small adults
Small adults
Large adults
Maximum
Minimum
Cooled
Heated
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec