Predatory and plant-use specialization by *Evarcha culicivora*, an East African salticid spider

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The time spent working toward my Ph.D. has included some of best moments in my life. I will close this Acknowledgment by dedicating thesis to all the people who taught me to love and to learn.

ABSTRACT

Using *Evarcha culicivora*, a salticid spider from East Africa, my goal was to understand some of the different specific ways in which predatory specialization might be expressed. This spider was already known for its unusual prey-choice behaviour. It feeds indirectly on vertebrate blood by actively choosing blood-carrying mosquitoes as preferred prey and, as its preferred mosquitoes, actively chooses *Anopheles*, the genus to which all-human malaria vectors belong. Here I investigated another two distinct contexts in which predatory specialization is expressed by this species, adaptive timing of predatory activity and reliance on specific nutrients during the first active life-history stage. Using sampling procedures and experiments, I found evidence that, for *E. culicivora*, the timing of specifically predatory activity, not activity in general, corresponds to the time of day when its unusual preferred prey tends to be most readily available in the field. For investigating the role of different nutrients, particular attention was given to another unusual characteristic of this predator. Besides feeding on mosquitoes, *E. culicivora* also visits plants and feeds on nectar. In a large series of rearing experiments, I considered the effects of different feeding regimes at on *E. culicivora* hatchlings. Type and number of prey, as well as well as the timing of prey meals, access to plants for nectar meals, access to artificial nectar made from different sugars and amino acids and combinations of access to prey and plants were shown to influence success at completing the first instar, completion time for spiders that did succeed and longevity for spiders that failed to complete the first instar.

CHAPTER ONE: INTRODUCTION

Credit: Robert Jackson

Male *Evarcha culicivora*

Using a small predator from East Africa, *Evarcha culicivora*, I investigated a large topic, specialization. Literature on predatory specialization is easy to find, but not always easy to understand because specialization seems to mean different things to different people in different situations. Compounding the problem, specialization is often discussed in a way that leaves the meaning of this expression implicit and obscure, sometimes with hypotheses about the consequences of becoming specialized seeming to be accepted as foregone conclusions and turned into part of an implicit meaning of specialization as a phenomenon. Finding one's way through this literature has been likened to "stepping into a conceptual jungle where we can easily get lost" (Jackson & Cross 2015).

The predator I investigated, *E. culicivora*, is a jumping spider (family Salticidae) known for being a mosquito specialist (Jackson & Cross 2015), but it seems to be easy to misconstrue what 'mosquito specialist' means. Many spiders and other predators eat mosquitoes (Jackson & Cross 2015), but eating mosquitoes or even eating primarily mosquitoes does not make a predator a mosquito specialist. With *E. culicivora*, we also have a predator that specializes at preying on a particular mosquito genus, *Anopheles*, this being a detail of unusual interest to people because all human malaria vectors belong to this mosquito genus. However, before going any further, I should emphasize that the basis for characterizing this spider as an *Anopheles*-specialist spider is not how many anopheline mosquitoes it eats compared to other prey in its environment. If we want to discuss specialization by predators on specific prey, then we need research specifically on the characteristics of a predator that make it proficient as a predator of the specified prey. There is no shortcut. Data on natural diet compared with prey availability does not suffice.

It is especially confusing when stenophagy and specialization are discussed as though they were the same thing. 'Stenophagy' is a convenient term for when a predator's natural diet is 'narrow', where 'natural diet' is what the predator eats in the field and 'narrow' means

a diet biased toward a specific 'selection' of the available prey. Stenophagy is of interest in the context of food webs and other topics in community ecology. However, the terms "choice" and "preference" are precisely the terms needed for discussing behaviour, motivation and cognition (Jackson & Cross 2011). Conclusions pertaining to choice and preference require data from experiments appropriately designed for detecting choice and preference.

Previous research (Jackson & Cross 2015) has given us a lot of information about *E. culicivora*'s prey-choice behaviour, but much less about this predator's natural diet. As an objective secondary to this thesis, I participated in research related to filling the gap in our understanding of *E. culicivora*'s natural diet and then considering the extent to which prey-choice behaviour and natural diet correspond (see Appendix 1). This work was more conventional, but it prepared me for my primary thesis objective, which was less conventional.

I set out to look more closely at the diversity of ways in which specialization can be expressed by a predator. For this, I decided to concentrate on two specific topics. One of these was the adaptive timing of predatory activity (Chapter 2) and the other was metabolic adaptation (Chapter 3). At the same time, I was interested in how different aspects of specialization might be interrelated.

It was already known that *E. culicivora*'s predatory and mating strategies are entangled. For understanding this, there is a need for background understanding of salticid sensory systems. The most distinctive characteristic of salticids in general is their unique eyes and exceptional capacity to see fine details of their prey and mates (Harland et al., 2012), but *E. culicivora* is also attracted to the odour of blood-carrying mosquitoes (Jackson et al. 2005) and the odour of potential mates (Cross & Jackson 2013). Mating strategy converges with

predatory strategy because both sexes of *E. culicivora* smell more attractive to mates after indirect blood meals (Cross et al. 2009).

However, the entanglement related to specialization I considered is related to yet another unusual characteristic of *E. culicivora*. Besides being attracted to the odour of prey and potential mates (Cross & Jackson 2013; Jackson et al. 2005), *E. culicivora* is also attracted to the odour of two particular plant species (Cross & Jackson 2009), *Lantana camera* and *Ricinus communes*. For one of these species, *L. camera*, the primary compounds that attract *E. culicivora* are known: (*E*)-β-caryophyllene and α-humulene (Nelson et al. 2012), which I will refer to as simply caryophyllene and humulene. In olfactometer experiments, caryophyllene and humulene are attractive to *E. culicivora* individuals from all active life-cycle stages (instars) (Nelson et al. 2012). However, after a 7-day fast, juveniles but not adults become more strongly attracted to the odour of caryophyllene and humulene (Nelson & Jackson 2013). Cold-anthrone testing, which detects the presence of specifically fructose, has demonstrated that juveniles in particular acquire nutrition from plants (Kuja et al. 2012) and it is known that, after feeding on artificial nectar, the smallest instars of *E. culicivora* become more effective at subduing blood-carrying mosquitoes as prey (Carvell et al. 2015). All of this background information suggests that feeding on nectar is especially important for the juveniles and this has been the rationale for deciding to focus on *E. culicivora* hatchlings.

'Hatchlings' are the smallest instars of *E. culicivora* and I propose that this is also a life-cycle stage in which nutritional factors are especially important to this predator. After emerging from their egg sacs, juveniles of *E. culicivora* can be thought of as facing the task of completing the first instar, which means staying alive, moulting and emerging as a second-instar juvenile. With rearing experiments, I investigated the effects of different feeding regimes on the capacity of hatchlings to complete this task (Chapter 3) and, besides

considering the effects of feeding on prey, I considered the effects of feeding on plants, with and without prey also being available.

In a general discussion at the end of the thesis (Chapter 4), my findings will be examined more fully in the context of understanding predatory specialization, and I will also consider the findings from my research in the context of our understanding of malaria. Strictly speaking, this thesis is about basic research on specialization, not applied work related to malaria. All the same, when doing research on a mosquito-specialist predator from Africa, especially when there is evidence of *Anopheles* in particular being the preferred mosquitoes, it would be difficult not to be interested in issues related to malaria.

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CHAPTER TWO: ADAPTIVE TIMING OF PREDATORY ACTIVITY BY A MOSQUITO-SPECIALIST PREDATOR

Credit: Robert Jackson

Female *Evarcha culicivora*

ABSTRACT

Evarcha culicovora, a salticid spider from East Africa, is a mosquito-specialist that feeds indirectly on vertebrate blood by actively choosing blood-carrying mosquitoes as preferred prey and by actively choosing *Anopheles* as preferred mosquitoes. Here, for the first time, we investigate whether specialization by this predator is also expressed in the timing of its predatory activity. With data from field sampling and from systematically observing *E. culicivora* under semi-field conditions, we show that predation tends to be concentrated in the early morning hours, this being when especially many night-feeding anthropophilic anopheline mosquitoes are resting while digesting blood acquired during the night. With data from experiments, we show that *E. culicivora* is significantly more responsive to prey in the morning than in the afternoon, where 'responsive' includes being significantly more inclined to eat living prey, choose the preferred prey, approach a source of prey odour and approach lures made from dead prey that can be seen but not touched or smelled. We found no significant diel pattern in the predator's inclination to mate and, although mate, plant and human odour are salient to *E. culicivora*, there was no significant diel pattern in response to these odours. Our findings suggest that *E. culicivora* has an innate activity pattern specific to predation which should facilitate encounters with its preferred prey.

1.0. Introduction

There has been considerable interest in predators that target specific types of prey, especially when the prey being targeted is a pest species or a disease vector (Murdoch et al. 1985; Poisot et al. 2011; Hodek et al. 2012), but blurring of the distinction between stenophagy and specialization often makes the literature confusing. A predator's natural diet is what it eats in the field and 'stenophagy' is a convenient term for instances of a predator's diet being biased toward a specific selection of the available prey (Pekár et al. 2011). Evidence of stenophagy can suggest hypotheses about predatory specialization, but a convincing case for characterizing a predator as specialized with respect to particular types of prey depends on having details that pertain to the particular adaptations which make the predator especially proficient at targeting the specified prey (West-Eberhard 2003; Huseynov et al. 2008; Pekár and Toft 2015).

We have been interested in clarifying how predatory specialization can be expressed, by *Evarcha culicivora* Wesolowska and Jackson, 2003, a mosquito-specialist predator from East Africa (Jackson and Cross 2015). This jumping spider (family Salticidae) feeds indirectly on vertebrate blood by actively choosing blood-carrying mosquitoes as preferred prey (Jackson et al. 2005) and by actively choosing *Anopheles* as its preferred mosquito genus (Nelson and Jackson 2006). Specialized feeding on this particular prey is also evident in *E. culicivora*'s prey-capture behaviour (Nelson et al. 2005), feature-detection mechanisms (Nelson and Jackson 2012a; Dolev and Nelson 2014) and systems for innate triggering of selective attention (Cross and Jackson 2009a, 2010a,b), along with other behavioural and cognitive capacities (Jackson and Cross 2011). Moreover, there are links between this unusual predatory strategy and *E. culicivora*'s mating strategy: both sexes become

more attractive to the opposite sex after feeding on blood-carrying mosquitoes (Cross et al. 2009).

Owing to their unique, complex eyes which support exceptional proficiency at identifying different kinds of prey by sight (Harland et al. 2012; Land and Nilsson 2012), salticids may come close to being ideal subjects in experiments related to specialized prey-choice behaviour. For example, when salticids are the test subjects, experiments can be carried out using dead prey (lures) mounted in life-like posture on cork discs and virtual prey generated by computer animation software (Harland and Jackson 2002; Nelson and Jackson 2006, 2012a; Dolev and Nelson 2014), thereby removing the risk of uncontrolled prey behaviour introducing confounding variables during experiments. Many salticids also have a well-developed sense of smell (Nelson et al. 2012a; Uhl 2013) and *E. culicivora* is known to be proficient at identifying blood-carrying mosquitoes even when restricted to using olfaction alone (Jackson et al. 2005).

It is particularly interesting that *E. culicivora* targets *Anopheles* as preferred mosquitoes because *Anopheles* is the genus to which all human-malaria vectors belong (Molina-Cruz et al. 2013) and malaria is an especially serious human-health problem in East Africa (Guerra et al. 2008; Murray et al. 2012), including in localities where *E. culicivora* is found. The one-cell parasites responsible for human malaria all belong to the genus *Plasmodium* (Garnham1966; Perez-Tris et al. 2005; Martinsen et al. 2008) and *P. falciparum*, the most lethal of these species, is the dominant agent of human malaria in East Africa.

There are about 500 named species in the genus *Anopheles*, with about 70 of these appearing to be competent human-malaria vectors (Harbach 2004; Godfray 2013). Discussing *An. gambiae*, the most notorious human-malaria vector in Africa

(Spielman and D'Antonio 2001; White et al. 2011), can be complicated because, instead of being a single species, this is a species complex (Coetzee et al. 2000) and molecular methods are necessary for distinguishing between the species in this complex (Fanello et al. 2002). One of these species, *An. gambiae sensu stricto* (Giles 1902), has characteristics that make it especially effective as a vector of *P. falciparum* (Sinka et al. 2010). These characteristics include a strong expression of anthropophagy (i.e. a strong predisposition to take blood meals primarily from people: White, 1974) and olfactory anthropophily (i.e. being attracted to human odour: Takken and Knols 1999; Carey et al. 2010). Although two chromosomal forms of *An. gambiae* s.s. (Coluzzi et al. 1985) are now recognized as being two distinct species (*An. gambiae* and *An. colluzii*), both of which are highly effective malaria vectors (Coetzee et al. 2013), distinguishing between these two cryptic species was unimportant in the present study.

There is no evidence to suggest that *E. culicivora* distinguishes between the odours of different *Anopheles* species or between the odours of mosquitoes from different genera (Jackson and Cross unpubl.), but there is a rationale for suggesting that, as an anthropophilic anopheline species, *An. gambiae s.s.* is particularly relevant to *E. culicivora*. For example, *E. culicivora* resembles *An. gambiae s.s.* by often being associated with human dwellings (Wesolowska and Jackson 2003) and perhaps this sharing of habitat facilitates frequent predation by *E. culicivora* on this particular mosquito species. However, the most striking convergence between *An. gambiae s.s.* and *E. culicivora* might be shared olfactory anthropophily. Like *An. gambiae s.s.*, *E. culicivora* is attracted to human foot odour. Dirty socks have often been used as a convenient human-odour source in experiments related to olfactory anthropophily by *An. gambiae s.s.* (Mukabana et al. 2002; Omolo et al. 2013) and an earlier study

(Cross and Jackson 2011) showed that *E. culicivora* is attracted to socks worn by the same individual whose socks were known to be especially attractive to *An. gambiae s.s.* (Njiru et al. 2006).

Unlike spiders (Foelix 2011), mosquitoes have specialized mouthparts for taking blood directly from vertebrates (Clements 1992) which means that, for *An. gambiae s.s.*, responding to human odour can be a step toward finding opportunities to take blood directly from people. By contrast, olfactory anthropophily for *E. culicivora* probably functions primarily in the context of locating habitats where opportunities for indirect blood meals (i.e., for feeding on blood-carrying anthropophilic mosquitoes) are common.

Anopheles gambiae s.s. and other anthropophilic anopheline mosquitoes tend to feed on blood especially at night and then rest on the walls of human dwellings in the mornings while digesting their evening blood meals (Ferguson et al. 2010; Gatton et al. 2013). Our objective here is to determine whether *E. culicivora*'s timing of predatory activity corresponds to the optimal time for finding its preferred prey. First we determined the prevalence of feeding on prey in the field at different times of the day and then we determined the daily feeding-activity patterns of individual spiders kept in cages under semi-field conditions but with mosquitoes being provisioned as prey throughout the day. As the findings from both of these objectives suggested a tendency to feed more often in the morning than later in the day, our next step was to determine whether *E. culicivora* is innately predisposed to express peak responsiveness to prey in the morning. For this, we carried out experiments in which *E. culicivora* could see, smell, touch and capture living prey, as well as experiments in which the prey-related cues were either visual alone or olfactory alone.

To determine whether peak responsiveness in the morning was specific to predation instead of being a general feature of *E. culicivora*'s activity pattern, we also carried out experiments using living mates instead of living prey, and using mate, human and plant odour instead of prey odour. The rationale for using these particular alternative odours was knowing that *E. culicivora* adults respond to the odour of opposite-sex conspecific individuals ('mates') (Cross and Jackson 2009b) and that juveniles as well as adults respond to the odour of *Lantana camara* and *Ricinus communis* (Cross and Jackson 2009c), these being plant species on which *E. culicivora* is typically found in the field. Findings from previous research suggest that *E. culicivora* juveniles visit plants especially for nectar meals (Kuja et al. 2012; Carvell et al. 2015) and that adults use this plant as a mating site (Cross et al. 2008). We used (*E*)-β-caryophyllene (hereafter referred to simply as caryophyllene) instead of living plants in our experiments because, besides being the dominant compound in the headspace of *Lantana camara* in Kenya, caryophyllene has been shown to attract *E. culicivora* in olfactometer experiments (Nelson et al. 2012; Nelson and Jackson 2013).

We carried out our field sampling and most of our experiments during the daytime because salticids are usually characterized as diurnal predators that rely primarily on vision to guide prey-capture (Foelix 2011) and there has been no evidence suggesting that *E. culicivora* is active under normal night-time ambient light levels. However, as a capacity for capturing prey in dim light (Penney and Gabriel 2009) and in total darkness (Forster 1982; Taylor et al. 1998; Guseinov et al. 2004) is known for a variety of salticid species, we also included experiments designed specifically for investigating whether *E. culicivora* practises predation during the night, including under total darkness.

2.0. Materials and Methods

2.1. General

Our field site and our laboratory were at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology in Mbita Point (western Kenya, elevation 1200 m above sea level, latitude 0◦25'S–0◦30'S, longitude 34◦10'E). Being close to the equator, there was a daytime period of 12–13 h throughout the year, with morning and evening civil twilight (i.e. the time when the geometric centre of the sun is 6° below the horizon) being at approximately 0630 h and 1900 h, respectively (http://time.unitarium.com/sunrise/114815).

As our rearing procedures and many of the details pertaining to experiments corresponded to those in earlier research on *E. culicivora* (see Jackson and Cross 2015), we provide only essential details here. For observations under semi-field conditions and for laboratory experiments, all *E. culicivora* individuals used as test spiders came from laboratory cultures (2nd and 3rd generation), with each individual maintained in its own plastic cage separate from other salticids. Laboratory photoperiod was 12 light:12 dark (lights on at 0700 h).

Adult spiders are called simply 'females' and 'males' and all adults used in experiments were virgins which matured 3–5 weeks before being used. All mosquitoes were *Anopheles gambiae s.s.* (hereafter referred to simply as *An. gambiae*) from stock cultures in Mbita Point. These mosquitoes had continuous access to a 6% glucose solution on cotton wool. The sugar solution was the only available food for male and 'no-blood female' mosquitoes, but 'blood female' mosquitoes also received a blood meal 4 h before being used in experiments. Mosquito body lengths (accurate to the nearest 0.5 mm) were always 4.0–4.5 mm, but test-spider body lengths were more variable.

2.2. Field sampling (Objective 1)

We searched for spiders by sight on or near the exterior walls of buildings occupied by people (i.e. in the kinds of habitat where *E. culicivora* was known to be prevalent). The area searched each time extended for 8 m along a wall at ground level, 1 m up the wall from the ground and 1 m out from the wall. There was grass growing against walls in all sites, and no plants within the area searched were ever more than 1 m high.

We sampled in the morning (0700–0900 hours), at midday (1200–1400 hours) and in the afternoon (1600–1800 hours). We collected each feeding *E. culicivora* individual and its prey in a plastic vial and then separated the spider from its prey by shaking the vial or by prodding the spider with a soft brush (prey transferred to a vial containing 80% ethanol and the vial labelled with a code number corresponding to the spider). The prey was usually a mosquito, but here we were interested solely in the timing of predation. Prey-identification data will be a part of another manuscript in preparation.

The sampling was carried out over an 8-year span (2004–2011), always in the same 4-month period (March–June). The time of day for each sample was decided at random until we had 25 samples from the morning, 35 from midday and 40 from the afternoon. More sampling later in the day compensated in part for an apparent trend of fewer spiders being found as the day progressed. Sometimes the same site was sampled more than once, but never more than once in the same year. We never sampled when it was raining or while vegetation was wet from earlier rainfall.

2.3. Daytime activity pattern under semi-field conditions (Objective 2)

Activity was recorded intermittently between morning and evening twilight for spiders kept one per plastic cage (66 mm long × 66 mm wide × 110 mm high) under an awning outside the south side of the laboratory building where the photoperiod and temperature regime were approximately the same as in the field. The awning shielded the cages from the heat of direct sunlight which we knew would kill the spiders and mosquitoes inside. The awning also shielded the spiders from rain, which we knew could flood the interior of cages. We did not record activity on rainy days because our impression was that, despite being sheltered by the awning, spiders were disinclined to feed when it rained.

We let all spiders feed to satiation on the day before observations began and then, throughout the following day while observations were being made, we ensured that 5–7 living mosquitoes were always in the spider's cage (i.e. whenever fewer than 5 living mosquitoes were present in a cage at the time of checking for activity, more living mosquitoes were added to bring the number in the cage back to 7). As with many salticids (Richman and Jackson 1992), *E. culicivora* builds cocoon-like silken nests for shelter and each individual of *E. culicivora* used for this objective had a nest in the cage on the day of observation.

Wanting to avoid findings that might have been influenced by diel changes in *E. culicivora*'s expression of a preference for blood-carrying female mosquitoes (see Objective 5), we used male mosquitoes as prey for this objective. Male mosquitoes do not normally feed on blood (Nikbakhtzadeh et al. 2015), but *E. culicivora* expresses a preference for male *Anopheles* when the alternative is not a blood-carrying female mosquito (Nelson and Jackson 2012b). Another consideration was that blood-carrying female mosquitoes put into cages early in the morning would have been digesting the

blood steadily through the day, thereby changing gradually from blood to no-blood females. We might have solved this problem by continually removing uneaten prey and substituting more blood-carrying females throughout the day, but removing mosquitoes from cages would have introduced an undesirable risk of altering spider activity.

With our goal being to acquire data each hour between morning and evening twilight, we assigned test spiders at random to three groups, with each group being defined by the times during the day when the spider's location and activity were recorded: Group A checked at 0700, 1000, 1300 and 1600 hours; Group B checked at 0800, 1100, 1400 and 1700 hours; Group C checked at 0900, 1200, 1500 and 1800 hours. There were no scheduled recordings before morning twilight or after evening twilight. However, when we occasionally checked cages during the 30-min intervals before morning twilight and after evening twilight, the spiders were always in their nests and none were feeding (also see Objective 3).

As *E. culicivora* always takes less than 3 h to complete feeding on an individual prey item, adopting a 3-h interval between checking times meant that, whenever we observed feeding by an individual at two successive checking times, we were confident that it was feeding on a different mosquito in two instances. That the spider might have been scavenging on a previously fed-upon prey was unlikely because the mosquito held in the spider's chelicerae always lacked the dried-out appearance typical of discarded prey carcasses.

Test spiders were females (4.5–5.5 mm), males (4.0–4.5 mm) and juveniles (1.5–3.0 mm). No juveniles moulted during the 5 days immediately preceding observation or the 5 days immediately after observation. We pooled the data from all observations because our objective did not encompass questions pertaining to whether

juvenile body length, the sex of adults or the adult-juvenile distinction had any influence on activity pattern, nor was our sample size large enough for considering these questions.

2.4. Night-time activity under semi-natural conditions (Objective 3)

Using a battery-operated headlamp, we checked spiders at 1900, 2000, 2100, 2200, 2300 and 2400 hours (i.e. at times when it was dark outside and at 1-h instead of 3-h intervals). Spiders were exposed to direct light only momentarily because it took no longer than a few seconds to determine the spider's location and whether there was any predatory activity. Otherwise, the methods adopted for this objective were the same as when making observations during the daytime (Objective 2).

2.5. Determining whether spiders killed prey while in total darkness (Objective 4)

Each spider was put into an individual cage at 2000 hours. The cage was then covered by a black cardboard box and kept in a windowless room in our laboratory with all lights turned off. Seven blood-carrying mosquitoes were put into each cage 30 min later. Immediately before transferring the mosquitoes to the cage, we removed the box and, using the battery-operated headlamp with a red light bulb, we removed the stopper from the top of the cage and transferred mosquitoes to the cage, after which we returned the stopper and the box, and turned off the headlamp. Spiders never ate any of the mosquitoes during the brief interval when the cage was lit by the headlamp. The cages were then left covered in the unlit room overnight. At 0600 hours the following morning, the laboratory lights were turned on, the box was removed and the

cage was checked for dead mosquitoes. Control trials were set up the same way except that there was no spider in the cage.

2.6. Morning-afternoon differences in predisposition to capture preferred prey (Objective 5)

Before a trial began, five blood-carrying mosquitoes were already in a cage (see Objective 2 for details concerning cages). The test spider was first put in a glass tube (diameter 15 mm; length 50 mm) with a stopper in each end of the tube. After 15 min, one of the stoppers was removed and, if the spider remained quiescent, this end of the tube was inserted into the introduction hole in the cage. We ensured that the open end of the tube was always the end that the spider was facing. The trial began when the test spider entered the cage. If the spider failed to walk spontaneously into the cage, we removed the other stopper and prodded the spider with a soft brush until it entered the cage. After the test spider entered the cage, the glass tube was removed and the introduction hole in the cage was plugged with a stopper. We then allowed 15 min for the test spider to capture the prey.

Each test spider was used in two successive trials (i.e. this experiment was based on a paired design): in the morning (1000–1200 h) and, on the next or previous day (decided at random), in the afternoon (1500–1700 h). Spiders were fed to satiation the day before they were used in the first of the two trials. During the first trial, we removed the spider from the cage as soon as it captured prey (i.e. it was allowed to eat only one prey item). For standardisation, we used only *E. culicivora* adults and we decided to use females instead of males because, compared with males, female salticids tend to be more strongly motivated to feed (Givens 1978; Jackson & Pollard 1996).

2.7. Morning-afternoon differences in predisposition to mate (Objective 6)

On the whole, our methods for this objective were the same as for Objective 5 except that, instead of a spider being in a cage with prey, a pair of adult spiders (one male and one female) was in each cage. We tested each male twice, on one day in the morning (1000–1200 hours) and on the next or previous day (decided at random) in the afternoon (1500–1700 hours). The female paired with the male on one day was always different from the female paired with the same male on the other day. The female was put into the cage 60 min before the male was put into the cage, and the pair was allowed 15 min to begin mating. No individual female was used in more than one trial and no individual male was used in more than one pair of trials. All spiders were 4.5 mm in body length. As a prerequisite for a successful trial, the female had to be outside of her nest when a trial began and the male had to be outside of his nest on both days when a trial began. We removed any spider that failed to meet these prerequisites from the experiment, replacing it with a substitute spider from our stock culture.

2.8. Morning-afternoon difference in responsiveness to lures made from preferred prey (Objective 7)

The testing apparatus (Fig. 1) was a transparent glass box (100 mm × 100 mm, walls 35 mm high) that sat centred on the top of a wooden platform (170 mm × 170 mm). There was a hole (diameter 16 mm) centred on each of the four sides of the box and another hole centred in the top of a removable glass lid (100 mm × 100 mm). A glass vial (length 50 mm, diameter 16 mm) plugged each hole in each side of the box (open end flush with the inside of the box; other end plugged with a rubber stopper). Eight lures were distributed around the box, one on each side of each glass tube. The

wooden platform, with the glass box on top, was surrounded by a 40-mm high wooden fence (painted white). The fence served as a background against which *E. culicivora* saw the lures.

Each lure was a dead blood-carrying female mosquito positioned in lifelike posture on the top of a cork disc (diameter 6 mm, thickness 2 mm). On the previous day, the mosquito had been immobilized using CO_2 and then kept in 80% ethanol overnight. For preservation, we sprayed the lure and the cork disc with a transparent plastic adhesive (Crystal Clear Lacquer, Atsco Australia Pty). Double-sided sticky tape on the bottom of the disc secured the lure to the wooden table top 10 mm away from the nearest side of the nearest vial and 15 mm out from the nearest side of the box, with the dead mosquito facing the vial.

Experiments were based on a paired design, with each test spider being used in one trial on one day in the morning and in another trial on the next or previous day in the afternoon (see Objective 5). Spiders were fed to satiation the day before they were used in the experiment. We began each trial by introducing a test spider (always an adult female) through the hole in the centre of the box lid. Once the spider was inside, the hole was plugged with a rubber stopper. The trial ended when the test spider entered one of the vials or, if the spider failed to enter a vial, when 15 min elapsed. In addition to fluorescent ceiling lamps providing ambient lighting, the apparatus was lit from 400 mm overhead by a lamp with a 100-W incandescent light bulb. Between trials, the lid was removed and the entire apparatus was washed with 80% ethanol followed by distilled water and then dried. For more detail about making lures and the experimental procedures, see Jackson et al. (2005).

2.9. Morning-afternoon differences in the expression of preference when tested with lures (Objective 8)

Instead of adopting a paired design for this objective, we used independent samples (i.e. the spiders used for trials in the morning were different from the spiders used for trials in the afternoon). In each trial, the test spider could choose between a blood meal (i.e. lures made from blood-carrying female mosquitoes) and a no-blood meal (i.e. lures made from male mosquitoes). Lures at adjacent walls were different and lures at opposite walls were the same. Otherwise, the methods were the same as for Objective 7 and it was particularly important that test spiders fed to satiation on the previous day as this is the prior-feeding condition under which *E. culicivora* is known to express the strongest preference (Nelson & Jackson 2012b).

2.10. Morning-afternoon differences in response to prey, mate, plant and human odour (Objective 9)

Using a Y-shaped glass olfactometer (Figs 2, 3; for details, see: Jackson et al. 2005; Cross and Jackson 2011), all experiments were based on a paired design (see Objective 5) and, for standardization, all test spiders were adult females when prey odour was used (see Objective 5 for rationale) and all test spiders were adult males when any other odour was used. All test spiders fed to satiation on the day before being used in experiments.

Except when using human odour (see below), air was pushed by a pump through two separate flowmeters (Matheson FM-1000 set at 1500 ml/min) into the two chambers, each of which was a glass cube made from 5-mm thick glass (inner dimensions, 70 × 70 × 70 mm, with a removable lid). There were two holes (diameter

20 mm) in the cube, positioned on opposite sides, and each of these holes was plugged with a rubber stopper. In each stopper, there was a hole and, inserted in each stopper hole, there was a glass tube (diameter 4 mm). Living prey and mates were confined to the chambers by putting nylon netting over the stoppers. New netting was used for each trial. Air moved independently through the stimulus chamber to the corresponding arm of the Y-shape olfactometer (i.e. the 'stimulus arm') and through the control chamber to the 'control arm' of the olfactometer, and then converged in the stem of the olfactometer (the 'test arm'). For each trial, we determined at random whether the stimulus arm would be on the left or right.

We put the odour source into the stimulus chamber 30 min before the trial began and we put a test spider in a holding chamber at the far end of the test arm 2 min before testing began. There were two slits in the holding-chamber roof and there was a removable metal grill in each slit, with the test spider confined to the space between the grills. The grill on one side blocked the spider's access to the test arm and the grill on other side ensured that the spider could not leave the holding chamber prematurely. At the end of the 2-min interval, we removed the grill from the slit closest to the test arm. Each trial began when the test spider entered the test arm and ended when the test spider responded, where 'respond' was defined as the test spider entering the stimulus arm and remaining there for 30 s. A maximum of 30 min was allowed for the test spider to respond. After each trial, the olfactometer was dismantled and cleaned with 80% ethanol followed by distilled water and then dried. No test spider and no odour source were used more than once.

Depending on the experiment, the odour source was 10 blood-carrying mosquitoes (prey odour: Jackson et al. 2005), one opposite-sex conspecific individual (mate odour: Cross and Jackson 2009b), human odour (see below) or a 4 µl sample of

caryophyllene (plant odour). The caryophyllene was added to 1.0 g of petroleum jelly in the centre of a small (diameter 30 mm) open Petri dish that sat on the centre of the chamber floor (Nelson et al. 2012b),

The source of human odour was a pair of white socks that had been worn for the previous 12 h by the same anonymous male volunteer who wore the socks used in earlier studies on *An. gambiae* (Njiru et al. 2006) and *E. culicivora* (Cross and Jackson 2011), with the control being a pair of clean but otherwise identical socks. All socks were 100% cotton (length 300 mm). There were two aluminium boxes (inner dimensions 150 × 130 × 130 mm), one for the pair of worn socks (stimulus chamber) and the other for the pair of clean socks (control chamber). There was a wire rack (110 × 100 mm, 90 mm high) inside each chamber, with one sock draped over the top of one 110-mm side and another sock over the top of the opposite 110-mm side of the rack (Fig. 3).

2.11. Data analysis

For details concerning the data analyses, see GraphPad, Prism version 6.00 for Mac OS X (GraphPad Software, La Jolla, California, USA, www.graphpad.com). We used Dunn's multiple comparisons and tests of independence for analyzing data from field sampling. For data from experiments based on a paired design, we used McNemar tests for significance of change (note: this statistical procedure considers only instances in which the outcome from testing in the morning was different from the outcome from testing in the afternoon). For Objective 8, where our data came from using independent samples, we first used tests of goodness of fit (null hypothesis 50/50) separately on data from the morning and the afternoon, after which the morning and afternoon samples were compared using tests of independence. Bonferroni adjustments were applied

whenever data sets were used in more than one comparison, but these adjustments never changed findings from significant to non-significant.

3.0. Results

3.1. Field sampling (Objective 1)

We found more spiders during sampling in the morning than at midday or in the afternoon (Fig. 4A) and the percentage of spiders feeding (Fig. 4B) was larger in the morning than at midday or in the afternoon (Dunn's comparisons for the percentages of spiders that were feeding: morning vs. midday, mean rank difference 35.32, $P<0.001$; morning vs. afternoon, 42.54, $P<0.001$; midday vs. afternoon, 7.22, $P=0.654$). Entire samples in which no spiders were feeding were scarce in the morning (12%), more common at midday (69%) and most common in the afternoon (87%) (tests of independence: morning compared with midday, $\chi^2=18.86$, $P<0.001$; morning compared with afternoon, $\chi^2=36.11$, $P<0.001$; midday compared with afternoon, $\chi^2=3.99$, $P=0.046$).

3.2. Daytime activity pattern under semi-field conditions (Objective 2)

We determined each individual's activity profile (i.e. when it was outside its nest and when it was feeding) and determined the number of individuals corresponding to each profile. At 0700 hours, 66% of the spiders were out of their nests and the percentage out of their nests then remained at 69% or higher until 1600 hours, after which it dropped to 45% at 1700 hours and then to 23% at 1800 hours (Fig. 5). About a third of the spiders (32%) were out of their nests every time they were checked; only 2% were in their nests every time; 38% were out of their nests three times, 18% only twice and 9% only once when checked. Most spiders (85%) were found out of their

nests at least once in the morning and at least once in the afternoon; 9% were found out of their nests only in the morning and 4% only in the afternoon.

After pooling data for all spiders at each time of the day, we used least-square linear regression for a rough characterization of diel trends in predatory activity (Fig. 6). Feeding was especially common in the early morning and then became progressively less often through the day. Of the 1281 spiders that we monitored, only 295 (23%) were ever seen feeding when checked and, of these 295 spiders, 75% were seen feeding only in the morning, 17% only in the afternoon and 8% in the morning and in the afternoon; 88.5% of these 295 spiders were seen feeding once, 10.2% twice and 1.3% three times. For these comparisons, morning defined as 0700–1200 hours and afternoon as 1300–1800 hours.

3.3. Activity and predation under dim light and in total darkness (Objectives 3 and 4)

When checked between 1900 and 2400 hours, all *E. culicivora* juveniles observed at night under semi-field conditions (n=38) were in their nests and no predation was ever seen. For trials in total darkness, we used 44 *E. culicivora* adults (33 females and 11 males) and, in each instance, no mosquitoes died during the testing period, and no mosquitoes from the 44 control trials (no spider present) died.

3.4. Morning-afternoon differences in response to prey and mates (Objectives 5 and 6)

Prey capture was significantly more common in the morning than in the afternoon (Table 1, row 1, McNemar test: χ^2=10.56, P=0.001), but there was no significant morning-afternoon difference for mating (Table 1, row 2, McNemar test: χ^2=0.00,

P=1.000). Significantly more spiders mated in the afternoon than captured prey in the afternoon (test of independence using only data from afternoon trials: χ^2=16.72, *P*<0.001).

3.5. Morning-afternoon difference in response to lures made from preferred prey (Objectives 7 and 8)

For spiders tested twice (once in the morning and once in the afternoon; Objective 7), responding to lures was significantly more common in the morning than in the afternoon (Table 1, row 3, McNemar test: χ^2=13.07, *P*<0.001). There were also significant time-of-day effects on spiders in choice tests (Objective 8; Table 2), where each spider was tested only once, either in the morning or in the afternoon: 93% of 30 spiders tested in the morning made a choice, but only 65% of 40 spiders tested in the afternoon made a choice (test of independence: χ^2=7.80, *P*=0.005). Considering only those spiders that made a choice, 93% of 28 chose the blood meal in the morning but only 62% of 26 chose the blood meal in the afternoon (test of independence: χ^2=7.65, *P*<0.001). Significantly more spiders chose blood instead of no-blood meals in the morning (test of goodness of fit, χ^2=20.57, *P*<0.001), but there was no significant choice in the afternoon (test of goodness of fit, χ^2=1.38, *P*=0.239).

3.6. Morning-afternoon differences in response to prey, mate, plant and human odour (Objective 9)

Response to prey odour was significantly more common in the morning than in the afternoon (Table 1, row 4, McNemar test: χ^2=12.50, *P*<0.001), but there was no significant morning-afternoon difference in response to any other odour (Table 1, row

5–7, McNemar tests; mate odour, χ^2=0.17, P=0.683; plant odour, χ^2=0.10, P=0.752; human odour, χ^2=0.00, P=1.000). In the afternoon, significantly more spiders responded to other odours than to prey odour (mate odour, χ^2=11.83, P<0.001; plant odour, χ^2=14.59, P<0.001; human odour, χ^2=7.52, P=0.006).

4.0. Discussion

Referring to a single preferred prey category can be convenient when making passing reference to examples of predatory specialization. There are, however, important distinctions between the way *E. culicivora* categorizes prey (i.e. this predator's own prey-classification system) and way scientists might categorize the same prey using Latin names from formal scientific taxonomy. When we say *E. culicivora* is a 'mosquito specialist', 'mosquito' means specifically the adult stage in this insect's life cycle. *Paracyrba wanlessi* is another mosquito-specialist salticid, but *P. wanlessi* uses different prey-capture methods depending on whether it is targeting aquatic juvenile mosquitoes or adult mosquitoes, these being substantially different types of prey (Jackson et al. 2014). In *P. wanlessi*'s prey-classification scheme, juveniles and adults of mosquitoes are two distinct preferred prey categories, whereas *E. culicivora* does not appear to classify juvenile mosquitoes as a prey category at all. Yet there are other distinctions which matter to *E. culicivora*, including whether the mosquito is carrying blood instead of not carrying blood, whether it is an anopheline instead of a culicine, and whether it is a female instead of a male (see Jackson and Cross 2015), with none of these categories appearing to matter to *P. wanlessi*. These two mosquito-eating predators illustrate how a deeper understanding of predatory specialization can come from considering preference profiles instead of simply naming a single preferred prey type for the predator.

It is also important to appreciate that prey-choice behaviour is not the only way in which specialization by predators on particular types of prey can be expressed. Our findings in the present study suggest that one of the ways in which *E. culicivora* expresses predatory specialization pertains to the adaptive timing of predatory activity, where 'predatory activity' refers to this predator's innate predisposition to engage in prey-capture and prey-choice behaviour.

There is an interesting correspondence between *E. culicivora*'s and *An. gambiae*'s activity patterns. Typically the activity pattern of *An. gambiae* females is summarized as feeding on blood late at night and in the early pre-dawn hours of the morning, and then resting on the walls of human dwellings while digesting nocturnal blood meals (Murray et al. 2012). This means that, when daylight arrives, *E. culicivora* encounters quiescent blood-carrying *An. gambiae* and other night-feeding malaria vectors on the walls of buildings.

Our field sampling data (Objective 1) revealed an early morning feeding peak for *E. culicivora*. Declining availability of preferred prey during the day or other factors extrinsic to the predator appear not to be the sole determinants of the feeding pattern revealed by sampling because, when spiders were kept under semi-field conditions, but with prey availability kept uniform through the day (Objective 2), the timing of peak feeding was similar to that found in the field. We also looked for, but found no evidence of, nocturnal predation (Objectives 3 and 4).

We proposed that, *E. culicivora* has an innate predisposition to be more responsive to prey in the morning and our experimental findings supported this hypothesis. When we used living prey (Objective 5), visual cues alone (lures made from dead prey: Objective 7) and olfactory cues alone (odour from prey in an

olfactometer: Objective 9), response to prey was significantly stronger in the morning than in the afternoon.

We considered the possibility that *E. culicivora*'s predisposition to be especially responsive to prey in the morning was a consequence of an innate predisposition to be more responsive in general to salient stimuli in the morning. However, in other experiments, no significant morning-afternoon difference was found when *E. culicivora* pairs were given the opportunity to mate (Objective 6) and we found no significant morning-afternoon difference between how many *E. culicivora* individuals responded to mate, human or plant odours (Objective 9). On this basis, we propose that this spider is adjusting activity specifically in the context of predation.

We also found a significant morning-afternoon difference in the expression of prey-choice behaviour (Objective 8). In our experiments, *E. culicivora* had simultaneous access to lures made from blood *Anopheles* females and lures made from *Anopheles* males. In the morning, but not in the afternoon, more spiders chose the blood meal. These findings suggested that *E. culicivora* is often sufficiently motivated to respond to prey in the afternoon without being sufficiently motivated to take the additional step of discriminating between the more preferred and the less preferred type of prey. The explanation for this difference might be partly related to the functioning of salticid eyes.

Salticids have eight eyes, but a large forward-facing pair, called the 'principal eyes', plays the major role in identifying prey. The corneal lenses of the principal eyes are fixed in place on the spider's carapace with the principal-eye retinas being located at the ends of long, slender eye tubes that extend deep into the salticid's cephalothorax. Using muscles attached to these eye tubes, the salticid can orchestrate

specific movement patterns (Land and Nilsson 2012), with ‘scanning’ being especially elaborate. A salticid scans when viewing an object of interest by moving its eye tubes in unison from side to side while also rotating them in alternating fashion clockwise and anti-clockwise. It has been proposed that, while scanning, the salticid carries out an active serial search for salient features in the image and, using this slow and effortful piece-by-piece rendering of a visual object, identifies what it is looking at (Land,1969; Harland et al. 2012).Scanning appears to be a part of the process by which salticids express selective visual attention and selective attention is generally envisaged as a cognitively demanding task governed by capacity limitations (e.g. see Dukas 2004). Our findings suggest that there is a morning-afternoon difference in *E. culicivora*’s willingness to undertake the effortful, attention-demanding task process required for discriminating by sight, in the absence of non-visual cues, between *Anopheles* individuals that are and are not carrying blood.

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Table 1 Response to prey, mates, lures and odours

Row		Response in morning but not in afternoon	Response in afternoon but not in morning	Response in morning and afternoon	No response in morning or afternoon
1	Captured prey	15	1	19	2
2	Mated	4	3	43	0
3	Moved to lure	15	0	12	1
4	Moved to prey odour	17	1	22	4
5	Moved to mate odour	3	3	41	5
6	Moved to plant odour	4	6	38	2
7	Moved to human odour	3	4	29	5

Table 2 Morning-afternoon difference in response to lures in choice tests. Blood meal: lures made from *Anopheles gambiae* females. No-blood meal: lures made from *Anopheles gambiae* males. Response: chose one of the two lure types. No response: failed to make a choice. Two groups of test spiders, one tested in morning and other in afternoon

	Response	No response	Chose blood meal	Chose no-blood meal
Tested in morning	28	2	26	2
Tested in afternoon	26	14	16	10

Fig. 1 Testing apparatus (not to scale) used for Objectives 7 and 8. Fence surrounding box not shown. Lures positioned on each side of each vial. In Objective 7, all lures were blood-carrying female mosquitoes. In Objective 8, lures at adjacent walls were a different type and lures at opposite walls were the same type. Test spider 'response': gets closer to lures by entering a vial

Fig. 2 Olfactometer (not drawn to scale) used for Objective 9 when testing *Evarcha culicivora* with prey odour, mate odour or plant odour. Arrows in silicone tubes indicate direction of airflow. Holding chamber (location of test spider at start of test): length 25 mm, internal diameter 25 mm. Start of test: test spider in holding chamber; grill removed, giving access to test arm, control arm and stimulus arm. Test spider enters stimulus arm to get closer to odour source in stimulus chamber (control chamber empty). Dimensions of test arm, control arm and stimulus arm: length 90 mm, internal diameter 20 mm. Opaque barriers prevent test spider from seeing odour source

Fig. 3 Olfactometer (not drawn to scale) used for Objective 9 when testing *Evarcha culicivora* with human odour coming from previously-worn socks. Arrows in silicone tubes indicate direction of airflow. Holding chamber (location of test spider at start of test): length 25 mm, internal diameter 25 mm. Start of test: test spider in holding chamber; grill removed, giving access to test arm, control arm and stimulus arm. Test spider enters stimulus arm to get closer to odour source (i.e. previously-worn socks) in stimulus chamber. Control chamber contained unworn socks. Dimensions of test arm, control arm and stimulus arm: length 90 mm, internal diameter 20 mm. Opaque

barriers prevent test spider from seeing odour source

Fig. 4 Findings from field sampling of *Evarcha culicivora* (Objective 1). (A) Number of *E. culicivora* individuals found during sampling. (B) Percentage of those *E. culicivora* individuals found during sampling that were feeding when found

Fig. 5 Percentage of spiders seen out of their nests at different times of the day under semi-field conditions (Objective 2)

Fig. 6 Percentage of spiders that were seen feeding on mosquitoes at different times of the day under semi-field conditions (Objective 2). Least-squared linear regression, r^2=0.810, y=21.32-1.19x

Fig. 1.

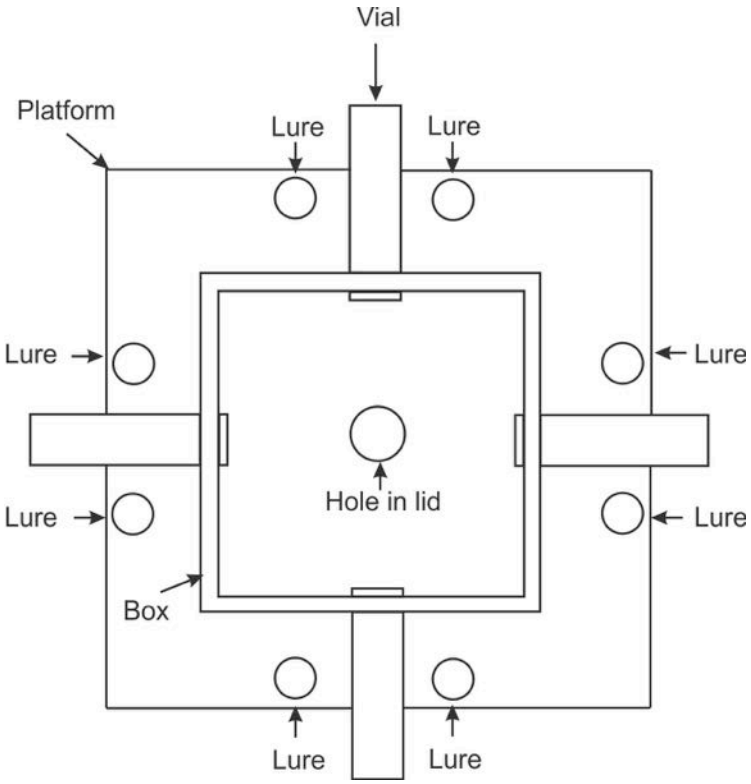

Vial
Platform
Lure
Lure
Lure
Lure
Hole in lid
Lure
Lure
Box
Lure
Lure

Fig. 2.

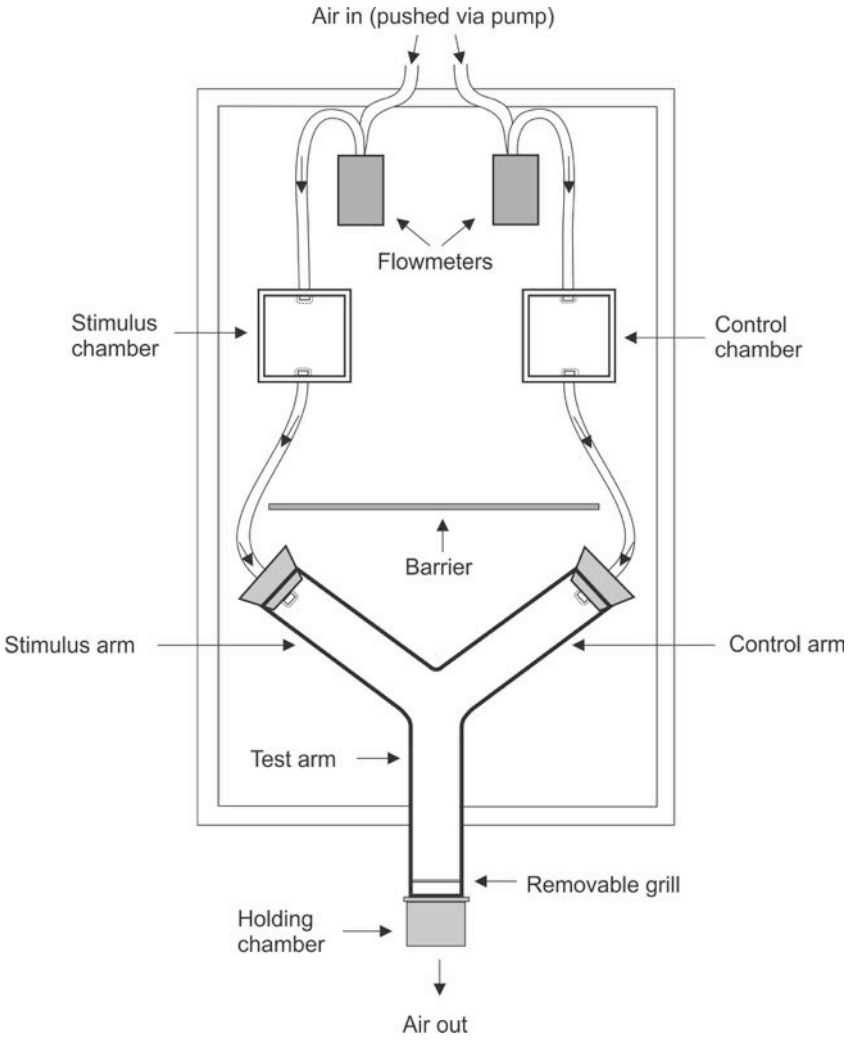

Air in (pushed via pump)
Flowmeters
Stimulus chamber
Control chamber
Barrier
Stimulus arm
Control arm
Test arm
Removable grill
Holding chamber
Air out

Fig. 3.

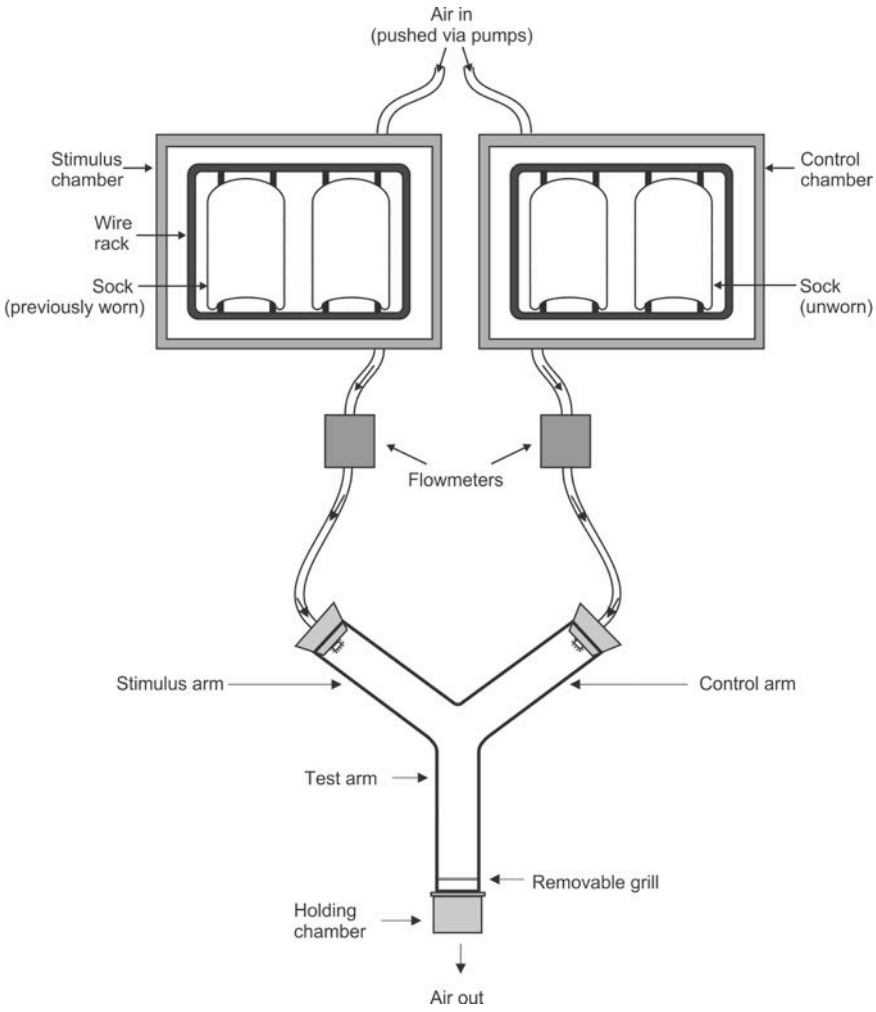

Air in
(pushed via pumps)
Stimulus
chamber
Wire
rack
Sock
(previously worn)
Control
chamber
Sock
(unworn)
Flowmeters
Stimulus arm
Control arm
Test arm
Removable grill
Holding
chamber
Air out

Fig. 4.

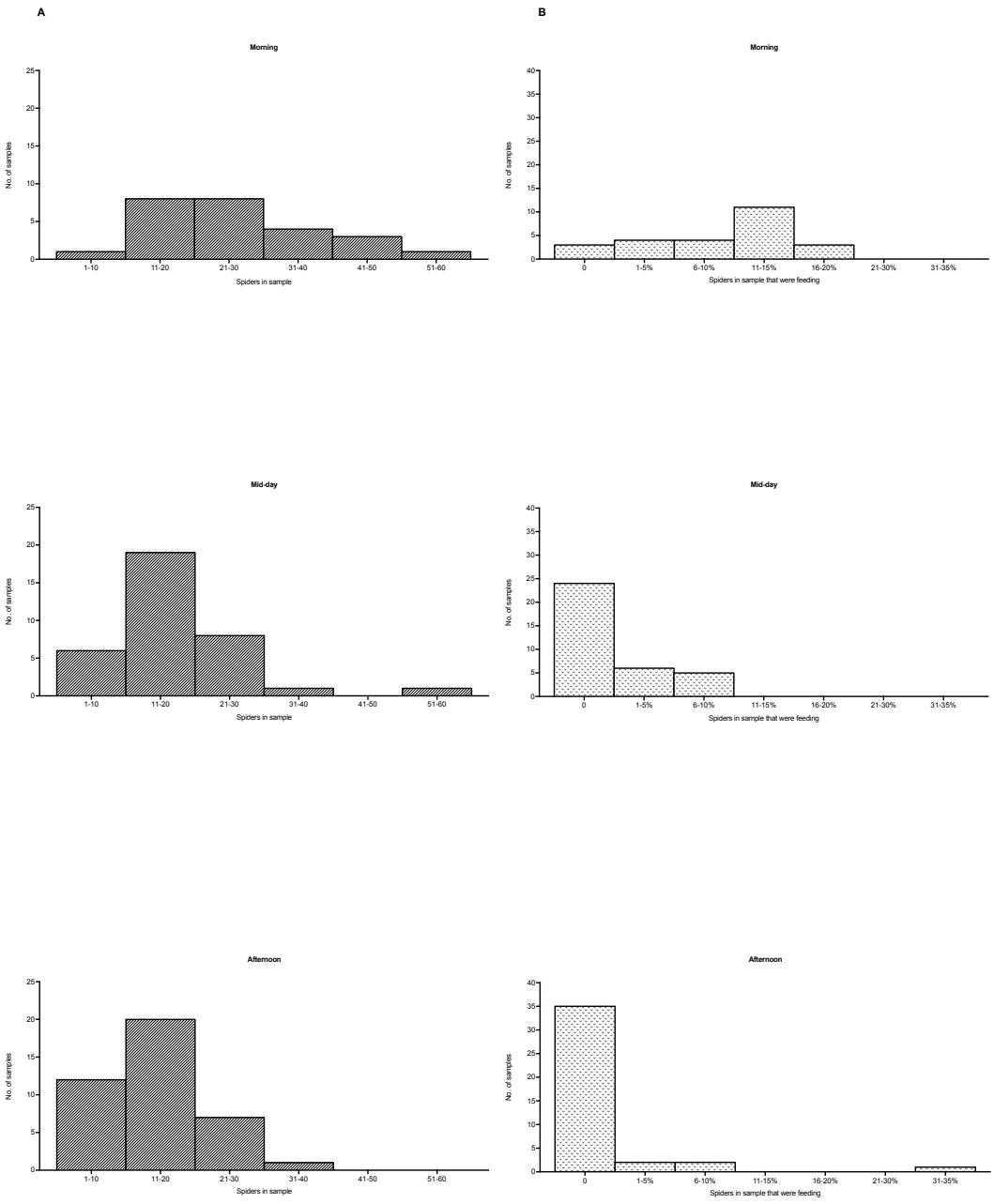

A
Morning
No. of samples
Spiders in sample
1-10
11-20
21-30
31-40
41-50
51-60
B
Morning
No. of samples
Spiders in sample that were feeding
0
1-5%
6-10%
11-15%
16-20%
21-30%
31-35%
Mid-day
No. of samples
Spiders in sample
Mid-day
No. of samples
Spiders in sample that were feeding
Afternoon
No. of samples
Spiders in sample
Afternoon
No. of samples
Spiders in sample that were feeding

Fig. 5.

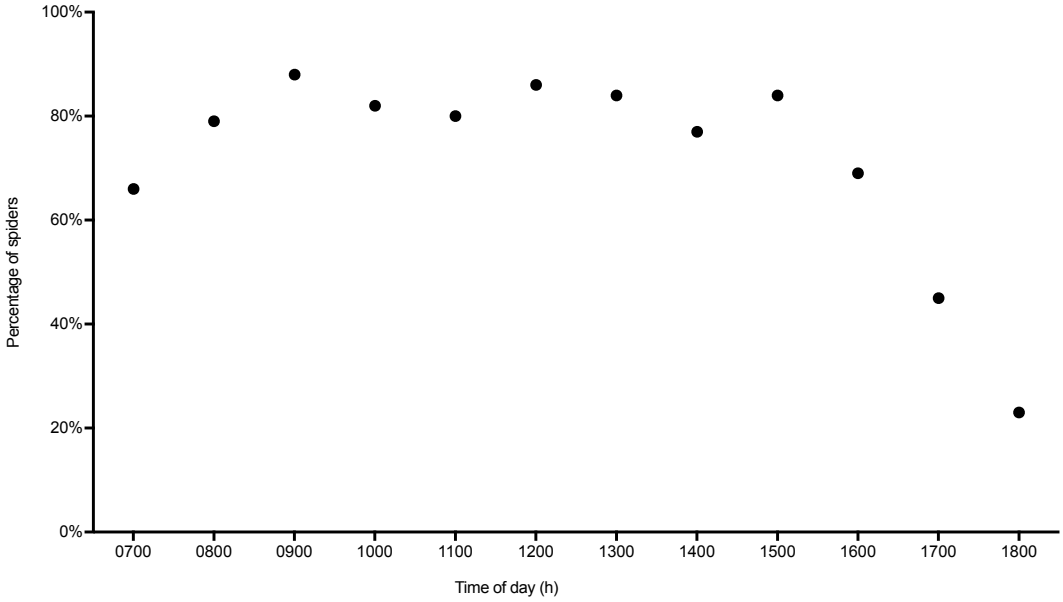
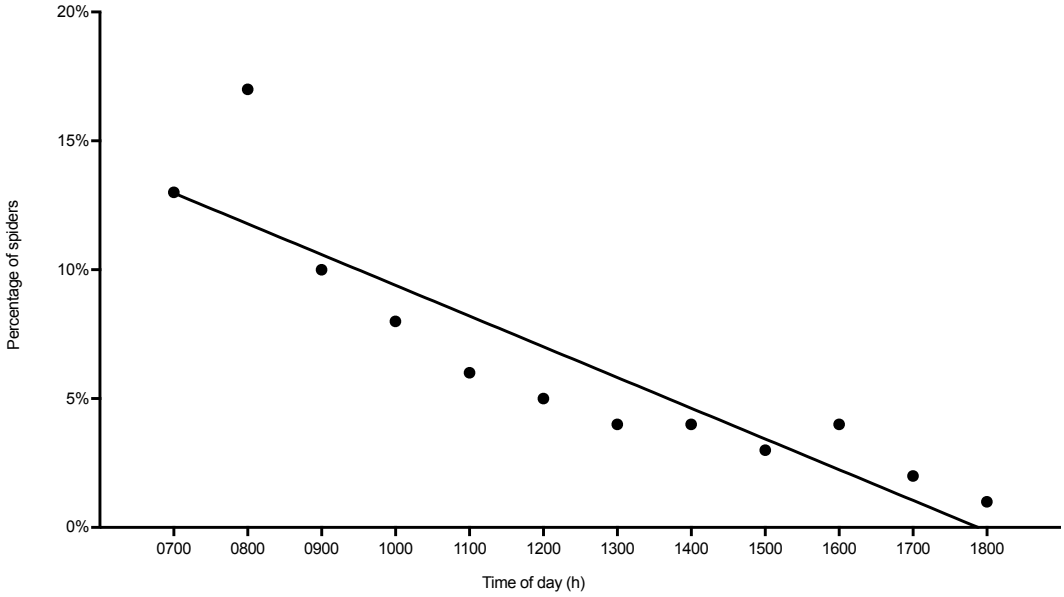

100%
80%
60%
40%
20%
0%
Percentage of spiders
0700
0800
0900
1000
1100
1200
1300
1400
1500
1600
1700
1800
Time of day (h)

Fig. 6.


20%
15%
10%
5%
0%
Percentage of spiders
0700
0800
0900
1000
1100
1200
1300
1400
1500
1600
1700
1800
Time of day (h)

CHAPTER THREE: EFFECTS OF PREY AND NECTAR MEALS ON THE CAPACITY OF A MOSQUITO-SPECIALIST PREDATOR TO COMPLETE THE FIRST ACTVIE STAGE IN ITS LIFE CYCLE

Credit: Robert Jackson

Juvenile *Evarcha culicivora*

Abstract

Evarcha culicivora is a mosquito-specialist salticid spider from East Africa that also feeds on nectar, but there has been no previous research pertaining to the roles nutrients from these two different sources might play in this unusual predator's life cycle. Here I investigate dietary effects on *E. culicivora*'s first active life cycle stage (hatchlings). The dietary variables in experiments were the type and number of mosquitoes eaten, the timing of prey availability, access to plants (with and without also eating prey) and access to artificial nectar made from known sugars and amino acids (with and without also receiving prey). The mosquitoes used as prey were *Anopheles gambiae s.s*. (males, blood-carrying females and females without blood) and the plants belonged to five species. The diet-related effects that I found pertained to the likelihood of completing the first instar ('completion successes), completion time of individuals that were successful and longevity of individuals that were unsuccessful at reaching the next instar. Prey meals appear to be especially important for completion success, but nutrients derived from plants can extend longevity and the hatchlings can then complete the first instar when prey later becomes available. Fructose appears to be the most beneficial sugar in nectar. Amino acids in nectar seem to have minimal effect.

Keywords: Nectarivory, omnivory, spider, Salticidae, *Evarcha culicivora*, mosquito, *Anopheles gambiae*, *Lantana camara*, *Ricinus communis*, *Parthenium hysterophorou*

1.0. Introduction

The origins and adaptive significance of omnivory (i.e., feeding at more than one trophic level) have often been discussed in the context of hypotheses about avoiding overexposure to toxins associated with otherwise superior food, capacities to rely on inferior food sources during periods when superior food is scarce and the acquisition of an optimal balance between the different nutrients provided by different food sources (Pimm & Lawton 1978; Singer & Bernays 2003; Hunter 2009). Omnivory is especially interesting when animals from a group of that has traditionally been envisaged as strictly predatory are shown also to take nutrients directly from plants (e.g., Coll & Guershon 2002). For example, there is a tradition of characterizing spiders as strictly predators, but *Bagheera kiplingi* (Meehan et al. 2009), a Central American jumping spider (Salticidae), is a striking exception. This salticid cohabits with ants (*Pseudomyrmex* spp.) on ant-acacias (*Vachellia* spp.), where it sometimes captures and eats insects, including ant larvae snatched out of the mandibles of attending workers, but the ants as well as *B. kiplingi* feed primarily on specialized leaf tips the ant-acacias (Heil et al. 2004).

Although plant products are not known to be the primary food any other spiders, many spider species may supplement a primarily predatory diet with direct or indirect plant products, including pollen, honeydew, seeds, sap and especially nectar, with the nectar being derived from extra-floral nectaries as well as flowers (Nyffeler 2016, Nyffeler et al. 2016). Although the prevalence of feeding on plant products raises questions about how plant-derived nutrients, either by themselves or combined prey, function in the spider's life cycle, most spider research has not gone much beyond the documenting that spiders feed on plant products. The primary exceptions have come from research on *Cheiracanthium mildei* (Miturgidae) and *Hibana velox*

(Anyphaenidae), two non-salticid spider species for which it is known that combining nectarivory with a predatory diet improves survival rate, growth and fecundity (Taylor & Bradley 2009; Taylor & Pfannenstiel 2009). However, a better understanding of the ways in which plant-related nutrients function in the lives of salticid spiders would be of particular interest because about 60% of the known examples of spiders feeding on plant products have come from this particular spider family (Nyffeler 2016).

That there are especially many examples of salticids feeding directly from plants might be surprising because salticids are known for having a particularly active style of predation (Jackson & Pollard 1996) and also for having unusual eyes (Land & Nilsson 2012; Harland et al. 2012) with which they can detect and identify prey from a distance, and then make highly specific vision-based prey-choice decisions (Nelson & Jackson 2011). For a spider already so well adapted for using vision-based behaviour as an active predator, it may seem as though feeding on plant products would be superfluous, but this is intuition, not an evidence-based conclusion.

Even by salticid standards, the level of prey-choice specificity expressed by *Evarcha culicivora*, the species I consider here, is remarkable (Cross & Jackson 2010a). This East African salticid feeds indirectly on vertebrate blood by actively choosing blood-carrying female mosquitoes as preferred prey (Jackson et al. 2005) and it also chooses species from a particular genus, *Anopheles*, as its preferred mosquitoes (Nelson & Jackson 2006, 2012a). As all human malaria vectors belong to this mosquito genus (Clements 1999; Sinka et al. 2010), *E. culicivora*'s preferences are of unusual interest to people.

Besides having an exceptional capacity for seeing detail, many salticids are also known for making extensive use of chemoreception, including olfaction (Nelson, Warui & Jackson 2012; Uhl 2013). However, the role of olfaction in the biology of *E.*

culicivora is especially complex (Cross & Jackson 2009a, 2010b, 2011, 2013, 2014) and perhaps the most surprising discovery related to this species' use of olfaction has come from research showing that *E. culicivora* responds to the odours of two of the most common plant species in its habitat (Cross et al. 2009b), *Lantana camara* and *Ricinus communis* (Cross et al. 2008).

In Kenya, two sesquiterpenes, β-caryophyllene and α-humulene, are the dominant volatile compounds from the headspace of *Lantana* and *E. culicivora* is known to be attracted to the odour from these particular compounds (Nelson et al. 2012). Pre-trial fasts make the early-instar juveniles, but not the adults, of *E. culicivora* even more strongly predisposed to move toward the source of these odours (Nelson & Jackson 2013) and there is evidence from cold-anthrone testing that small *E. culicivora* juveniles in the field ingest sugar from plants more often than is the case for larger individuals (Kuja et al. 2012). Prior nectar meals have also been shown to improve the smaller juveniles' capacities to overpower mosquitoes that are considerably larger than themselves (Carvell et al. 2015).

This combination of findings suggests that nectar meals are especially important for the smaller juveniles of *E. culicivora*. On this basis, I decided to investigate diet effects on specifically the first active stage in *E. culicivora*'s life cycle. I refer to spiders in this stage as 'hatchlings' and my primary hypothesis is that plant-derived nutrients are especially important for hatchlings. In a series of experiments, I considered this hypothesis by recording the effects of feeding regimes during which hatchlings have access to prey, plants and artificial nectar prepared from known compounds, as well as combinations of prey with plants or artificial nectar.

2.0. Methods and Materials

2.1. General

Arachnologists sometimes refer to the non-feeding postembryonic stages in the life cycles of spiders with various terms, including 'larva' and 'postembryo'. The first active feeding stage, which is normally the stage at which the juvenile leaves the egg sac, is sometimes called 'nymph 1', with similar terms for the stages after each successive moult (Foelix 2011). There appears to be no consensus pertaining to the use of the expression 'instar'. As postembryonic individuals moult one or more time before leaving the egg sac, it is logical to call the stage after each moult a different instar and yet it is also common practice to call 'nymph 1' the 'first instar'. All of this can be confusing for non-arachnologists and for arachnologists (Foelix 2011) and, in most instances, by using the expression 'hatchlings', I will avoid these terminological issues. When it is awkward to avoid specifying an instar, I will refer to the hatchling as the first instar, as this is perhaps the easiest usage for non-arachnologists.

By relying on laboratory cultures derived from individuals collected at the field site in Mbita Point, Western Kenya (elevation 1200m above sea level; latitude 0◦30'S; longitude 34◦10'E), I had access to large numbers of *E. culicivora* hatchlings. The basic methods for rearing, maintaining and testing spiders that I adopted were as in earlier studies (e.g., Cross & Jackson 2013) and I provide only essential details here.

As is commonplace for salticids (e.g., Jackson 1978), *E. culicivora* females put their eggs in silk egg sacs situated inside cocoon-like nests. Hatchings emerge from the nest and spread about in the cage at roughly the same time. On the day of emergence, I isolated the hatchlings into separate cages and assigned them at random to different groups, each group being defined by a specific feeding regime (prey-only

regimes, Table 1; plant-only and prey + plant regimes, Table 2; artificial-nectar regimes, Table 3).

Each cage was cylindrical (height 55 mm, diameter 45 mm) and made from clear plastic. Irrespective of feeding regime, each hatchling had continual access to water by means of a cotton roll ('dental wick') that was inserted into a hole in the bottom of the cage and positioned so that it extended into a water-filled plastic pot below the cage. For ventilation, there was a hole in the top of the cage, which was covered by metal screening. For introducing prey, there was a second hole in the top of the cage. Except when being used for introducing prey, this hole was plugged with a rubber stopper. All holes were 8 mm in diameter.

For each experiment, I recorded how many hatchlings completed the first instar ('completion success') and the longevity of each hatchling that died without completing the first instar. For hatchlings that completed the first instar, I recorded completion time (i.e., the time elapsing between emerging from the nest and being successful at moulting). Longevity and completion time were recorded accurate to the nearest day.

2.2. Prey and plants

The mosquitoes used as prey in experiments were always *Anopheles gambiae sensu strictu* from stock cultures (see Mukabana et al. 2002). Three categories were used: males, blood females and no-blood females. All mosquitoes had access to 6% glucose prior to use in experiments ('sugar meals'). Males and no-blood females received only the sugar meals, but blood females also received a blood meal 4 h before use.

Cuttings from the plants were taken as needed from the field on the day when they were used. The plants were *Lantana camara* (Verbenaceae) and *Ricinus*

communis (Euphorbiaceae), these being the two species known to attract *E. culicivora* in olfactometer experiments (Cross & Jackson 2009b), and also *Lippia kituensis* (Verbenaceae), *Parthenium hysterophorus* (Asteraceae) and *Hibiscus rosa-sinensis* (Malvaceae). Although *L. kituensis* and *R. communis* are native to Kenya, *L. camara* and *P. hysterophorus* are invasive weed species of American origin that have become widespread in Africa and much of the world (Ghisalberti 2000; Beentje 1994; Mcconnachie et al. 2010). The other species, *H. rosa-sinensis* is a widespread horticultural species (Dharani 2011). All of these plants came from, and were common in, the field site.

2.3. Artificial nectar

The sugar and amino acid content of *L. camara* nectar is known on the basis of a personal communication from Irene Baker to Alm et al. (1990): sucrose 187.25 g/L, fructose 57.00 g/L, glucose 55.8 g/L, proline 0.256 g/L, glutamine 0.136 g/L, glycine 0.178 g/L, serine 0.144 g/L, alanine 0.64 g/L, arginine 0.032 g/L, asparagines 0.056 g/L, glutamic acid 0.048 g/L, threonine 0.080 g/L, tyrosine 0.040 g/L, valine 0.016 g/L). Using these sugars and a selection of these amino acids, I made artificial nectar for use in experiments. Two of these were blends approximating the ratios for sugars or for sugars plus amino acids reported by Alm et al. (1990).

Full blend: sucrose 187.3 g/L, fructose 57.0 g/L, glucose 55.8 g/L, proline 0.3 g/L, glutamine 0.1 g/L, glycine 0.2 g/L, serine 0.1 g/L.

Sugar-only blend: sucrose 187.3 g/L, fructose 57.0 g/L, glucose 55.8 g/L.

The other artificial nectars were based on using, in different concentrations of single compounds and using specific combinations of compounds (Table 3). I also wanted to compare the performance hatchlings when using sugars known to be

present in *Lantana* nectar with a sugar that is not known to be present in this plant's nectar. For this, I chose maltose, which is known to be present in the petals and other floral tissue of *Ricinus* and *Parthenium* (Nyasembe et al. 2012), but is not prevalent in the nectar of plants (Nicolson & Thornburg 2007).

2.4. Control trials

The performance of hatchlings in some of the experimental trials was compared with the performance of hatchlings in water-only control trials. The procedures for these control trials were the same as for the experimental trials except that no prey, plants or artificial nectar were provided. Hatchlings in the control trials for prey-only, plant-only and prey+plant regimes (C1) had access to water from the cotton roll and juveniles in the control trials for artificial nectar (C2) had access to water-only artificial flowers in addition to the cotton roll.

2.5. Methods specific to prey-only feeding regimes

In each instance of feeding a hatchling, I first removed the stopper from the hole in the top of the hatchling's cage and then, using an aspirator, I put two mosquitoes in the cage, after which I returned the stopper to the hole. Both of the mosquitoes were of the type specified for the group on that day. On the specified day or days for an experiment, I always introduced the mosquitoes into the cage at 0800 h. Each hatchling was then allowed 60 min in which to capture and begin feeding on one of the two mosquitoes. My rationale for having two mosquitoes present was knowing from preliminary work that having two mosquitoes in a cage at the same time ensures that hatchlings almost always capture a mosquito in the allowed time (i.e., when there

are two mosquitoes, but not when there is only one, hatchlings almost always captured a mosquito within 60 min). As my methods stipulated that the test spider would eat only one mosquito, I removed the other mosquito from the cage after the first was captured. Whenever the hatchling failed to capture a mosquito during the 60-min interval on any one of the scheduled prey-feeding days, I removed this hatchling from the experiment and took a substitute hatchling from stock culture.

2.6. Methods specific to plant-only and prey+plant feeding regimes

For each hatchling, a cutting was taken from a living plant in the field at 0700 hours and then held in a closed plastic box under 100% carbon dioxide for 10 min, after which it was examined under a microscope for any arthropods that might have remained. On rare occasions, a few tiny mites were seen under the microscope and, using forceps, I removed them. I then put the cutting inside the cage at 0800 hours. The cut end of the stem was the only incision or wound on the plant and this cut end remained outside the cage (i.e. the stem, positioned alongside the cotton roll, went through the hole in the bottom of the cage so that the cut end was in the pot of water below the cage). The remainder of the plant (stems, flowers and leaves) was inside, almost filling, the cage.

For plant-only feeding regimes, I removed the plant from the cage at 1800 hours and then, by repeating the procedure, I put a new plant cutting of the same plant species in the cage at 0800 on the next and each successive morning. For prey+plant feeding regimes, the procedure was the same except that there was no plant in the cage on the day when mosquitoes were provided.

2.7. Methods specific to feeding regimes based on using artificial nectar

For providing hatchlings with artificial-nectar meals, I made artificial flowers, each being a disc (diameter 5 mm, thickness 2 mm) cut from a clean kitchen sponge. At 0730 hours, I submerged the disc for 10 s in the solution specified for the particular feeding regime. The control was an artificial flower that had been soaked in water only (see Kuja et al. 2012).

Next the sponge was attached with a pin to the centre top of a clean, dry cotton roll (i.e., the sharp end of the pin was inserted into the cotton) to form the artificial flower. At 0800 hours, the cotton roll that had been providing water to the hatchling was removed from the cage and replaced with the artificial flower (i.e., the lower end of the clean cotton roll went through the hole in the bottom of the cage and sat in a water-filled pot below the cage so that the cotton became damp). The sponge disc was positioned horizontal (i.e., with its long axis aligned with the floor and ceiling of the cage) at the blunt end of the pin, and it was 25 mm above the floor and 30 mm below the ceiling of the cage. At 1800 hours, I removed the artificial flower and then I repeated this procedure on each successive day (i.e., artificial nectar was available to the juvenile from 0800 to 1800 hours on ever day stipulated by the feeding regime).

2.8. Data analyses

I used Prism (GraphPad Software, San Diego, CA, USA) when analyzing data on completion time and longevity, and I display completion-time and longevity data as survival curves derived by using Kaplan-Meier methods. These methods are based on calculating the fraction of the original number of juveniles that were still alive (longevity) or still in the first instar (completion time) on each successive day. For determining whether there were significant treatments effects, I used log-rank Mantel-

Cox tests (also known as Gehan-Breslow-Wilcoxon tests) for pair-wise comparisons. For determining whether the feeding regime that was adopted affected completion success, I used pair-wise tests of independence (based on chi-square). I applied Bonferroni adjustments (Sokal & Rohlf 1995; Hardin et al. 1996) whenever multiple comparisons were made using the same data sets.

2.9. The use of terms

Sometimes I use the expressions 'prey-only' (solely mosquitoes provided), plant-only (solely cuttings from plants were provided), 'solution-only' (solely artificial solutions provided) and 'plant+prey' (plant cuttings and mosquitoes provided). When referring to plants, sometimes I use the genus name with the species being implicit.

3.0. Results and discussion

3.1. Performance in experimental trials and performance in control trials compared

No hatchling from control trials completed the first instar (Table 4, Rows 1 & 2). Longevity in control trials was significantly shorter than longevity of hatchlings in experimental trials in which hatchlings fed on even a single mosquito, had access to only a plant or had access to only artificial nectar (Table 5).

3.2. Hatchlings that ate a single mosquito and had no access to plants or artificial nectar

As the mosquitoes used in all experiments were 4.5 mm in body length and considerably larger than *E. culicivora* hatchlings (1.5 mm in body length), I initially predicted that hatchlings could complete the first instar after eating a single mosquito.

However, contrary to this prediction, no hatchlings in my experiments completed the first instar when their only meal was from a single mosquito on Day 1, regardless of whether the mosquito was a male, a no-blood female or a blood female (Table 4, rows 3-5). Yet these hatchlings lived significantly longer than hatchlings in water-only control trials (Table 5).

These findings suggest that one prey is not sufficient, but there is an alternative hypothesis that should also be considered: that hatchling can complete the first instar after eating a single mosquito, but this meal must come later than Day 1 because, on Day 1, the hatchling's digestive tract is insufficiently developed. However, contrary to this hypothesis, hatchlings that fed for the first time on Day 5 still failed to complete the first instar (Table 4) and there was no significant effect on longevity (i.e., the longevity of hatchlings that ate a single mosquito on Day 5 was not significantly different from the longevity of hatchlings that ate a single mosquito on Day 1; Table 6).

As previous research (Jackson et al. 2005) has shown that even the smallest juveniles of *E. culicivora* express an active preference for blood meals, I predicted that a single blood female mosquito would be the one-prey feeding regime that would have the most beneficial effects on hatchling longevity. However, my data did not corroborate this prediction (i.e., there were no significant differences related to whether the single prey on Day 1 was a blood female, a no-blood female or a male) (Table 6).

When I compared groups in which the type of prey eaten by the spider stayed the same but the number of prey varied, I found that significantly more hatchlings completed the first instar after feeding twice than after feeding only once and I also found that significantly more of the hatchlings completed the first instar after eating

three times instead of only twice when the mosquito eaten each time was a no-blood female or a male, but not when it was a blood female (Table 7).

When hatchlings fed twice, there was no significant difference in completion success related to whether the mosquitoes were blood or no-blood females (Table 7). However, before Bonferroni adjustments were applied, completion success for hatchlings that ate two males would have been significantly less than the completion success of hatchlings that ate two females, regardless of whether or not the females were carrying blood. However, when juveniles fed three times, there was no significant difference related to the type of the prey (Table 7).

Completion times for hatchlings that ate three blood female mosquitoes was significantly shorter than completion times for hatchlings that ate three males (Table 8), but there were no other instances of completion times being significantly different depending on the type of mosquito eaten (completion times ranged from 7 to 16 days, but with the median latency being 10 or 11 in most instances).

Completion times for hatchlings that ate three mosquitoes were not significantly different from completion times for hatchlings that ate only two mosquitoes. Hatchlings that fed on two mosquitoes and still failed to complete the first instar lived significantly longer than hatchlings that ate only one mosquito. However, when the longevity of hatchlings that ate the same number of prey (one, two or three mosquitoes) was compared, no significant effect of prey type was found (Table 6)

3.3. Hatchlings that had access to plants but did not eat mosquitoes

First I considered whether *E. culicivora* hatchlings could complete the first instar when the only nutrients available to them had to be taken directly from plants. There

was evidence of this with two plant species: 25% of the hatchlings kept with *Lantana* and 38% of the hatchlings kept with *Lippia* completed the first instar (Table 4, rows 13 & 14). The number of hatchlings that completed the first instar when kept with *Lantana* was not significantly different from the number that completed the first instar when kept with *L. kituensis* (Table 7). No hatchlings kept with *Hibiscus, Parthenium* or *Ricinus* completed the first instar (Table 4, rows 15-17). After Bonferroni adjustment, completion times for hatchlings kept with *Lippia* were not significantly different from completion times of hatchlings kept with *Lantana* (Table 8).

Irrespective of the identity of the plant, the longevity of hatchlings from plant-only feeding regimes was significantly better (i.e., they lived significantly longer) than the longevity of hatchlings in water-only control trials (Table 5). Moreover, the hatchlings we kept with *Lantana* and *Lippia* had significantly better longevity than the hatchlings from prey-only feeding regimes that ate only one mosquito (Table 6). Longevity with *Parthenium* was not significantly different from longevity with one mosquito in prey-only regimes, but hatchlings that ate a single prey in prey-only regimes had significantly better longevity than hatchlings kept with *Ricinus* and *Hibiscus* in the absence of prey (Table 6).

3.4. Hatchlings that ate mosquitoes and also had access to plants

The findings from prey-only regimes implied that, for completing the first instar when the only food came from eating mosquitoes, a single mosquito does not suffice, but sometimes hatchlings completed the first instar after eating two mosquitoes (i.e., two mosquitoes was the minimum requirement for completion success in prey-only trials). The findings from the plant-only regimes implied that nutrients taken directly from *Lantana* or *Lippia*, but none of the other plants, were sometimes sufficient food for

enabling hatchlings to complete the first instar. Here I take a next step by considering how the hatchling's performance might be affected by combining feeding on prey with feeding from plants.

An initial prediction was that combining feeding from plants with a single mosquito meal would make hatchlings more successful at completing the first instar. However, contrary to this prediction, the number of hatchlings that completed the first instar after having access to plants and also eating a single no-blood female mosquito was not significantly different from the number that completed the first instar while having access to the same plants but without eating a mosquito (Table 7). The completion times of hatchlings kept solely with either *Lantana* or *Lippia* (i.e., provided no mosquitoes as prey) were not significantly different from the completion times of hatchlings kept with these same plants after eating a single no-blood female. I also found no significant difference in completion times for hatchlings that were kept with *Lantana* and hatchlings that were kept with *Lippia* after eating a single no-blood mosquito.

The type of plant hatchlings were kept with had a significant effect on the completion success of hatchlings after they ate two no-blood female mosquitoes. Almost 100% of the juveniles that ate two female mosquitoes, irrespective of whether these were blood or no-blood females, completed the first instar when the plants were *Lantana* or *Lippia* (Table 4), these being the two plant species on which, in the absence of prey, hatchlings performed best in plant-only feeding regimes. No significant difference was found when *Lantana* and *Lippia* were compared, but hatchlings sometimes had significantly better completion success when the plant was *Lantana* or *Lippia* instead of *Parthenium* or *Ricinus.* There were no significant differences when *Parthenium* was compared with *Ricinus* (Table 7).

Completion times were significantly longer when hatchlings had access to *Lantana* or *Lippia* after eating a single no-blood female instead of eating two no-blood females without having any access to a plant. I also found that completion times were significantly longer when hatchlings had access to *Lantana* after eating one instead of two no-blood female mosquitoes and when hatchlings had access to *Lippia* after eating one instead of two no-blood female mosquitoes.

When hatchlings ate two no-blood female mosquitoes and also had access to plants, their completion times were significantly shorter than when they ate two no-blood female mosquitoes without having any opportunity for taking plant meals. However, when the two mosquitoes were blood females instead of no-blood females, completion times were not significantly different. Hatchlings that ate two mosquitoes and were then given access to the plant *Lantana* or *Lippia* had completion times that were significantly shorter than the completion times of hatchlings that had access to these same plants but without eating mosquitoes.

I found that the type of mosquito had a significant effect on completion time when the hatchlings that ate two mosquitoes were then given continuous access to *Lantana* or *Lippia*. The completion times for these hatchlings were significantly shorter when the mosquitoes were no-blood females instead of blood females, but there was no significant difference between no-blood and blood females when the plant was *Parthenium* or *Ricinus* (Table 8)

When hatchlings ate two mosquitoes and then were given continuous access to plants, there were no significant difference in the type of plant on completion times for hatchlings that ate the same type of mosquito (Table 8). There are no significant differences of the completion time when hatchlings ate two mosquitoes and were then kept with different plants.

When hatchlings failed to complete the first instar, I did comparisons of longevity. With one exception, hatchlings that had access to a plant after eating a single no-blood mosquito lived significantly longer than hatchlings that ate a single no-blood mosquito and then had no access to a plant (Table 6). The exception was *Hibiscus*.

In some instances, significant plant-species differences in longevity were found when hatchlings ate a single no-blood female mosquito and were then given continuous access to the plant (Table 6). I found that the hatchlings kept with *Hibiscus* had longevity that was significantly shorter than that of hatchlings kept with *Lantana*, *Lippia* or *Parthenium*. I also found that hatchlings kept with *Ricinus* had significantly shorter longevity than hatchlings that were kept with *Lippia*.

3.5. Hatchlings that had access to artificial nectar but no prey

Juveniles kept on feeding regimes in which the only food source was artificial nectar never completed the first instar (Table 4), but the type of artificial nectar sometimes had a significant effect on longevity (Table 6). When using sucrose, the dominant sugar in *Lantana* nectar (Alm et al. 1990), I varied concentration over a range from 1% to 40%. When using sucrose-only artificial nectar, I found that even at 1%, hatchlings had better longevity than in the water-only control trials (Table 5), but the longevity of hatchlings kept with 1% sucrose were significantly shorter than the longevity of hatchlings kept with 5% sucrose. I found no significant differences when the longevity of hatchlings kept with 5% sucrose was compared to the longevities of hatchlings kept with any of the higher concentrations of sucrose.

The type of sugar used for making sugar-only artificial nectar also mattered. When the concentration was constant at 20%, longevity with maltose was decidedly

shorter than longevity with any of the other sugars (Table 6). I also found that longevity with sucrose was significantly better than longevity with glucose. Sucrose appears to be the most beneficial sugar, followed by fructose, then glucose and then maltose.

For glucose and for fructose, there was no significant difference between longevity related to whether the concentration was 5% instead of 20% (Table 6), and I also found no significant difference between 5% fructose and 5% sucrose. Longevity when hatchlings were kept with 5% sucrose was significantly longer than when hatchlings were kept with 5% glucose. The longevity of hatchlings kept with 5% fructose was significantly longer than the longevity of hatchlings kept with 5% glucose, but there was no significant fructose-glucose difference when the sugar concentration was 20%.

Sucrose is a disaccharide, with the component monosaccharides being glucose and fructose (i.e., splitting each sucrose molecule generates one glucose and one fructose molecule, the resulting total concentration of sugar being twice what it had been when the disaccharide was intact). With this as my rationale, I decided to compare the longevities of hatchlings kept with 5%, 10% and 20% sucrose with the longevities of hatchlings kept with blends corresponding to the concentrations of the two monosaccharides separated out instead of being bound together in the disaccharide (i.e., I used glucose-fructose blends: 5% glucose + 5% fructose, 10% glucose + 10% fructose, 20% glucose + 20% fructose). When I made these comparisons, I found no significant longevity differences between disaccharide and the corresponding two-monosaccharide blend at any concentration (Table 6**).**

3.6. Hatchlings that had access to artificial nectar containing amino acid as well as sugar

Being interested in whether the small volumes of amino acid typically found in nectar might affect the longevity of *E. culicivora* hatchlings, I compared the longevity of hatchlings kept on a 4% amino-acid blend with the longevity of hatchlings from control trials and found that, even in the absence of prey or sugar, hatchling longevity was significantly longer when kept with the amino acid blend instead of water alone (Table 5).

I was also interested in whether small volumes of amino acid combined with sugar in would be better for hatchlings than sugar alone. For this, I used a blend of 20% sugar combined with 4% amino-acid. With one exception, no significant differences were found in longevity when I varied the type of sugar that went into the blend (Table 6). The exception was maltose. When hatchlings were kept with 20% maltose combined with 4% amino acid, their longevity was significantly longer than when hatchlings were kept with 20% maltose alone.

At 27 days, median longevity of hatchlings kept with glucose combined with amino acid was the highest among the sugars and this median of 27 days was more than twice the median longevity of hatchlings kept with 20% glucose alone. The longevity of hatchlings kept with 20% glucose combined with amino acids was significantly longer than the longevity of hatchlings kept with 20% maltose combined with same amino acids. When hatchlings were kept with 20% glucose combined with amino acids, longevity was not significantly different from when hatchlings were kept with 20% sucrose or fructose combined with same amino acids. Good performance on glucose seems to depend on having amino acid as well in the meal.

3.7. Performance when kept on full-blend artificial nectar

The longevity of the hatchlings that failed to complete the first instar when they had continuous access to *Lantana* was not significant different from the longevity of hatchlings had access only to the full-blend artificial nectar (i.e., the blend that was based on the reported content of *Lantana* nectar), the sugar-only blend (i.e., the blend that was based solely on the reported sugar content of *Lantana* nectar), 20% fructose, 20% sucrose or the blend of sucrose plus amino acid (Table 6).

I also found no significant difference in the longevity when hatchlings that were kept with full blend were compared with the longevity of hatchlings kept with the sugar-only blend or hatchlings kept with 20% sucrose. However, hatchlings kept with the sugar-only blend had significantly shorter longevity than hatchlings that were kept with 20% sucrose.

3.8. Hatchlings that fed on mosquitoes in addition to having access to artificial nectar

No hatchlings that ate only one mosquito and had no access to any other food and no hatchlings that had access to artificial nectar but did not eat mosquitoes completed the first instar. However, hatchlings that had access to cuttings from *Lantana* or *Lippia* after eating a single mosquito sometimes completed the first instar. On this basis, I predicted that hatchlings kept on artificial nectar after eating a single mosquito might complete the first instar.

When hatchlings ate two mosquitoes and then had access to the same artificial nectars, some completed the first instar and completion success of these hatchlings was significantly better than that of the hatchlings that fed on one or no prey (Table 7). When the full blend was used, 60% and 50% of the hatchlings completed the first instar after eating either two no-blood females or two blood females, respectively

(Table 4, row 54 & 55); however, there was no significant effect of prey type on completion success or completion time of these hatchlings (Tables 7 & 8).

Completion success was significantly less and completion time was significantly longer for hatchlings kept on the full blend after eating two mosquitoes instead of being kept with *Lantana* cuttings after eating two mosquitoes (Table 7 & 8). However, for completion success and for completion time, whether hatchlings that ate two blood female mosquitoes were then kept with the full- blend artificial nectar or with *Lantana* cuttings had significant effect.

Regardless of whether they ate two mosquitoes, only one mosquito or no mosquitoes and regardless of whether the mosquitoes they ate were no-blood or blood females, there were no significant differences in longevity when the hatchlings had continuous access to the full blend (Table 6). However, hatchlings kept with the full blend after eating one or two mosquitoes lived significantly longer than hatchlings that ate same number and type of mosquitoes but with the mosquitoes being their only food (Table 6).

Although there was no significant longevity difference between hatchlings kept with only the full blend and hatchlings kept with only *Lantana* cuttings, hatchlings kept with full blend after eating a single no-blood female mosquito lived significantly longer than hatchling kept with *Lantana* cuttings after eating a single no-blood female mosquito (Table 6). This suggests that the nutrients provided on the artificial flowers were more abundant or more easily accessed than the nutrients from the cutting.

3.9. Hatchlings that received unlimited access to mosquitoes after being kept for 15 days with only artificial nectar

When we supplied hatchlings with only water, they died without completing the first instar about a week later. When only mosquitoes were provided as food, a minimum of two mosquitoes was necessary before hatchlings completed the first instar. When plant cuttings, but no mosquitoes, were provided, some hatchlings completed the first instar when the plants were *Lantana* or *Lippia*, but not when any other plants were used. No hatchlings completed the first instar when provided with only artificial nectar, even when it was the full blend. However, even though they failed to complete the first instar, hatchlings tended to live significantly longer than on water alone when they had access to plant cuttings, artificial nectar or insufficient numbers of mosquitoes as prey. For example, the median longevity of hatchlings that failed to complete the first instar while kept with *Lantana*, *Lippia* or the full blend in the absence of prey was 22, 21 and 26 days, respectively, which was three times longer than the median longevity of hatchlings in the water-only control trials.

We can characterize hatchlings that did not complete the first instar as experiencing "hard times". Any feeding regime that extended the longevity of these hatchlings significantly beyond that of hatchlings that received water alone might be characterized as a feeding regime that supports better performance (i.e., better than in control trials). However, the expression "better" would appear to be adaptively irrelevant if the hatchling, despite living longer, becomes incapable of completing the first instar even should a good diet become available before it dies. My hypothesis is that, during hard times (e.g., times when prey is scarce), extending longevity by feeding on food source that will not be adequate for completion of the first instar has an important adaptive benefit. When opportunity comes to eat what they need for

completing the first instar, these hatchlings that stayed alive on a subsistence diet are proficient at taking advantage of the opportunity and completing the first instar (i.e., "better late than never").

I carried out an experiment designed to consider a specific version of this more general hypothesis. I considered specifically the capacity of hatchlings that stayed alive on a diet of full-blend artificial nectar alone (i.e., no prey) retained the capacity to complete the first instar when sufficient prey became abundant at a later time. An alternative hypothesis would be that these hatchlings are merely slow to die, meaning that they have lost the capacity to complete the first instar even should prey become abundant while they are in their extended life span.

My findings corroborated the prediction from the first hypothesis. After surviving for 15 days on the full-blend artificial nectar alone, 46% of 84 hatchlings provided with unlimited from day 16 onward succeeded at completing the first instar. The median completion time for these hatchlings was 29 days. This meant that the median time elapsing between prey becoming available and the first instar being completed was two weeks (14 days), which was similar to the median completion times for hatchlings that ate two or three mosquitoes in prey-only feeding (Table 4).

4.0. General discussion

4.1. Prey quantity versus prey quality

Food-related stress has been frequently discussed in the literature on spiders (Foelix 2011), but the role of food quantity has been more often emphasized instead of food quality. For example, spiders are often characterized as predators that are unusually well adapted to surviving through periods of low prey availability (Wise 1993). The resting metabolic rate of spiders tends to be lower than that of most similar-sized land

arthropods, after taking body size into account (e.g., Kawmoto et al. 2011; Canals et al. 2015; but see Lighton et al. 2001), and spiders are well known for their capacity to reduce metabolic rate dramatically and sometimes survive fasts lasting for months at a time (Anderson 1970). However, there is also evidence that spiders sometimes encounter food stress in the context of food quality and imbalances in the nutrient composition of the spiders' intake (Greenstone 1979; Maynst & Toft 2001; Maynst et al. 2003; Wilder 2011). These findings are of interest in the context of understanding the prey-choice and prey-handling behaviour of spiders, with the normal assumption being that the spider's behaviour in relation to a specific prey type pertains to direct adaptation to that prey type, with this adaptation leading to the maximization of the spider's fitness.

Prey-choice behaviour is especially pronounced, and unusual, in the biology of *E. culicivora*, a salticid which has been characterized as a "mosquito-specialist predator". This salticid has remarkable expertise at identifying particular types of prey by sight and also by odour. It appears to be unique as a predator in its active preference mosquitoes, including a particular preference for the mosquito genus *Anopheles*. This salticid is one of the few spider species for which we have a large body of data on prey-choice behaviour from laboratory experiments complemented by a large body of data on natural diet in the field (see Appendix 1). Based on 1115 records of *E. culicivora* feeding in the field, we have evidence that mosquitoes dominate this predator's natural diet; the prey was a mosquito for 80.2% of these 1115 records. These field records along with data from prey-choice experiments were my rationale for using mosquitoes as the prey in all of my experiments.

However, *E. culicivora* is also known for being attracted to plants and it is known that especially the smaller juveniles ingest plant-derived nutrients when they

visit these plants (Jackson & Cross 2015). This was my rationale for including access to plants and to artificial nectar consisting of known compounds in some of my experiments, and also to focus on how variation in diet specifically affects the smallest active stage in *E. culicivora*'s life cycle (i.e., 'hatchlings'). My underlying hypothesis was that fulfilling nutrition requirements is an especially urgent need for the hatchling stage. My first step was to consider the effects of prey quality, prey quantity and these two factors together on hatchling performance.

4.2. Effects of the number of mosquitoes eaten when prey is the only food source

When using prey-only feeding regimes, I found evidence that, in the range 1-3, the number of mosquitoes eaten by hatchlings had a pronounced effect on hatchling performance. However, despite a single mosquito being much larger than the *E. culicivora* hatchling, feeding on one mosquito did not suffice for letting hatchlings complete the first instar. Eating at least two mosquitoes appears to be a prerequisite for completion success.

These findings suggest that the size disparity between the mosquito and the *E. culicivora* hatchling gave me an unrealistic impression of what a hatchling can acquire by eating a single mosquito. That spiders practise extra-oral digestion (Cohen 1995) needs to be taken into consideration when trying to understand what is meant by saying a hatchling ate a mosquito. If the predator had been a toad, for example, then "eating a mosquito" would have meant ingesting the mosquito's entire body in one gulp. However, we have no clear understanding of precisely how much of the mosquito's content and what compounds in particular are ingested when an *E. culicivora* hatchling is using its procedure of extra-oral digestion. The smallness of the hatchling's digestive tract relative to the size of the mosquito suggests that the

quantity of nutrients ingested in a single feeding event is considerably less than the quantity of nutrients a larger *E. culicivora* juvenile or an adult could acquire from the same mosquito. As *E. culicivora* is not a predator that saves uneaten prey remains for later meals, the hatchling acquires only what it can take in a single feeding event. Regardless of what this might be in quantity and quality, it is apparently not as much as the hatchling needs for completing the first instar.

Although two mosquitoes sometimes sufficed for completing the first instar when no other food source was available, most hatchlings appeared to need three. When hatchlings were given three mosquitoes, almost 100% completed the first instar. However, it seems that a feeding regime insufficient for completing the first instar can have other potentially beneficial consequences for the hatchling. It was with longevity that we found the evidence suggesting this. For all prey-only feeding regimes, hatchlings that failed to complete the first instar nonetheless lived longer than hatchlings from water-only control trials. There was also a significant effect of the number of mosquitoes eaten on longevity, with unsuccessful hatchlings, which ate more mosquitoes living longer. This suggests as a hypothesis that a diet which is insufficient for completing the first instar can benefit the hatchling by keeping it alive until a better diet becomes available and that, when the better diet arrives, even though it is late, the hatchling is still competent to complete the first instar. I found evidence that seems to corroborate this hypothesis (Section 4.5), but this was in the context of hatchlings feeding on plant products instead of an inferior prey-only regime.

Toft and Wise (1999) used the expression “high quality prey” for prey that allows a spider to complete its full life cycle with low mortality even when the diet is monotypic. This is not quite the same as what I investigated because I considered only

the hatchling stage, but there is a basis for calling *Anopheles gambiae* "high quality prey" for *E. culicivora*. In the Kenya laboratory, healthy cultures of *E. culicivora* have frequently been maintained for a generation on a diet of midges (Chironomidae and Chaoboridae, known locally as "lake flies"), only to die off in the 2nd and 3rd generation. However, when supplied with *Anopheles* as prey, healthy *E. culicivora* cultures in Kenya have been sustained indefinitely. Healthy cultures of *E. culicivora* have also been sustained for one generation in the New Zealand laboratory, where there was no reliable access to *Anopheles* or other mosquitoes, only to die off in the second and third generation (Jackson & Cross 2015). That *Anopheles gambiae* appears to be a high-quality prey for *E. culicivora* was my rationale for using this species as the prey in my experiments.

4.3. Evidence that female mosquitoes are superior to males as prey for E. culicivora hatchlings

Indirect feeding on vertebrate blood may be the most surprising characteristic of *E. culicivora*. This is something *E. culicivora* achieves by choosing blood-carrying female mosquitoes as preferred prey. This preference is expressed even when the only available prey-choice cues in experiments are visual and also even when the only available prey-choice cues in experiments are olfactory (Jackson et al. 2005). We also know that *E. culicivora* is innately predisposed to express stronger preference for blood-carrying female mosquitoes in the morning than in the afternoon, morning being when this specific prey is most available (Chapter 2).

This predator's unusual prey-choice behaviour has links to unusual mate-choice behaviour. The adult males as well as the adult females of *E. culicivora* express distinctive mate-choice behaviour (Cross & Jackson 2007) and it is known

that both sexes acquire a diet-related odour when they feed on blood female mosquitoes and that this odour makes males more attractive to females and females more attractive to males (Cross et al. 2009).

Even when the mosquitoes are not carrying blood, adults and juveniles of *E. culicivora* discriminate between female and male mosquitoes and express a preference for the females (Nelson & Jackson 2012a). Female and male mosquito antennae differ in appearance and this difference is a major cue used by *E. culicivora* for identifying the sex of the mosquitoes during predator sequences (Nelson & Jackson 2012b). These preferences suggest that eating females, even in the absence of blood, might be more beneficial than eating males throughout this predator's life cycle.

This was my rationale in the present study, for investigating a hypothesis that, for hatchlings, female mosquitoes, even in the absence of blood, are superior to male mosquitoes in the context of completion time. However, my findings failed to corroborate this hypothesis. Although my results showed that hatchlings took significantly fewer days to complete the first instar when they ate three blood females instead of three male mosquitoes, no significant difference in completion time was found when hatchlings on a diet of blood females were compared to hatchlings on a diet of no-blood females or hatchlings on a diet of no-blood females were compared to hatchlings on a diet of male mosquitoes. However, before Bonferroni adjustments were made, completion time for hatchlings on both the blood female and the no-blood female diet was significantly shorter than completion time for hatchlings on the male mosquito diet when they ate two mosquitoes (X^2=6.94, p=0.008), suggesting that increasing the sample size for this comparison would be of interest. If a larger sample size were to reveal a significant difference, then it would be of interest to investigate the basis for this difference.

The diet of the adult mosquitoes appears to be unimportant, as females and males were both fed solely a glucose solution. If the nutrients in these adult females and these adult males differed in a way that influenced hatchling completion time, then it would be useful to try to determine whether, as larvae, females and males foraged differently and, as a consequence of this difference, they had nutrient composition as adults.

4.4. Performances of hatchlings on different plant-only diets

We know *E. culicivora* is often found on plants in the field, that all life-cycle stages of *E. culicivora* are attracted to the odour of *Lantana* and *Ricinus* in olfactometer experiments (Cross & Jackson 2009b) and that juveniles in particular frequently ingest fructose (plant sugar) in the field and the laboratory (Kuja et al. 2012). From earlier work, it was also known already that taking nutrients directly from plants was not a necessity when rearing *E. culicivora* because normal rearing was based on providing prey alone and yet cultures have been sustained for generation after generation year after year in the laboratory without plants being part of the feeding regime. My own data also show that hatchlings complete the first instar when fed mosquitoes in the absence of access to plants.

As a next step, it might be tempting to propose that, for hatchlings, prey meals are an absolute prerequisite for completing the first instar. However, this hypothesis also appears to be refuted by my data. In the plant-only rearing regimes, 25% and 38% of the hatchlings completed the first instar when the plant was *Lantana* and *Lippia*, respectively. No hatchlings kept with plants other than *Lantana* and *Lippia* completed the first instar. When using plant cuttings, an effort was made to eliminate any access to prey and certainly the hatchlings in these trials had no access to

mosquitoes or any other large prey. We cannot entirely rule out the possibility that there were some tiny arthropods (e.g., mites) on *Lantana* and *Lippia* that we missed and that it was nutrients hatchlings got from eating these unknown, unseen arthropods that account for some individuals completing the first instar, but this is probably a far-fetched hypothesis. It seems more likely that the factor that lets 25% and 38% of hatchlings complete the first instar was nectar or some other plant products that comes directly these two species from the family Verbenaceae.

For the five plant species, the apparent ranking of as a food source *Ricinus* and *Hibiscus* as the least beneficial, *Parthenium* intermediate and *Lantana* and *Lippia* best. All plants seemed to have a beneficial effect on longevity. Compared to longevity in the control trials, longevity of hatchlings that had access to plants of all species was significantly longer. Whether access to a plant alone was better or worse than eating one prey and receiving no other food depended on the plant species. When *Lantana, Lippia* or *Parthenium* was the plant species in plant-only feeding regimes, hatchlings survived significantly longer than hatchlings in prey-only feeding regimes fed on a single mosquito, but hatchlings that fed on a single mosquito in prey-only feeding regimes survived significantly longer than hatchling kept on *Ricinus* and *Hibiscus* in plant-only feeding regimes.

These findings suggest that the identity of the plant species hatchlings visit matters, but the characteristics of the different plants that mattered are not clear. If nectar was the primary plant-derived food for hatchlings, then the volume and chemical composition of different plant species' nectar would be factors that we might consider. However, although there is information for *Lantana*, we know little about nectar volume or content for the other species.

When on *Ricinus*, *E. culicivora* juveniles press their mouthparts against drops of nectar on the surface of the conspicuous extra-floral nectaries (EFNs) that are characteristic of this species (Baker et al. 1978, van Rijn & Tanigoshi 1999) and yet even *Ricinus* has, besides its large, conspicuous EFNs, other EFNs that are evident only with magnification (Reed 1923). Conspicuous extra-floral nectaries are not characteristic of *Lantana, Lippia*, *Parthenium* and *Hibiscus*, but EFNs are widespread among plants and often they are not conspicuous (Heil 2013). When on *Lantana*, *E. culicivora* juveniles only rarely go into flowers and yet they become positive for fructose (Kuja et al. 2012). The way *E. culicivora* feeds when on *Lantana* and *Lippia* appears to be by pressing its mouthparts against petals and stems of these plants. This might suggest *E. culicivora* finds and feeds from inconspicuous EFNs on *Lantana* and *Lippia*. This in turn suggests that, instead of thinking about nectar volume, perhaps we should be giving more attention to understanding the manner in which a plant can deliver nectar to hatchlings when attempting to explain the ranking of plants.

Poor performance by hatchlings kept with *Hibiscus* might be explained at least in part by this plant's nectar seeming to be especially sticky. Hatchlings kept with this plant were sometimes seen with nectar appearing to be stuck on their mouthparts. This problem seems to go beyond being a situation where it is instructive to say *Hibiscus* nectar is of low quality and instead is a situation that is more accurately described as *Hibiscus* nectar being actively dangerous for hungry hatchlings.

Butterflies in particular seem to visit *Lantana* frequently (Weiss 1997; Andersson & Dobson 2003; Barp et al. 2011, Mukherjee et al. 2015) and feed on this plant's floral nectar (Baker & Baker 1983). However, a butterfly uses its long proboscis for taking nectar from the flowers. Not having a proboscis, it might be that hatchlings would have considerable difficulty taking nectar from the *Lantana* flowers

in particular and maybe flowers in general. A food source on petals and stems may be particularly advantageous to hatchlings.

4.5. The roles of plant nutrients play when prey is scare or arrive late

Questions concerning how *E. culicivora* might benefit by visiting *Lantana* are of particular interest because this is one of the two plants that ranked highest in my plant-only rearing regimes and *E. culicivora* is frequently found on *Lantana* in the field. We also know that (*E*)-β-caryophyllene, which I will simplify to "caryophyllene", is the dominant volatile compounds in the headspace of *Lantana* in Kenya and that *E. culicivora* is attracted to caryophyllene odour (Nelson et al. 2012). Knowing that pre-trial fasts make the early-instar juveniles of *E. culicivora* even more strongly attracted to caryophyllene (Nelson & Jackson 2013) and knowing that, in the field and during laboratory experiments, *E. culicivora* juveniles often, but larger individuals comparatively rarely, ingest nectar. Kuja et al. (2012) suggest that, for hatchlings, there are important nutrition-related benefits to be gained by visiting *Lantana*, as well as the closely related plant *Lippia*. A short-term benefit was demonstrated in an earlier study where prior nectar meals improved the smaller juveniles' capacities to overpower mosquitoes that are considerably larger than themselves (Carvell et al. 2015). This effect was expressed on the next day after the nectar meal. My objective was different because I was interested in longer-term nutrition-related effects.

The findings from the plant-only feeding regime showed that *Lantana* and *Lippia* have the capacity sometimes to serve as an alternative to eating mosquitoes and enable hatchlings to complete the first instar. For those hatchlings that failed to complete the first instar, access to plants extended longevity and, as demonstrated

using artificial nectar instead of plant cuttings, hatchlings that survive well past what their longevity would have been on water alone can complete the first instar when unlimited prey eventually become available.

Using plant+prey feeding regimes, I found evidence of a third way hatchlings can gain nutrition-related benefits when visiting plants. Access to *Lantana* and *Lippia* can compensate for having fed on a suboptimal number of mosquitoes. When hatchlings were kept with the good-performance plants *Lantana* and *Lippia*, almost 100% completed the first instar after eating two mosquitoes whereas only about half of the hatchlings completed the first instar after eating two mosquitoes and then having no access to a plant.

4.6. The effects of specific sugars and amino acids

It is known from numerous studies that sugar and amino acids acquired by feeding on nectar has beneficial effects on growth, survival and reproduction of insects (e.g. Vrzal et al. 2010, Portillo et al. 2012, Cahenzli & Erhardt 2012, Choate & Lundgren 2013), but my experiments were unusual because they were designed to investigate benefits that apply during a particular phase in the life of a spider, namely the phase immediately after emerging from egg sacs and before having a first prey meal. We have been especially interested in the roles plant-meals derived nutrients might have in improving the capacity of *E. culicivora* hatchlings to complete the first instar. However, it soon became apparent that, for understanding the consequences of plant-related feeding, we needed a baseline understanding of how different prey-only and prey+plant feeding regimes affected the juvenile performance.

The results from these experiments seem to give us a precise understanding of the nutritional requirement of the first-instar spider and they are particularly useful for

clarifying the benefits of plant-derived nutrients from this specialized predator. I was interested in the relevance to the hatchlings of specific sugars and amino acids. For *Lantana* but not for any of the other plants, we had details concerning nectar content from Baker (Alm et al. 1990). However, caution is needed because Baker's work was done using *Lantana* in California and it would not be surprising to discover that composition and volume of *Lantana* nectar in Kenya is different. Unfortunately, it was not realistic in my study to extract enough fresh *Lantana* nectar to do the chemical analysis needed for determining the specific nutrient composition and volume. However, I was able to investigate the performance of hatchlings when they had access to different sugars, amino acids and blends.

My experiments revealed that sugar and amino acid in nectar both matter to the hatchlings. Although no hatchling completed the first instar when the only food source was artificial nectar containing only sugar, only amino acid or combinations of sugar and amino acid, artificial nectar did improve hatchling longevity.

As determined by Baker, the dominant compound in the nectar of *L. camara* is sucrose (18.7% of the total weight: see Alm et al. 1990) and my experiments showed that sucrose improves hatchling longevity. The amount of sucrose present in artificial nectar also appears to be important. At the lowest concentration (1%) of sucrose, hatchling longevity was significantly less than at higher sucrose concentrations. However, no significant differences in longevity were found when concentrations within the range 5%-30% were compared to each other. This suggests that, when ample sucrose is available, a hatchling can avail itself of the time needed to extract as much sucrose as needed for significant extension of longevity.

Evaporation effects were not investigated, but they might have been important. During the 24-h period during which the sponge holding the solution was in the

spider's cage, evaporation might have resulted in substantial changes in the concentration of sucrose. Higher sugar concentrations seem to create difficulties for hatchlings. When I used solutions with higher sucrose concentration (30% and 40%), the sponge disc became noticeably sticky and sometimes hatchlings seemed to be experiencing problems with their mouthparts getting gummed up. Sometimes we could see that the hatchlings were sticking to the sponge discs. However, the longevity of hatchlings that survived when provided with these high-concentration solutions was not significantly different from the longevity of hatchlings kept on 20% sucrose.

4.7. The metabolism of different sugars and combined with amino acids

The disaccharide sucrose, and its component monosaccharides fructose and glucose, dominate the nectar of most plants (Pate et al. 1985). Other sugars, such as the disaccharide maltose, can also be present, but often in only trace amounts and these other sugars are generally considered to be of minor nutritional importance to insects (Baker & Baker 1982, 1983; Barker & Lehner 1974).

In our experiments, we used four sugars. When we reared hatchlings with a single sugar at a concentration of 20%, longevity was highest with sucrose followed by fructose, then glucose and then maltose. No significant difference in longevity was found between the two monosaccharides (fructose & glucose), each being a six-carbon sugar (hexose). However, when a single sugar at a concentration of 5% was used, the hatchlings kept with fructose lived significantly longer than hatchlings kept with glucose. At lower concentration, fructose seems to be an especially beneficial sugar.

Fructose and glucose have the same molecular formula, $C_6H_{12}O_6$, but they differ in molecular structure and tend to be metabolized differently by invertebrates. Fructose metabolism relies on fructokinase whereas glucose mechanism relies on glucokinase or hexokinase. Although fructose has a lower glycemic index than glucose, it creates a much higher glycemic load. The precise mechanism by which sugars are transported within the spider's body is unknown. When referring to mammals, glucose is sometimes called "blood sugar" and glucose is the primary initial fuel used by most organisms for energy production in the form of ATP. However, fructose significantly increases levels of postprandial triglyceride levels (a type of fat) (Bray 2007). This might explain why hatchlings fed with sucrose in my experiments performed better than hatchlings fed maltose at the same concentration. Although sucrose and maltose are both disaccharides, a sucrose molecule consists of a fructose plus a glucose molecule, but each maltose molecule consisting of a pair of glucose molecules. By being proficient in the hydrolysis of sucrose, the hatchling can acquire glucose and fructose, which gives it two potential metabolic pathways for energy derivation. However, the consistently poor performance of hatchlings kept with maltose alone suggests that hatchlings have poor capacity for the hydrolysis of this sugar.

Besides considering the effect of sugar when it is by itself, I also considered the effect of amino acid by itself in artificial nectar. My data show that amino acids by themselves let hatchlings live significantly longer than hatchlings in the water-only control trials. Organisms use amino acids for synthesizing proteins and other biomolecules, but amino acids can, when oxidized to carbon dioxide, serve as an energy source. Although sugars are more important and direct as energy sources, than amino acids, proline is unique because it can stimulate the salt cell and has the

potential to modify insect behaviour by stimulating insect chemosensory receptors (Hansen et al. 1998; Wacht et al. 2000). Proline is known from *Lantana* nectar and is also common in the nectar of many plants. It may be that, when in a difficult situation, hatchlings can consume amino acids from nectar and use them as an energy source. Proline is an especially efficient fuel in short term, as it produces 71% of the levels of ATP that are produced by glucose, but glucose is a far superior fuel in the long term (Carter et al. 2006).

After adding amino acids in small amounts to a blend with sugar, hatchlings fed a blend of 20% maltose with amino acids lived significantly longer than hatchlings fed 20% maltose by itself. There was no significant difference between 20% maltose and 20% glucose, but hatchlings lived significantly longer when kept with glucose instead of maltose combined with amino acid. Amino acid combined with glucose should give hatchlings the basic elements needed for synthesizing protein essential for the hatchlings development, but hatchlings never completed the first instar when they got their sugar and amino acid from artificial nectar. Yet hatchlings sometimes completed the first instar when kept with *Lantana* or *Lippia* cuttings. If the food acquired from the cuttings was nectar, then there are various hypotheses that could be investigated as possible explanation for the different outcomes when using plant cuttings instead of artificial nectar. For example, perhaps some critical component of real nectar was missing from artificial nectar, perhaps the delivery system for nectar on the plant was considerably superior to the artificial nectar or perhaps yeast and bacteria degraded the artificial nectar. The plant cuttings might have replenished nectar throughout the day or the plant cutting might have guarded in some way against yeast and bacteria (see Vannette & Fukami 2016).

4.8. Diet of 15 days on full blend nectar followed by unlimited prey

The hypothesis considered here is that, although full blend *Lantana* nectar does not suffice for completing the first instar, it does keep the hatchling alive and, when mosquitoes then become available, the hatchling can feed on the mosquitoes and complete the first instar. With water alone, hatchlings only lived around seven days, but hatchlings that had access to the full-blend artificial nectar survived significantly longer (median longevity 26 days) than the hatchlings in the water-only control trials (about one week). The critical question I considered was whether the hatchlings that were kept alive on artificial nectar for much longer than they could have lived on water alone still had the capacity to complete the first instar. The alternative hypothesis was that they were just slow to die when kept on artificial nectar, having lost the capacity to complete the first instar even should mosquitoes become abundant.

My data show that, consistent with the first hypothesis, 46% of 84 hatchlings completed the first instar after they were given unlimited prey from day 16 and afterwards. That about half of the hatchlings died despite receiving unlimited prey from day 16 onwards suggests that there the long spell of feeding only on the artificial nectar rendered many hatchlings too weak or impaired to complete the first instar despite unlimited prey being provided. Additional experiments in which the time with only artificial nectar is reduced stepwise from 15 days would be of interest.

Even with success being 46% instead of 100%, this is an important finding. It suggests that, in the field, during difficult times when prey might be scarce, hatchlings could, by relying on nectar, hold on for considerably longer than with water alone and then complete the first instar when prey becomes abundant.

4.9. Nutrient balance

A reasonable expectation is that a predator's prey-choice and prey-handling behaviour, and the methods of food utilization in general, can be understood as adaptations by which fitness is maximized. How this maximization might be achieved by *E. culicivora* is of particular interest. This is the only predator known to feed indirectly on vertebrate blood by expressing a pronounced preference for blood-carrying female mosquitoes, but its preference profile goes beyond this and also includes a choice of *Anopheles* as the preferred mosquitoes and a preference for female instead of male mosquitoes even when the female is not carrying blood. In addition to this unusual style of predatory behavior, *E. culicivora* is also unusual because of the way particular plant species also feature in its feeding repertoire. For some time now, there has been a sense of mystery concerning the role of plants in this predator's life. It might have appeared likely to be complex and interesting, but rearing studies have been lacking until now.

I considered specifically the hatchling stage in this predator's life, where the primary task can be envisaged as completing the first instar. My data show that feeding on three *Anopheles* mosquitoes, in the absence of any other food source, is enough to let almost 100% of the hatchlings complete the first instar. A few complete the first instar after eating only two mosquitoes, but plants give this predator an alternative to prey as a source of nutrients that can help it complete the first instar. If the plant is *Lantana* or *Lippia*, some hatchlings manage to complete the first instar without eating any mosquitoes, demonstrating that eating mosquitoes is not an absolute prerequisite for completing the first instar.

It is particularly interesting that, within limits, nutrients taken from plants and nutrients taken from mosquitoes are interchangeable. For example, if three

mosquitoes are not available, but *Lantana* or *Lippia* are provided, two mosquitoes plus the plant lets close to 100% of the hatchlings complete the first instar. The plant seems to be a good substitute for the third mosquito. There are other effects arising from mosquito-plant pairings. For example completion time was significantly reduced when the hatchling could get a plant meal instead of the third mosquito.

The findings from these experiments suggest that an imbalance in nutrients can be compensated for by taking nutrients from a plant or by eating more mosquitoes. That the plants with the strongest effect were *Lantana* and *Lippia* suggests that there are specific nutrients that hatchlings can acquire especially effectively from these particular plants.

When thinking only about *E. culicivora* as a predator, it is easy to characterize this species as stenophagous, although this is somewhat misleading because, if we focus on how this predator instead of human taxonomists, classify prey, *E. culicivora* starts to seem more appropriately characterized as euryphagous (Appendix 1; Jackson & Cross 2015). When we take into consideration feeding from plants as well as feeding as a predator, the case for characterizing *E. culicivora* as euryphagous becomes even stronger. We just need to allow for euryphagy to apply to feeding at more than one trophic level. Yet, when we focus on *E. culicivora* as a spider that feeds on plant products, a case can be made for stenophagy because *Lantana* and *Lippia* appear to be of special importance. The lesson to learn from *E. culicivora* is perhaps that we should not expect it always to be the case that we can justify simply saying a species is stenophagous or simply euryphagous. Real animals in the real world are more complicated than that.

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Table 1. Experimental groups of *Evarcha culicivora* hatchlings kept on prey-only feeding regimes. Prey always *Anopheles gambiae s.s*. Day 1: day when prey was put into cage. C1: see text. Groups defined by mosquitoes eaten

Description of group	Number of prey eaten	Feeding days specified for this group	Type of mosquito eaten
Water-only control (C1)	0	Nil	Nil
Male on Day 1	1	1	Male
Blood female on Day 1	1	1	Blood female
No-blood female on Day 1	1	1	No-blood female
No-blood female on Day 5	1	5	No-blood female
Male on Day 1 & Day 5	2	1 & 5	Male on both days
Blood female on Day 1 & Day 5	2	1 & 5	Blood female both days
No-blood female on Day 1 & Day 5	2	1 & 5	No-blood female both days
Male on Day 1, 5 & 9	3	1, 5 & 9	Male each day
Blood female on Day 1, 5 & 9	3	1, 5 & 9	Blood female each day
No-blood female on Day 1, 5 & 9	3	1, 5 & 9	No-blood female each day

Table 2. Experimental groups of *Evarcha culicivora* hatchlings kept on plant-only and prey+plant regimes. Prey always *Anopheles gambiae s.s*. Day 1: day when juvenile is put into cage. Groups defined by plant or plant & mosquitoes was provided

Description of group	Plant species	Number of prey eaten	Feeding days with mosquitoes specified for this group	Type of mosquito eaten
Lantana camara, no prey	*Lantana camara*	0	Nil	Nil
Lippia kituensis, no prey	*Lippia kituensis*	0	Nil	Nil
Parthenium hysterophorus, no prey	*Parthenium hysterophorus*	0	Nil	Nil
Ricinus communis, no prey	*Ricinus communis*	0	Nil	Nil
Hibiscus rosa-sinensis, no prey	*Hibiscus rosa-sinensis*	0	Nil	Nil
Lantana camara, no-blood female on Day 1	*Lantana camara*	1	Day 1	No-blood female mosquito
Lippia kituensis, no-blood female on Day 1	*Lippia kituensis*	1	Day 1	No-blood female mosquito
Parthenium hysterophorus, no-blood female on Day 1	*Parthenium hysterophorus*	1	Day 1	No-blood female mosquito
Ricinus communis, no-blood female on Day 1	*Ricinus communis*	1	Day 1	No-blood female mosquito
Hibiscus rosa-sinensis, no-blood female on Day 1	*Hibiscus rosa-sinensis*	1	Day 1	No-blood female mosquito
Lantana camara, no-blood female on Day 1 & 5	*Lantana camara*	2	Days 1 & 5	No-blood female mosquito each day
Lantana camara, blood female on Day 1 & 5	*Lantana camara*	2	Days 1 & 5	Blood-carrying female mosquito
Lippia kituensis & ate a no-blood female on Day 1 & Day 5	*Lippia kituensis*	2	Days 1 & 5	No-blood female mosquito each day
Lippia kituensis, blood female on	*Lippia kituensis*	2	Days 1 & 5	Blood-carrying female mosquito each day

Day 1 & 5				

Table 3. Experimental groups of *Evarcha culicivora* hatchlings kept on artificial-nectar regimes. Artificial nectar always present in cage. In some instances, prey is also eaten. Prey always *Anopheles gambiae s.s*. Day 1: day when juvenile is put into cage. Groups defined by type of artificial nectar present & mosquitoes eaten

Description of group	Details
Water-only control (C2)	Water-only artificial flower available continuously
1% sucrose, no prey	1% sucrose in artificial flower available continuously
5% sucrose, no prey	5% sucrose in artificial flower available continuously
10% sucrose, no prey	10% sucrose in artificial flower available continuously
20% sucrose, no prey	20% sucrose in artificial flower available continuously
30% sucrose, no prey	30% sucrose in artificial flower available continuously
40% sucrose, no prey	40% sucrose in artificial flower available continuously
20% glucose, no prey	20% glucose in artificial flower available continuously
5% glucose, no prey	5% glucose in artificial flower available continuously
20% fructose, no prey	20% fructose in artificial flower available continuously
5% fructose, no prey	5% fructose in artificial flower available continuously
20% maltose, no prey	20% maltose in artificial flower available continuously
1% amino-acid blend, no prey	1% of each amino acid (glutamine, glycine, serine, proline) in artificial flower available continuously
Glucose+amino-acid blend, no prey	20% glucose & 1% of each amino acid (glutamine, glucine, serine, proline) in artificial flower available continuously
Fructose+amino-acid blend, no prey	20% fructose & 1% of each amino acid (glutamine, glucine, serine, proline) in artificial flower available continuously
Maltose+amino-acid blend, no prey	20% maltose & 1% of each amino acid (glutamine, glucine, serine, proline) in artificial flower available continuously
Sucrose+amino-acid blend, no prey	20% sucrose & 1% of each amino acid (glutamine, glucine, serine, proline) in artificial flower available continuously
5% glucose & 5% fructose blend, no prey	5% glucose & 5% fructose in artificial flower available continuously

10% glucose & 10% fructose blend, no prey	10% glucose & 10% fructose in artificial flower available continuously
20% glucose & 20% fructose blend, no prey	20% glucose & 20% fructose in artificial flower available continuously

Table 4. Descriptive statistics from experiments using different feeding regimes (groups) for *Evarcha culicivora* hatchlings. Groups defined by: mosquitoes eaten; plant or plant & mosquitoes provided; type of artificial nectar present & mosquitoes eaten. Completion success (CS): number that completed 1st instar. Completion time (CT): median (range) of days elapsing before successful hatchlings became 2nd-instar juveniles. Longevity (L): median (range) of days elapsing before unsuccessful hatchlings died

Row	Group	N for group	CS	CT	Fig. 1 for CT	N for L	L	Fig. 2 for L
1	Water-only control for prey & plants (C1)	260	0	Nil		260	7 (2-15)	L1
2	Water-only control for artificial nectar (C2)	240	0	Nil		240	7 (4-16)	L2
3	No-blood female on Day 1	30	0	Nil		30	13 (9-22)	L3
4	Blood female on Day 1	30	0	Nil		30	14 (8-17)	L4
5	Male on Day 1	30	0	Nil		30	12 (8-19)	L5
6	No-blood female on Day 5	30	0	Nil		30	14 (9-23)	L6
7	No-blood female on Day 1 & Day 5	30	17	11 (10-13)	CT7	13	22 (16-23)	L7
8	Blood female on Day 1 & Day 5	30	17	11 (9-15)	CT8	13	21 (13-24)	L8
9	Male on Day 1 & Day 5	30	7	10 (10-12)	CT9	23	19 (14-26)	L9
10	No-blood female on Day 1, 5 & 9	60	55	11 (8-15)	CT10	5	24 (24-26)	L10
11	Blood female on Day 1, 5 & 9	60	48	10 (7-15)	CT11	12	23 (19-26)	L11
12	Male on Day 1, 5 & 9	60	48	12 (9-16)	CT12	12	21 (15-28)	L12
13	*Lantana camara*, no prey	100	25	15 (9-57)	CT13	75	22 (4-69)	L13
14	*Lippia kituensis*, no prey	60	23	33 (16-64)	CT14	37	21 (7-83)	L14
15	*Parthenium hysterophorus*, no prey	100	0	Nil		100	15 (5-38)	L15
16	*Ricinus communis*, no prey	30	0	Nil		30	10 (7-15)	L16
17	*Hibiscus rosa-sinensis*, no prey	30	0	Nil		30	10 (4-25)	L17
18	*Lantana camara*, no-blood female on Day 1	30	7	22 (11-34)	CT18	23	20 (5-40)	L18
19	*Lippia kituensis*, no-blood female on Day 1	30	7	30 (7-56)	CT19	23	22 (9-68)	L19

20	*Parthenium hysterophorus*, no-blood female on Day 1	30	1	Nil		29	18 (8-38)	L20
21	*Ricinus communis*, no-blood female on Day 1	30	1	Nil		29	18 (2-32)	L21
22	*Hibiscus rosa-sinensis*, no-blood female on Day 1	30	1	Nil		29	13 (8-25)	L22
23	*Lantana camara*, no-blood female on Day 1 & Day 5	30	29	10 (7-11)	CT23	1	22	
24	*Lantana camara*, blood female on Day 1 & Day 5	30	27	10 (8-17)	CT24	3	4, 17, 19	
25	*Lippia kituensis*, no-blood female on Day 1 & Day 5	30	28	8 (8-13)	CT25	2	8, 18	
26	*Lippia kituensis*, blood female on Day 1 & Day 5	30	29	10 (9-19)	CT26	1	25	
27	*Parthenium hysterophorus*, no-blood female on Day 1 & 5	30	15	9 (8-17)	CT27	15	23 (19-28)	L27
28	*Parthenium hysterophorus*, blood female on Day 1 & 5	30	16	10 (9-14)	CT28	14	24 (17-35)	L28
29	*Ricinus communis*, no-blood female on Day 1 & 5	30	10	10 (7-24)	CT29	20	31 (9-35)	L29
30	*Ricinus communis*, blood female on Day 1 & 5	30	21	10 (9-16)	CT30	9	28 (22-32)	L30
31	1% sucrose, no prey	30	0	Nil		30	15 (9-33)	L31
32	5% sucrose, no prey	30	0	Nil		30	28 (8-43)	L32
33	10% sucrose, no prey	30	0	Nil		30	26 (9-48)	L33
34	20% sucrose, no prey	30	0	Nil		30	30 (11-46)	L34
35	30% sucrose, no prey	30	0	Nil		30	24 (3-45)	L35
36	40% sucrose, no prey	30	0	Nil		30	21 (4-48)	L36
37	20% glucose, no prey	30	0	Nil		30	11 (4-41)	L37
38	20% fructose, no prey	30	0	Nil		30	19 (7-45)	L38
39	20% maltose, no prey	30	0	Nil		30	10 (3-21)	L39
40	5% glucose, no prey	30	0	Nil		30	15 (5-26)	L40
41	5% fructose, no prey	30	0	Nil		30	28 (9-45)	L41
42	5% glucose-fructose blend, but no prey	30	0	Nil		30	31 (11-53)	L42
43	10% glucose-fructose blend, but no prey	30	0	Nil		30	29 (11-49)	L43
44	20% glucose-fructose blend, but no prey	30	0	Nil		30	20 (3-50)	L44
45	4% amino-acid blend (Glu,Gly,Pro,Ser) but no prey	30	0	Nil		30	8 (6-16)	L45
46	20% glucose+amino-acid blend, but no prey	30	0	Nil		30	27 (5-44)	L46
47	20% fructose+amino-acid blend, but no prey	30	0	Nil		30	22 (10-36)	L47
48	20% maltose+amino-acid blend, but no prey	30	0	Nil		30	15 (7-35)	L48

49	20% sucrose+amino-acid blend, but no prey	30	0	Nil		30	23 (11-37)	L49
50	Sugar-only *L. camara* blend, but no prey	30	0	Nil		30	22 (8-40)	L50
51	Full *L. camara* blend, but no prey	30	0	Nil		30	26 (12-61)	L51
52	Full blend & ate one no-blood female	30	0	Nil		30	37 (7-58)	L52
53	Full blend & ate one blood female	30	0	Nil		30	29 (10-53)	L53
54	Full blend & ate two no-blood females	30	18	11 (8-17)	CT54	12	38 (15-56)	L54
55	Full blend & ate two blood females	30	15	11 (9-12)	CT55	15	37 (26-43)	L55

Table 5. Comparisons of longevities of *Evarcha culicivora* hatchlings kept on different experimental feeding regimes with hatchlings in water-only control trials (C1 for prey-only, plant-only & plant+prey feeding regimes; C2 for artificial-nectar regimes. See Table 4 for descriptive statistics pertaininig to experimental groups and controls. For each row, df=1. All comparisions significant by adjusted alpha for 0.05 (Bonferroni).

Experimental group	Row in Table 4	Adjusted alpha	Mantel-Cox test
No-blood female on Day 1	3	0.002	X^2=79.82, p<0.001
Blood female on Day 1	4	0.005	X^2=87.18, p<0.001
Male on Day 1	5	0.005	X^2=63.51, p<0.001
Lantana camara, no prey	13	0.003	X^2=205.50, p<0.001
Lippia kituensis, no prey	14	0.004	X^2=107.90, p<0.001
Parthenium hysterophorus, no prey	15	0.004	X^2=183.10, p<0.001
Ricinus communis, no prey	16	0.004	X^2=23.39, p<0.001
Hibiscus rosa-sinensis, no prey	17	0.004	X^2=20.57, p<0.001
1% sucrose, no prey	31	0.008	X^2=86.21, p<0.001
5% glucose, no prey	40	0.006	X^2=84.16, p<0.001
5% fructose, no prey	41	0.006	X^2=103.90, p<0.001
20% maltose, no prey	39	0.006	X^2=36.95, p<0.001
4% amino-acid blend, no prey	45	0.010	X^2=25.73, p<0.001

Table 6. Comparisons of longevities of *Evarcha culicivora* hatchlings kept on different experimental feeding regimes. See Table 4 for descriptive statistics pertaining to experimental groups and controls. For each row, df=1. *Comparision significant by adjusted alpha for 0.05 (Bonferroni). When significant, Group 1 value for longevity larger

Group 1	Group 2	Rows in Table 4	Adjusted alpha	Mantel-Cox test
No-blood female on Day 1	No-blood female on Day 5	3 & 6	0.003	X^2=2.00, p=0.157
No-blood female on Day 1	Blood female on Day 1	3 & 4	0.003	X^2=0.40, p=0.529
No-blood female on Day 1	Male on Day 1	3 & 5	0.003	X^2=2.20, p=0.138
Blood female on Day 1	Male on Day 1	4 & 5	0.007	X^2=3.00, p=0.084
No-blood female on Day 1 & 5	No-blood female on Day 1	3 & 7	0.003	X^2=26.04, p<0.001*
Blood female on Day 1 & 5	Blood female on Day 1	4 & 8	0.008	X^2=23.75, p<0.001*
Male on Day 1 & 5	Male on Day 1	5 & 9	0.008	X^2=40.49, p<0.001*
Blood female on Day 1 & 5	No-blood female on Day 1 & 5	7 & 8	0.010	X^2=0.42, p=0.518
No-blood female on Day 1 & 5	Male on Day 1 & 5	7 & 9	0.010	X^2=4.20, p=0.041
Blood female on Day 1 & 5	Male on Day 1 & 5	8 & 9	0.010	X^2=1.79, p=0.181
No-blood female on Day 1, 5 & 9	Blood female on Day 1, 5 & 9	10 & 11	0.017	X^2=3.48, p=0.062
No-blood female on Day 1, 5 & 9	Male on Day 1, 5 & 9	10 & 12	0.017	X^2=0.13, p=0.720
Blood female on Day 1, 5 & 9	Male on Day 1, 5 & 9	11 & 12	0.017	X^2=0.22, p=0.640
Lantana camara, no prey	*Lippia kituensis*, no prey	13 & 14	0.003	X^2=0.754, p=0.385
Lantana camara, no prey	*Parthenium hysterophoru*, no prey	13 & 15	0.003	X^2=18.72, p<0.001*
Lantana camara, no prey	*Ricinus communis*, no prey	13 & 16	0.003	X^2=75.46, p<0.001*
Lantana camara, no prey	*Hibiscus rosa-sinensis*, no prey	13 & 17	0.003	X^2=69.92, p<0.001*
Lippia kituensis, no prey	*Parthenium hysterophorus*, no prey	14 & 15	0.005	X^2=11.91, p<0.001*
Lippia kituensis, no prey	*Ricinus communis*, no prey	14 & 16	0.005	X^2=36.12, p<0.001*
Lippia kituensis, no prey	*Hibiscus rosa-sinensis*, no prey	14 & 17	0.005	X^2=34.34, p<0.001*

Parthenium hysterophorus, no prey	*Ricinus communis*, no prey	15 & 16	0.005	X^2=33.69, p<0.001*
Parthenium hysterophorus, no prey	*Hibiscus rosa-sinensis*, no prey	15 & 17	0.005	X^2=29.47, p<0.001*
Ricinus communis, no prey	*Hibiscus rosa-sinensis*, no prey	16 & 17	0.005	X^2=0.02, p=0.879
Lantana camara, no prey	No-blood female on Day 1	13 & 3	0.002	X^2=36.97, p<0.001*
Lippia kituensis, no prey	No-blood female on Day 1	14 & 3	0.003	X^2=15.05, p<0.001*
Parthenium hysterophoru, no prey	No-blood female on Day 1	15 & 3	0.003	X^2=7.51, p=0.006
Ricinus communis, no prey	No-blood female on Day 1	16 & 3	0.003	X^2=28.46, p<0.001*
No-blood female on Day 1	*Hibiscus rosa-sinensis*, no prey	17 & 3	0.003	X^2=15.86, p<0.001*
Lantana camara, no-blood female on Day 1	No-blood female on Day 1	18 & 3	0.003	X^2=25.89, p<0.001*
Lippia kituensis, no-blood female on Day 1	No-blood female on Day 1	19 & 3	0.003	X^2=17.52, p<0.001*
Parthenium hysterophorus, no-blood female on Day 1	No-blood female on Day 1	20 & 3	0.003	X^2=23.33, p<0.001*
Ricinus communis, no-blood female on Day 1	No-blood female on Day 1	21 & 3	0.003	X^2=11.93, p<0.001*
Hibiscus rosa-sinensis, no-blood female on Day 1	No-blood female on Day 1	22 & 3	0.003	X^2=0.015, p=0.902
Lantana camara, no-blood female on Day 1	*Lippia kituensis*, no-blood female on Day 1	18 & 19	0.005	X^2=3.47, p=0.062
Lantana camara, no-blood female on Day 1	*Parthenium hysterophorus*, no-blood female on Day 1	18 & 20	0.005	X^2=1.54, p=0.215
Lantana camara, no-blood female on Day 1	*Ricinus communis*, no-blood female on Day 1	18 & 21	0.005	X^2=5.05, p=0.025
Lantana camara, no-blood female on Day 1	*Hibiscus rosa-sinensis*, no-blood female on Day 1	18 & 22	0.005	X^2=16.67, p<0.001*
Lippia kituensis, no-blood female on Day 1	*Parthenium hysterophorus*, no-blood female on Day 1	19 & 20	0.006	X^2=5.75, p=0.017
Lippia kituensis, no-blood female on Day 1	*Ricinus communis*, no-blood female on Day 1	19 & 21	0.006	X^2=8.95, p=0.003*
Lippia kituensis, no-blood female on Day 1	*Hibiscus rosa-sinensis*, no-blood female on Day 1	19 & 22	0.006	X^2=16.39, p<0.001*
Parthenium hysterophorus, no-blood female on Day 1	*Ricinus communis*, no-blood female on Day 1	20 & 21	0.006	X^2=1.04, p=0.307
Parthenium hysterophorus, no-blood female on Day 1	*Hibiscus rosa-sinensis*, no-blood female on Day 1	20 & 22	0.006	X^2=13.78, p<0.001*
Ricinus communis, no-blood female on Day 1	*Hibiscus rosa-sinensis*, no-blood female on Day 1	21 & 22	0.006	X^2=6.29, p=0.012
5% sucrose, no prey	1% sucrose, no prey	31 & 32	0.008	X^2=21.66, p<0.001*
10% sucrose, but no prey	5% sucrose, no prey	32 & 33	0.007	X^2=0.01, p=0.905
20% sucrose, but no prey	10% sucrose, no prey	33 & 34	0.004	X^2=0.26, p=0.610
30% sucrose, but no prey	20% sucrose, no prey	34 & 35	0.005	X^2=1.13, p=0.288
40% sucrose, but no prey	30% sucrose, no prey	35 & 36	0.025	X^2=0.52, p=0.472
20% sucrose, no prey	20% fructose, no prey	34 & 38	0.003	X^2=3.86, p=0.050

20% sucrose, no prey	20% glucose, no prey	34 & 37	0.004	X^2=13.67, p<0.001*
20% sucrose, no prey	20% maltose, no prey	34 & 39	0.004	X^2=49.40, p<0.001*
20% fructose, no prey	20% glucose, no prey	38 & 37	0.005	X^2=3.40, p=0.065
20% fructose, no prey	20% maltose, no prey	38 & 39	0.006	X^2=19.06, p<0.001*
20% glucose, no prey	20% maltose, no prey	37 & 39	0.006	X^2=5.34, p=0.021
5% frucotse, no prey	20% fructose, no prey	41 & 38	0.006	X^2=0.93, p=0.334
5% glucose, no prey	20% glucose, no prey	40 & 37	0.006	X^2=0.01, p=0.905
5% fructose, no prey	5% sucrose, but no prey	41 & 32	0.006	X^2=0.62, p=0.433
5% frucotse, no prey	5% glucose, no prey	41 & 40	0.006	X^2=17.77, p<0.001*
5% sucrose, but no prey	5% glucose, no prey	32 & 40	0.006	X^2=24.93, p<0.001*
5% fructose & 5% glucose blend, no prey	5% sucrose, no prey	42 & 32	0.010	X^2=1.51, p=0.219
10% fructose & 10% glucose blend, no prey	10% sucrose, no prey	43 & 33	0.017	X^2=2.43, p=0.119
20% fructose & 20% glucose blend, no prey	20% sucrose, no prey	44 & 34	0.005	X^2=0.53, p=0.468
Fructose+amino-acid blend, no prey	20% fructose, no prey	47 & 38	0.006	X^2=1.39, p=0.238
Glucose+amino-acid blend, no prey	20% glucose, no prey	46 & 37	0.006	X^2=6.51, p=0.011
Maltose+amino-acid blend, no prey	20% maltose, no prey	48 & 39	0.007	X^2=12.85, p<0.001*
Sucrose+amino-acid blend, no prey	20% sucrose, no prey	49 & 34	0.004	X^2=7.28, p=0.007
Fructose+amino-acid blend, no prey	Glucose+amino-acid blend, no prey	47 & 46	0.007	X^2=5.83, p=0.016
Fructose+amino-acid blend, no prey	Maltose+amino-acid blend, no prey	47 & 48	0.007	X^2=2.98, p=0.084
Sucrose+amino-acid blend, no prey	Fructose+amino-acid blend, no prey	47 & 49	0.006	X^2=1.18, p=0.278
Glucose+amino-acid blend, no prey	Maltose+amino-acid blend, no prey	46 & 48	0.007	X^2=13.07, p<0.001*
Sucrose+amino-acid blend, no prey	Glucose+amino-acid blend, no prey	46 & 49	0.006	X^2=2.79, p=0.095
Sucrose+amino-acid blend, no prey	Maltose+amino-acid blend, no prey	48 & 49	0.006	X^2=9.72, p=0.002*
Lantana camara, no prey	20% sucrose, no prey	13 & 34	0.003	X^2=1.44, p=0.230
Lantana camara, no prey	20% fructose, no prey	13 & 38	0.003	X^2=0.69, p=0.406
Lantana camara, no prey	Sucrose+amino-acid blend, no prey	13 & 49	0.003	X^2=0.65, p=0.422
Lantana camara, no prey	Full blend, no prey	13 & 51	0.003	X^2=1.72, p=0.180
Lantana camara, no prey	Sugar-only *L. camara* blend, no prey	13 & 50	0.004	X^2=2.57, p=0.109
20% sucrose, no prey	Full blend, no prey	51 & 34	0.003	X^2=0.36, p=0.550

20% sucrose, no prey	Sugar-only blend, no prey	50 & 34	0.004	X^2=9.36, p=0.002*
Full blend, no prey	Sugar-only blend, no prey	51 & 50	0.006	X^2=7.49, p=0.006
Full blend & ate one no-blood females	Full blend & ate one blood females	52 & 53	0.006	X^2=5.794, p=0.016
Full blend & ate one no-blood females	Full blend, no prey	52 & 51	0.005	X^2=2.60, p=0.107
Full blend & ate one blood females	Full blend, no prey	53 & 51	0.005	X^2=0.03, p=0.870
Full blend & ate two no-blood females	Full blend, no prey	54 & 51	0.005	X^2=3.39, p=0.066
Full blend & ate two blood females	Full blend, no prey	55 & 51	0.005	X^2=0.69, p=0.406
Full blend & ate two no-blood females	Full blend & ate one no-blood females	54 & 52	0.006	X^2=0.70, p=0.404
Full blend & ate two blood females	Full blend & ate one blood females	55 & 53	0.007	X^2=1.76, p=0.185
Full blend & ate two no-blood females	Full blend & ate two blood females	55 & 54	0.007	X^2=3.36, p=0.067
Full blend & ate one no-blood females	No-blood female on Day 1	52 & 3	0.003	X^2=58.14, p<0.001*
Full blend & ate one blood females	Blood female on Day 1	53 & 4	0.007	X^2=51.79, p<0.001*
Full blend & ate two no-blood females	No-blood female on Day 1 & 5	54 & 7	0.008	X^2=18.32, p<0.001*
Full blend & ate two blood females	Blood female on Day 1 & 5	55 & 8	0.008	X^2=30.10, p<0.001*
Full blend & ate one blood females	*Lantana camara* & ate one no-blood females	52 & 18	0.005	X^2=22.04, p<0.001*

Table 7. Completion success of *Evarcha culicivora* hatchlings. Note: no hatchlings that ate only one prey completed the first instar. For each row, df=1. *Comparision significant by adjusted alpha for 0.05 (Bonferroni). When significant, Group 1 value for completion success larger.

Group 1	Group 2	Rows in Table 4	Adjusted alpha	Test of Independence
No-blood female on Day 1 & 5	No-blood female on Day 1	3 & 7	0.013	X^2=23.72, p<0.001*
Blood female on Day 1 & 5	Blood female on Day 1	4 & 8	0.013	X^2=23.72, p<0.001*
Male on Day 1 & 5	Male on Day 1	5 & 9	0.013	X^2=7.92, p=0.005*
No-blood female on Day 1, 5 & 9	No-blood female on Day 1 & 5	7 & 10	0.008	X^2=15.31, p<0.001*
Blood female on Day 1, 5 & 9	Blood female on Day 1 & 5	8 & 11	0.008	X^2=5.43, p=0.020
Male on Day 1, 5 & 9	Male on Day 1 & 5	9 & 12	0.008	X^2=27.02, p<0.001*
No-blood female on Day 1 & 5	Blood female on Day 1 & 5	7 & 8	0.007	X^2=0.00, p=1.000
No-blood female on Day 1 & 5	Male on Day 1 & 5	7 & 9	0.007	X^2=6.94, p=0.008
Blood female on Day 1 & 5	Male on Day 1 & 5	8 & 9	0.007	X^2=6.94, p=0.008
No-blood female on Day 1, 5 & 9	Blood female on Day 1, 5 & 9	10 & 11	0.010	X^2=3.36, p=0.067
No-blood female on Day 1, 5 & 9	Male on Day 1, 5 & 9	10 & 12	0.010	X^2=3.36, p=0.067
Blood female on Day 1, 5 & 9	Male on Day 1, 5 & 9	11 & 12	0.010	X^2=0.00, p=1.000
Lantana camara, no prey	*Lippia kituensis*, no prey	13 & 14	0.010	X^2=3.17, p=0.075
Lantana camara, no-blood female on Day 1	*Lantana camara*, no prey	18 & 13	0.013	X^2=0.03, p=0.853
Lippia kituensis, no-blood female on Day 1	*Lippia kituensis*, no prey	19 & 14	0.025	X^2=2.03, p=0.155
Lantana camara, no-blood female on Day 1 & 5	*Lippia kituensis*, no-blood female on Day 1 & 5	23 & 25	0.008	X^2=0.35, p=0.550
Lantana camara, blood female on Day 1 & 5	*Lippia kituensis*, blood female on Day 1 & 5	24 & 26	0.008	X^2=1.07, p=0.301
Lantana camara, no-blood female on Day 1 & 5	*Parthenium hysterophorus*, no-blood female on Day 1 & 5	23 & 27	0.008	X^2=16.70, p<0.001*
Lantana camara, no-blood female on Day 1 & 5	*Ricinus communis*, no-blood female on Day 1 & 5	23 & 29	0.008	X^2=26.45, p<0.001*
Lippia kituensis, no-blood female on Day 1 & 5	*Parthenium hysterophorus*, no-blood female on Day 1 & 5	25 & 27	0.010	X^2=13.87, p<0.001*
Lippia kituensis, no-blood female on Day 1 & 5	*Ricinus communis*, no-blood female on Day 1 & 5	25 & 29	0.010	X^2=23.25, p<0.001*

Lantana camara, blood female on Day 1 & 5	*Parthenium hysterophorus*, blood female on Day 1 & 5	24 & 28	0.008	X^2=9.93, p=0.002*
Lantana camara, blood female on Day 1 & 5	*Ricinus communis*, blood female on Day 1 & 5	24 & 30	0.008	X^2=3.75, p=0.053
Lippia kituensis, blood female on Day 1 & 5	*Parthenium hysterophorus*, blood female on Day 1 & 5	26 & 28	0.010	X^2=15.02, p<0.001*
Lippia kituensis, blood female on Day 1 & 5	*Ricinus communis*, blood female on Day 1 & 5	26 & 30	0.010	X^2=7.68, p=0.006*
Parthenium hysterophorus, no-blood female on Day 1 & 5	*Ricinus communis*, no-blood female on Day 1 & 5	27 & 29	0.010	X^2=1.71, p=0.190
Parthenium hysterophorus, blood female on Day 1 & 5	*Ricinus communis*, blood female on Day 1 & 5	28 & 30	0.010	X^2=1.76, p=0.184
Lantana camara, no prey	20% sucrose, no prey	13 & 34	0.013	X^2=9.29, p=0.002*
Lantana camara, no prey	Full blend, no prey	13 & 51	0.013	X^2=9.29, p=0.002*
Full blend & ate two no-blood females	Full blend & ate one no-blood females	54 & 52	0.017	X^2=25.71, p<0.001*
Full blend & ate two blood females	Full blend & ate one blood females	55 & 53	0.017	X^2=20.00, p<0.001*
Full blend & ate two no-blood females	Full blend & ate two blood females	54 & 55	0.010	X^2=0.61, p=0.436
Lantana camara & ate two no-blood females	Full blend & ate two no-blood females	54 & 23	0.008	X^2=11.88, p<0.001*
Lantana camara & ate two blood females	Full blend & ate two blood females	55 & 24	0.008	X^2=11.43, p<0.001*

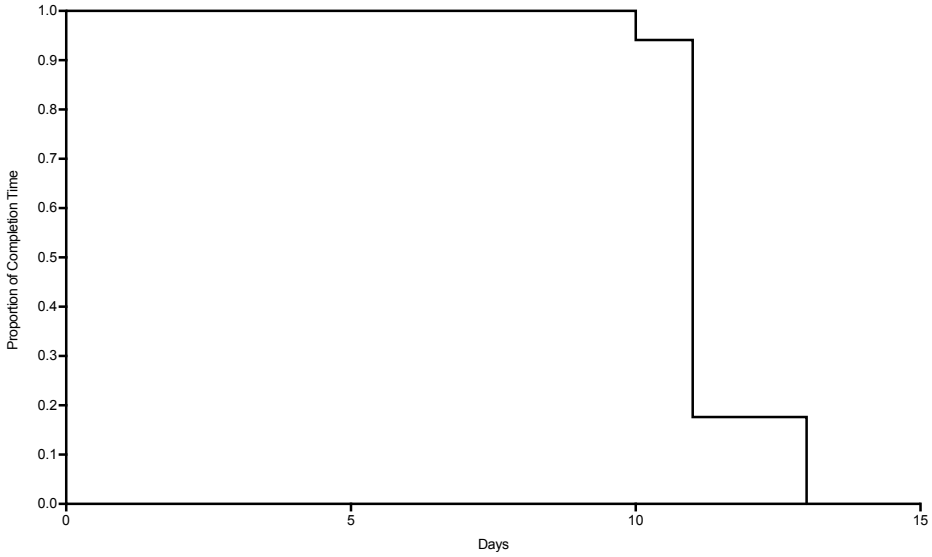
Table 8. Comparison of completion times for *Evarcha culicivora* hatchlings that were successful at completing first instar. Prey-only feeding regimes. For each row, df=1. *Comparision significant by adjusted alpha for 0.05 (Bonferroni). When significant, Group 1 value for completion time smaller

Group 1	Group 2	Rows in Table 4	Adjusted alpha	Mantel-Cox test
No-blood female on Day 1 & 5	No-blood female on Day 1, 5 & 9	7 & 10	0.003	X^2=0.71, p=0.401
Blood female on Day 1 & 5	Blood female on Day 1, 5 & 9	8 & 11	0.004	X^2=0.15, p=0.696
Male on Day 1 & 5	Male on Day 1, 5 & 9	9 & 12	0.01	X^2=4.22, p=0.040
No-blood female on Day 1 & 5	Blood female on Day 1 & 5	7 & 8	0.003	X2=1.31, p=0.253
No-blood female on Day 1 & 5	Male on Day 1 & 5	7 & 9	0.004	X2=5.76, p=0.016
Blood female on Day 1 & 5	Male on Day 1 & 5	8 & 9	0.005	X2=0.48, p=0.490
No-blood female on Day 1, 5 & 9	Blood female on Day 1, 5 & 9	10 & 11	0.004	X2=5.45, p=0.020
No-blood female on Day 1, 5 & 9	Male on Day 1, 5 & 9	10 & 12	0.006	X2=0.25, p=0.620
Blood female on Day 1, 5 & 9	Male on Day 1, 5 & 9	11 & 12	0.006	X2=7.20, p=0.007*
Lantana camara, no prey	*Lippia kituensis*, no prey	13 & 14	0.007	X^2=6.10, p=0.014
Lantana camara, no-blood female on Day 1	*Lantana camara*, no prey	18 & 13	0.007	X^2=0.00, p=0.997
Lippia kituensis, no-blood female on Day 1	*Lippia kituensis*, no prey	19 & 14	0.007	X^2=0.02, p=0.88
Lantana camara, no-blood female on Day 1	*Lippia kituensis*, no-blood female on Day 1	18 & 19	0.007	X^2=2.06, p=0.15
No-blood female on Day 1 & 5	*Lantana camara*, no-blood female on Day 1	18 & 7	0.004	X^2=16.06, p<0.001*
No-blood female on Day 1 & 5	*Lippia kituensis*, no-blood female on Day 1	19 & 7	0.004	X^2=9.20, p=0.002*
Lantana camara, no-blood female on Day 1 & Day 5	*Lantana camara*, no-blood female on Day 1	18 & 23	0.004	X^2=20.79, p<0.001*
Lippia kituensis, no-blood female on Day 1 & Day 5	*Lippia kituensis*, no-blood female on Day 1	19 & 25	0.005	X^2=14.96, p<0.001*
Lantana camara, no-blood female on Day 1 & Day 5	No-blood female on Day 1 & 5	23 & 7	0.003	X^2=29.43, p<0.001*
Lippia kituensis, no-blood female on Day 1 & Day 5	No-blood female on Day 1 & 5	25 & 7	0.003	X^2=25.57, p<0.001*
Parthenium hysterophorus, no-blood female on Day 1 & 5	No-blood female on Day 1 & 5	27 & 7	0.003	X^2=11.22, p<0.001*
Ricinus communis, no-blood female on Day 1 & 5	No-blood female on Day 1 & 5	29 & 7	0.003	X^2=9.47, p=0.002*

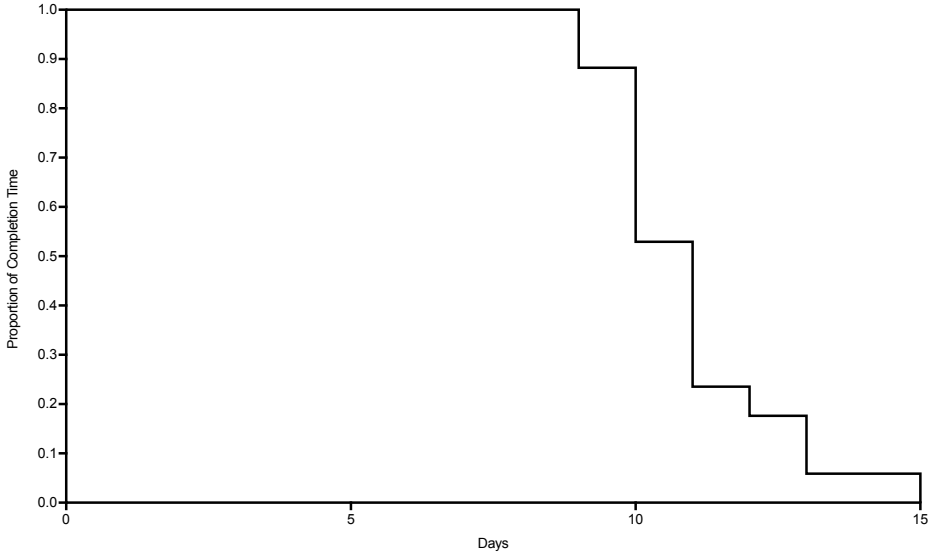
Lantana camara, blood female on Day 1 & Day 5	blood female on Day 1 & 5	24 & 8	0.003	X^2=0.25, p=0.614
Lippia kituensis, blood female on Day 1 & Day 5	blood female on Day 1 & 5	26 & 8	0.004	X^2=0.73, p=0.392
Parthenium hysterophorus, blood female on Day 1 & 5	blood female on Day 1 & 5	28 & 8	0.004	X^2=1.97, p=0.161
Ricinus communis, blood female on Day 1 & 5	blood female on Day 1 & 5	30 & 8	0.004	X^2=0.03, p=0.871
Lantana camara, no-blood female on Day 1 & Day 5	No-blood female on Day 1, 5 & 9	23 & 10	0.003	X^2=17.33, p<0.001*
Lippia kituensis, no-blood female on Day 1 & Day 5	No-blood female on Day 1, 5 & 9	25 & 10	0.004	X^2=37.06, p<0.001*
Parthenium hysterophorus, no-blood female on Day 1 & 5	No-blood female on Day 1, 5 & 9	27 & 10	0.004	X^2=6.44 p=0.011
Ricinus communis, no-blood female on Day 1 & 5	No-blood female on Day 1, 5 & 9	29 & 10	0.004	X^2=2.00, p=0.157
Lantana camara, blood female on Day 1 & Day 5	Blood female on Day 1, 5 & 9	24 & 11	0.004	X^2=1.82, p=0.178
Lippia kituensis, blood female on Day 1 & Day 5	Blood female on Day 1, 5 & 9	26 & 11	0.004	X^2=9.56e-01, p=0.998
Parthenium hysterophorus, blood female on Day 1 & 5	Blood female on Day 1, 5 & 9	28 & 11	0.004	X^2=0.47, p=0.494
Ricinus communis, blood female on Day 1 & 5	Blood female on Day 1, 5 & 9	30 & 11	0.004	X^2=0.17, p=0.681
Lantana camara, no-blood female on Day 1 & Day 5	*Lantana camara*, no prey	23 & 13	0.004	X^2=41.870, p<0.001*
Lantana camara, blood female on Day 1 & Day 5	*Lantana camara*, no prey	24 & 13	0.005	X^2=20.360, p<0.001*
Lippia kituensis, no-blood female on Day 1 & Day 5	*Lippia kituensis*, no prey	25 & 14	0.005	X^2=55.940, p<0.001*
Lippia kituensis, blood female on Day 1 & Day 5	*Lippia kituensis*, no prey	26 & 14	0.005	X^2=56.930, p<0.001*
Lantana camara, no-blood female on Day 1 & Day 5	*Lantana camara*, blood female on Day 1 & Day 5	23 & 24	0.003	X^2=11.71, p<0.001*
Lippia kituensis, no-blood female on Day 1 & Day 5	*Lippia kituensis*, blood female on Day 1 & Day 5	25 & 26	0.004	X^2=24.10, p<0.001*
Parthenium hysterophorus, no-blood female on Day 1 & 5	*Parthenium hysterophorus*, blood female on Day 1 & 5	27 & 28	0.005	X^2=0.78, p=0.38
Ricinus communis, no-blood female on Day 1 & 5	*Ricinus communis*, blood female on Day 1 & 5	29 & 30	0.005	X^2=0.51, p=0.47

Lantana camara, no-blood female on Day 1 & 5	*Lippia kituensis*, no-blood female on Day 1 & 5	23 & 25	0.003	X^2=6.115, p=0.013
Lantana camara, blood female on Day 1 & 5	*Lippia kituensis*, blood female on Day 1 & 5	24 & 26	0.004	X^2=0.61, p=0.434
Lantana camara, no-blood female on Day 1 & 5	*Parthenium hysterophorus*, no-blood female on Day 1 & 5	23 & 27	0.004	X^2=0.09, p=0.764
Lantana camara, blood female on Day 1 & 5	*Parthenium hysterophorus*, blood female on Day 1 & 5	24 & 28	0.004	X^2=2.98, p=0.084
Lantana camara, no-blood female on Day 1 & 5	*Ricinus communis*, no-blood female on Day 1 & 5	23 & 29	0.004	X^2=0.40, p=0.526
Lantana camara, blood female on Day 1 & 5	*Ricinus communis*, blood female on Day 1 & 5	24 & 30	0.004	X^2=0.50, p=0.479
Lippia kituensis, no-blood female n Day 1 & 5	*Parthenium hysterophorus*, no-blood female on Day 1 & 5	25 & 27	0.004	X^2=2.63, p=0.105
Lippia kituensis, blood female on Day 1 & 5	*Parthenium hysterophorus*, blood female on Day 1 & 5	26 & 28	0.004	X^2=1.39, p=0.238
Lippia kituensis, no-blood female on Day 1 & 5	*Ricinus communis*, no-blood female on Day 1 & 5	25 & 29	0.004	X^2=2.21, p=0.137
Lippia kituensis, blood female on Day 1 & 5	*Ricinus communis*, blood female on Day 1 & 5	26 & 30	0.004	X^2=0.17, p=0.679
Parthenium hysterophorus, no-blood female on Day 1 & 5	*Ricinus communis*, no-blood female on Day 1 & 5	27 & 29	0.005	X^2=0.35, p=0.556
Parthenium hysterophorus, blood female on Day 1 & 5	*Ricinus communis*, blood female on Day 1 & 5	28 & 30	0.005	X^2=1.57, p=0.210
Full blend & ate two no-blood females	No-blood female on Day 1 & 5	54 & 23	0.004	X^2=2.01, p=0.157
Full blend & ate two blood females	Blood female on Day 1 & 5	55 & 24	0.005	X^2=0.01, p=0.934
Full blend & ate two no-blood females	*Lantana camara*, no-blood female on Day 1 & 5	54 & 7	0.005	X^2=25.09, p<0.001*
Full blend & ate two blood females	*Lantana camara*, blood female on Day 1 & 5	55 & 8	0.005	X^2=0.00, p=0.947
Full blend & ate two no-blood females	Full blend & ate two blood females	54 & 55	0.010	X^2=2.35, p=0.126

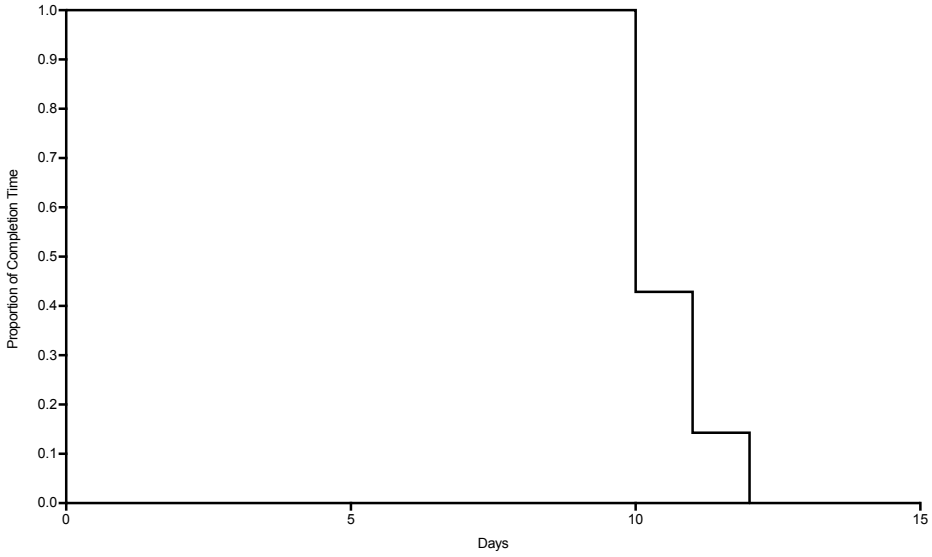
CT7. No-blood female on Day 1 & Day 5



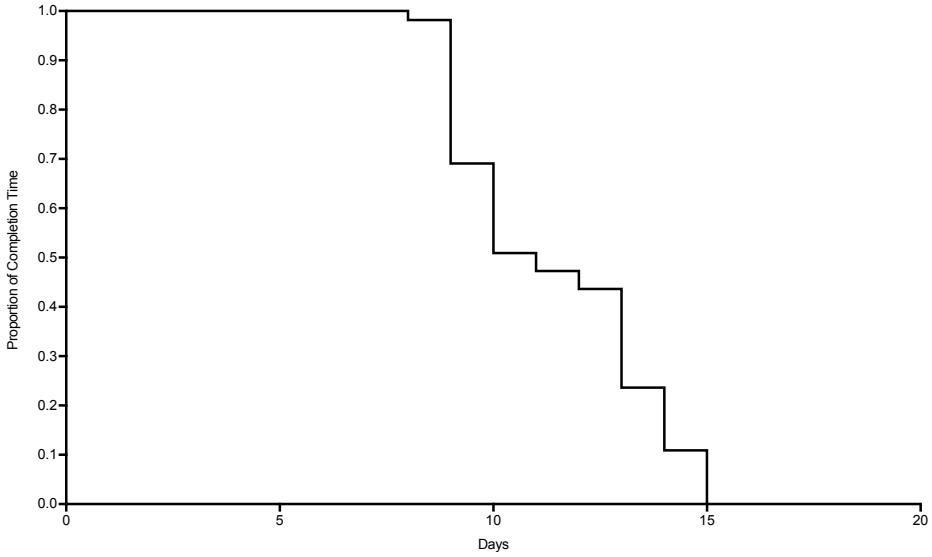
CT8. Blood female on Day 1 & Day 5



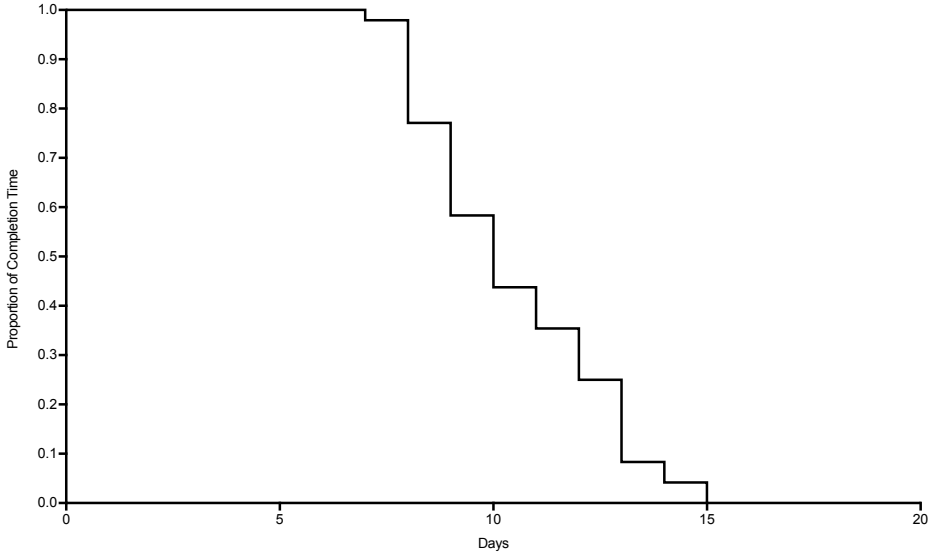
CT9. Male on Day 1 & Day 5



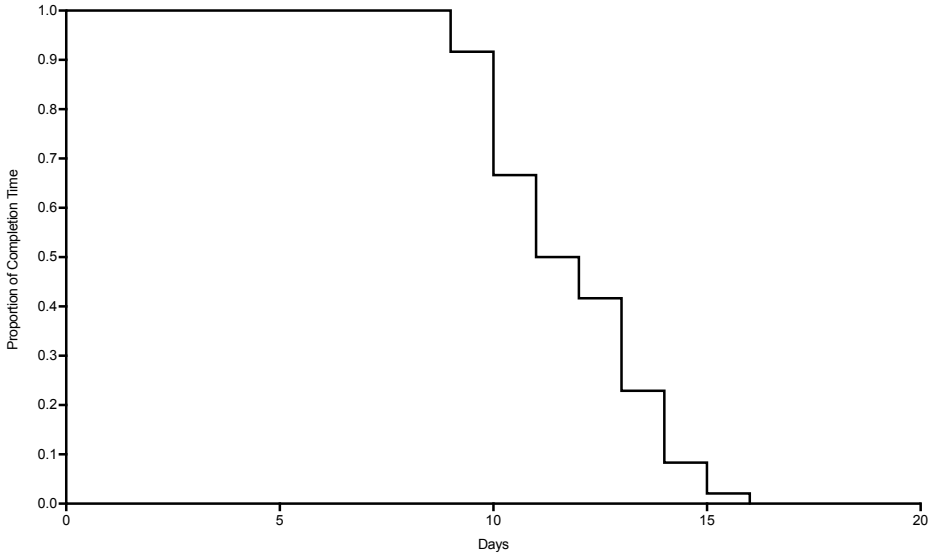
CT10. No-blood female on Day 1, 5 & 9



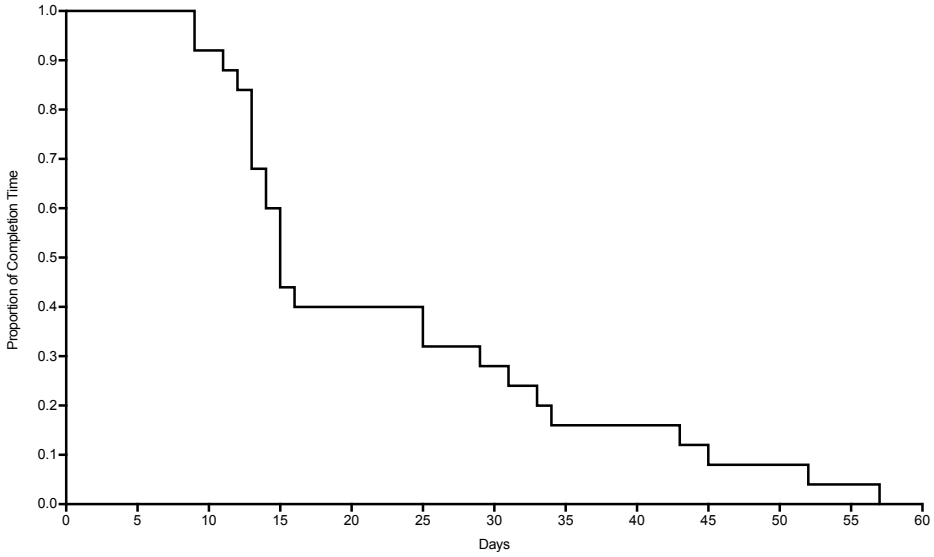
CT11. Blood female on Day 1, 5 & 9



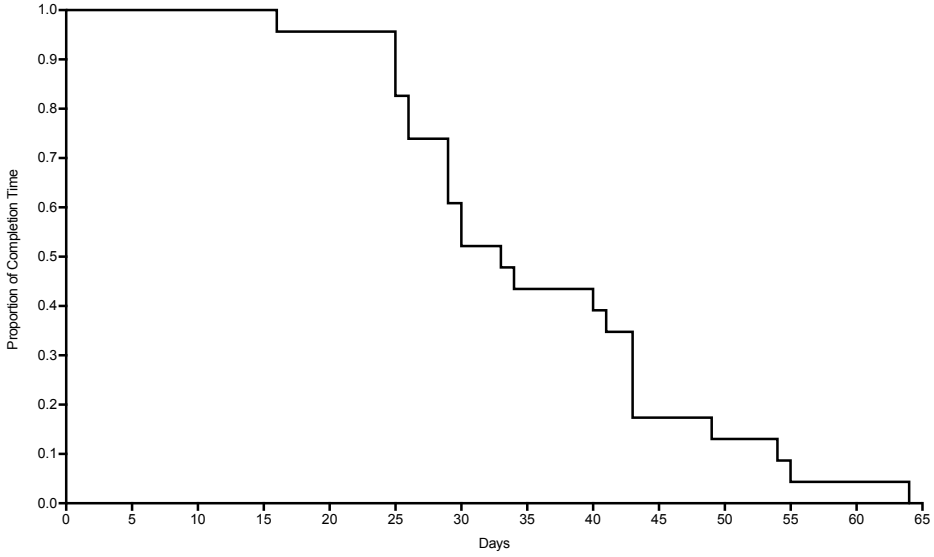
CT12. Male on Day 1 & Day 5



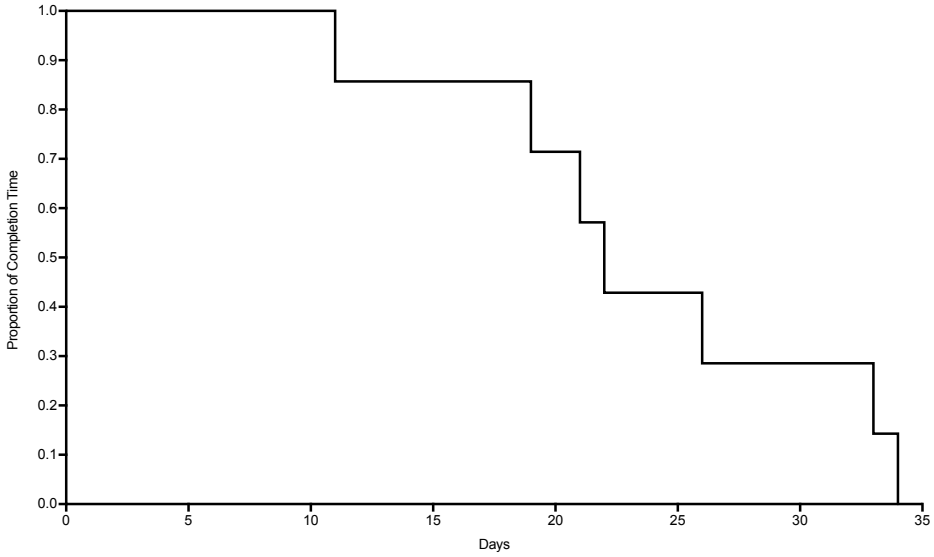
CT13. *Lantana camara*, no prey



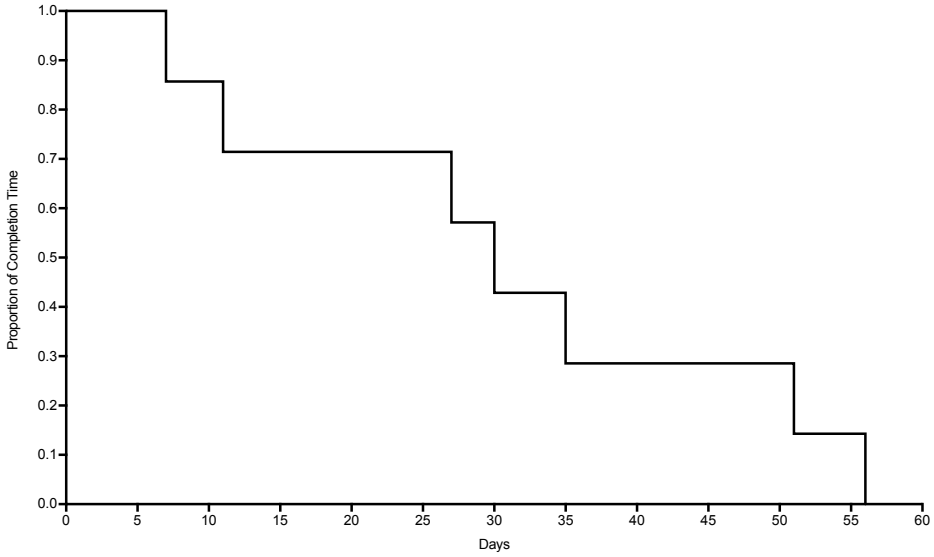
CT14. *Lippia kituensis*, no prey



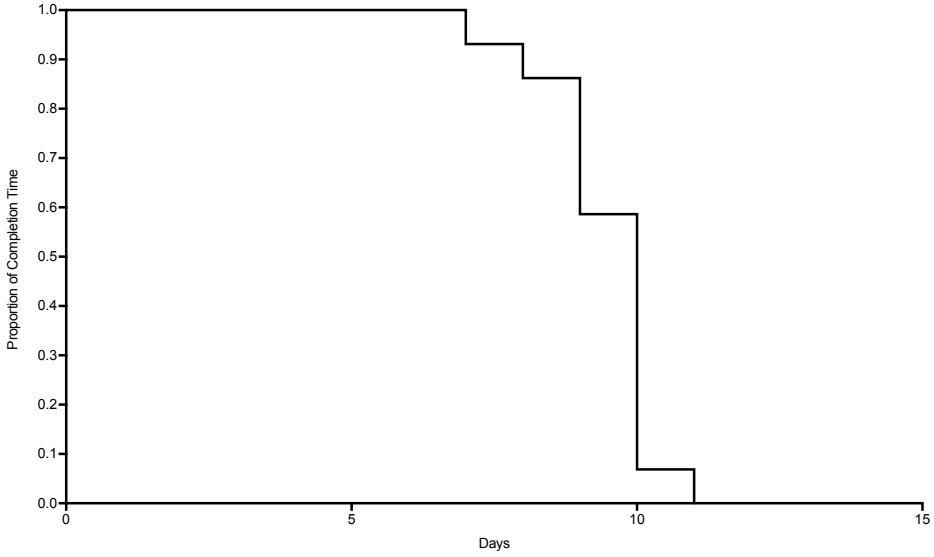
CT18. *Lantana camara*, no-blood female on Day 1



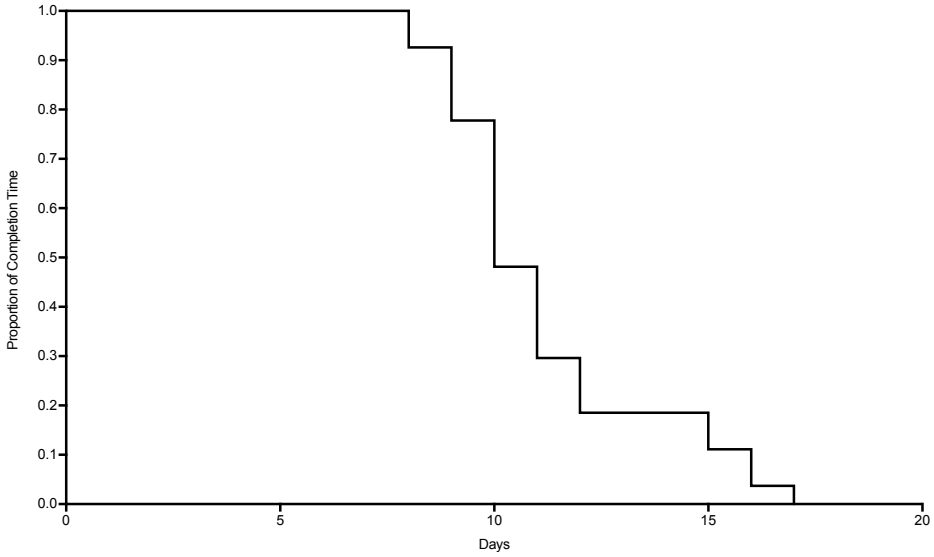
CT19. *Lippia kituensis*, no-blood female on Day 1



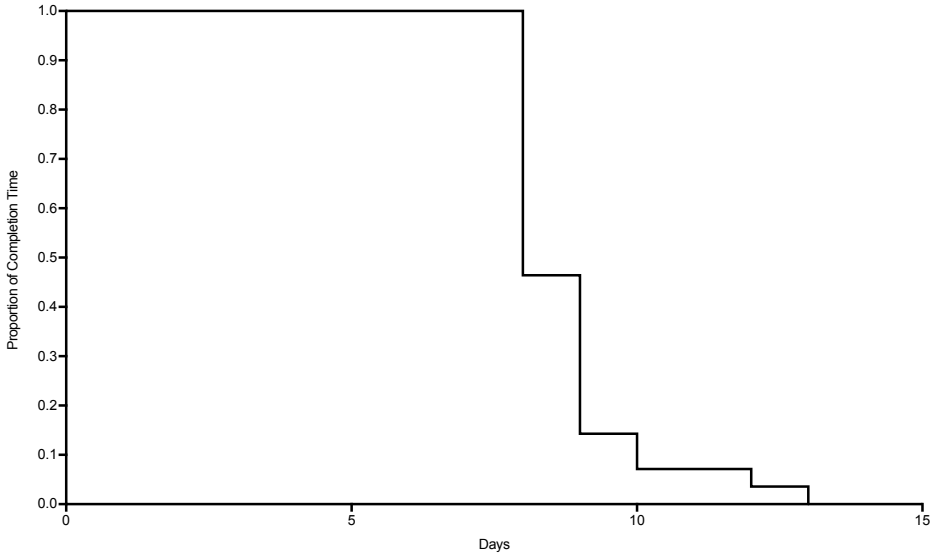
CT23. *Lantana camara*, no-blood female on Day 1 & Day 5



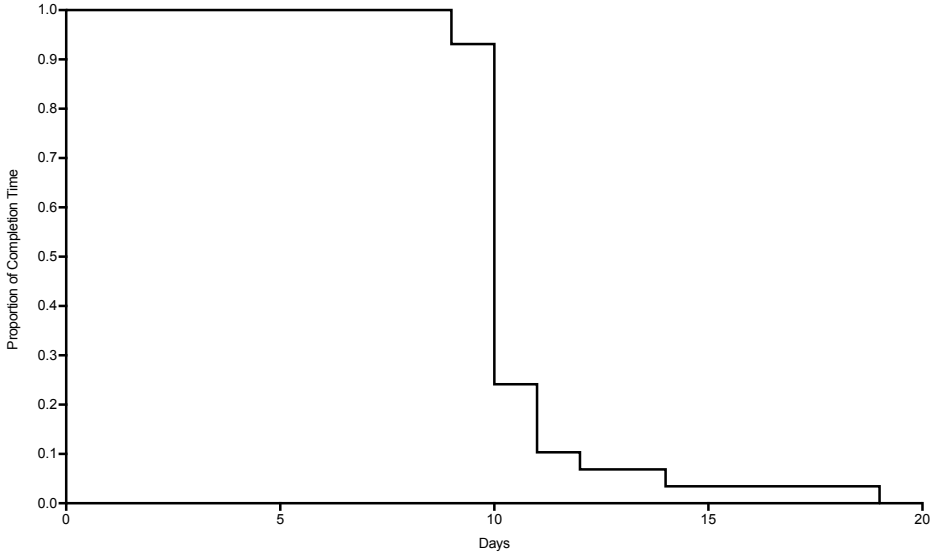
CT24. *Lantana camara*, blood female on Day 1 & Day 5



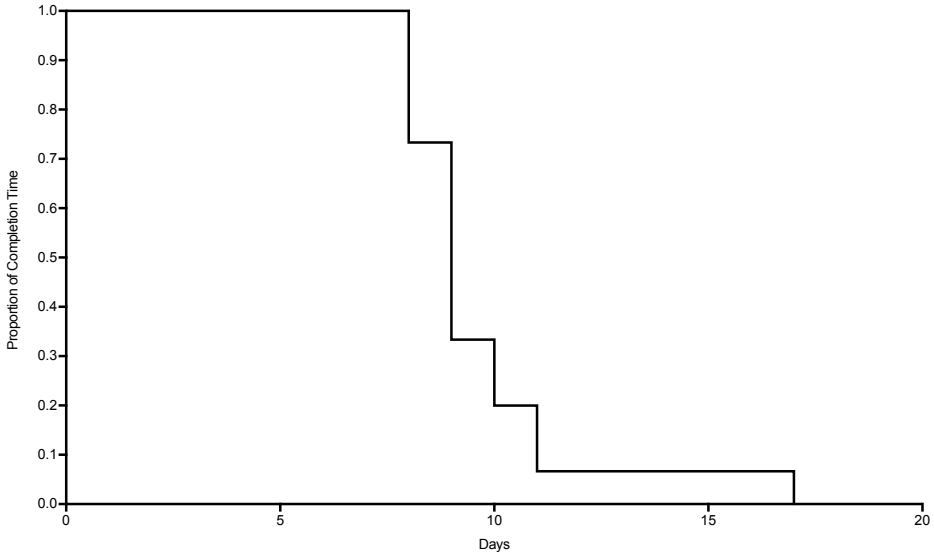
CT25. *Lippia kituensis*, no-blood female on Day 1 & Day 5



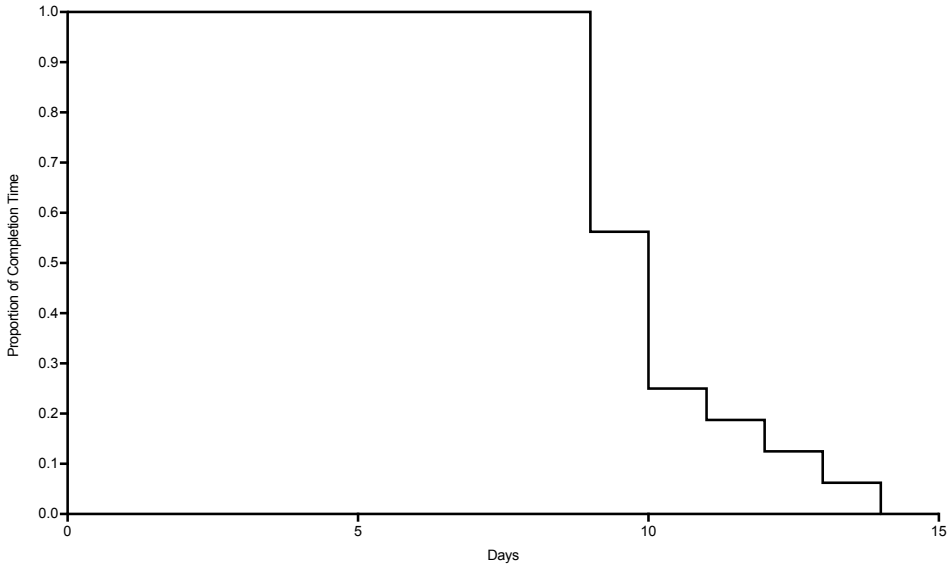
CT26. *Lippia kituensis*, blood female on Day 1 & Day 5



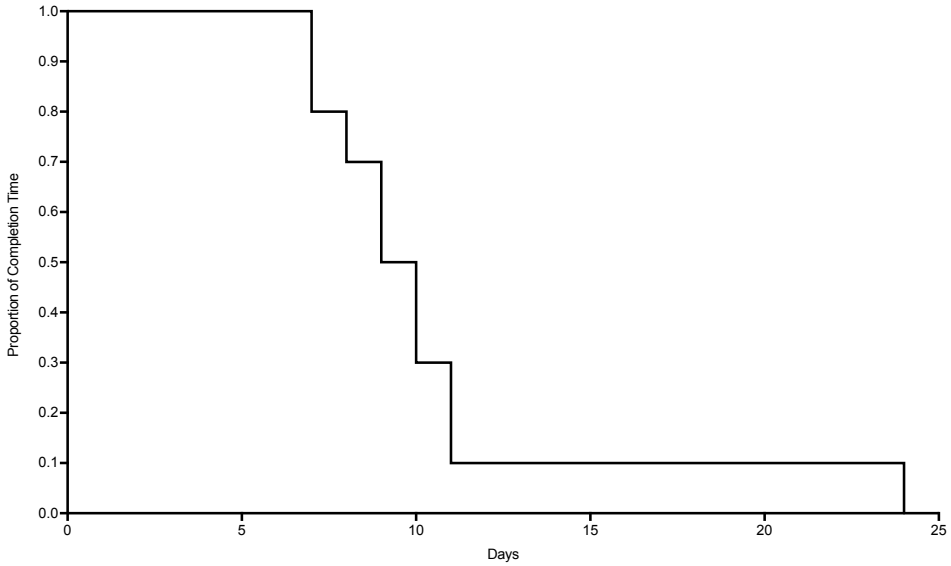
CT27. *Parthenium hysterophorus*, no-blood female on Day 1 & 5



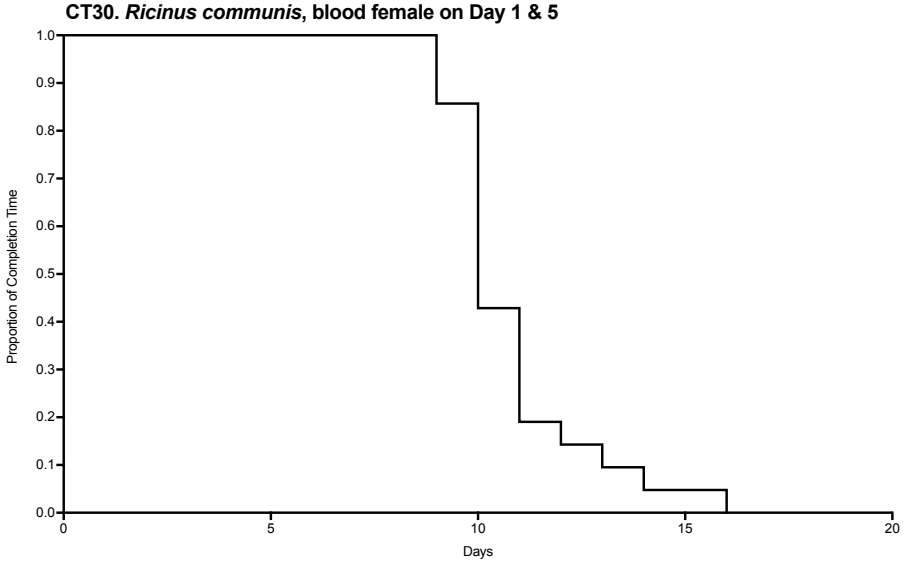
CT28. *Parthenium hysterophorus*, blood female on Day 1 & 5



CT29. *Ricinus communis*, no-blood female on Day 1 & 5



CT30. *Ricinus communis*, blood female on Day 1 & 5



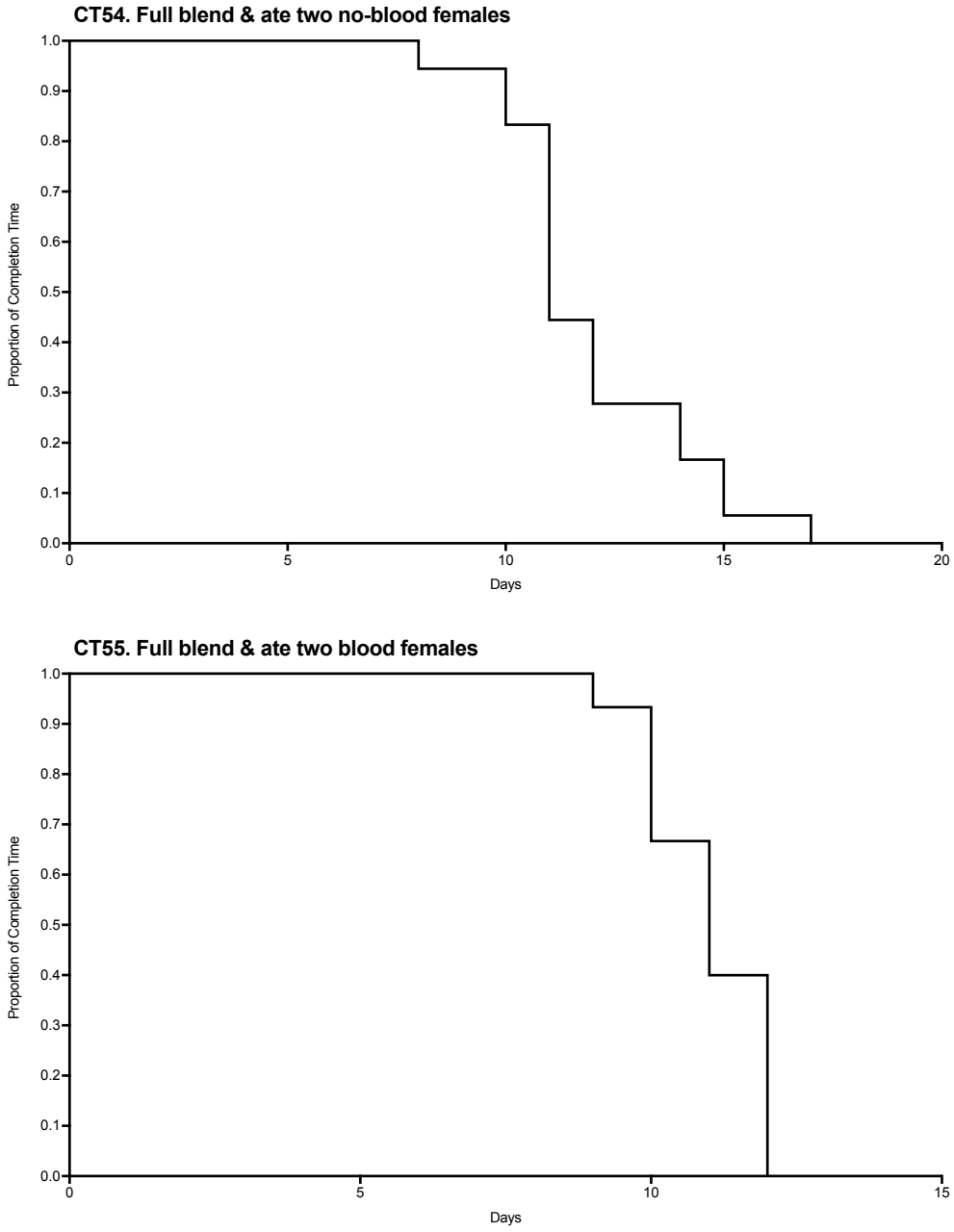
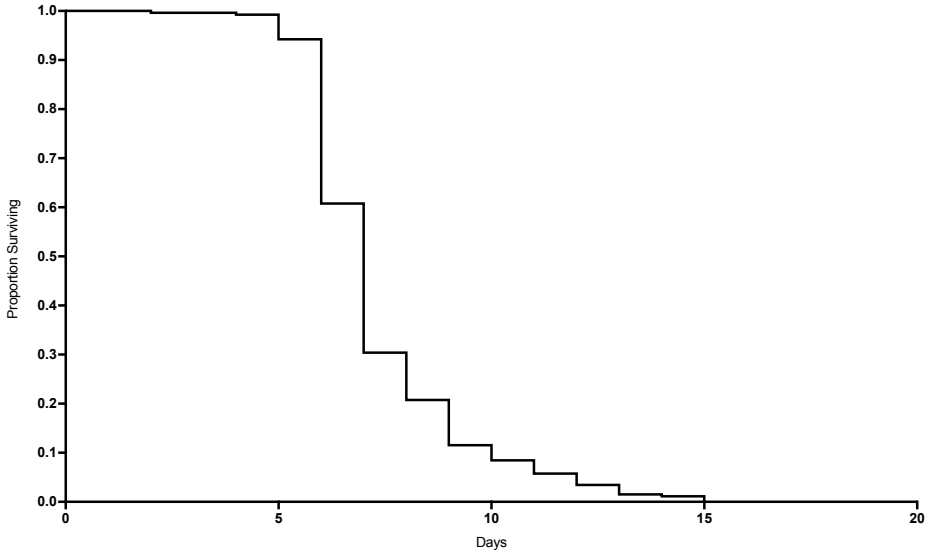
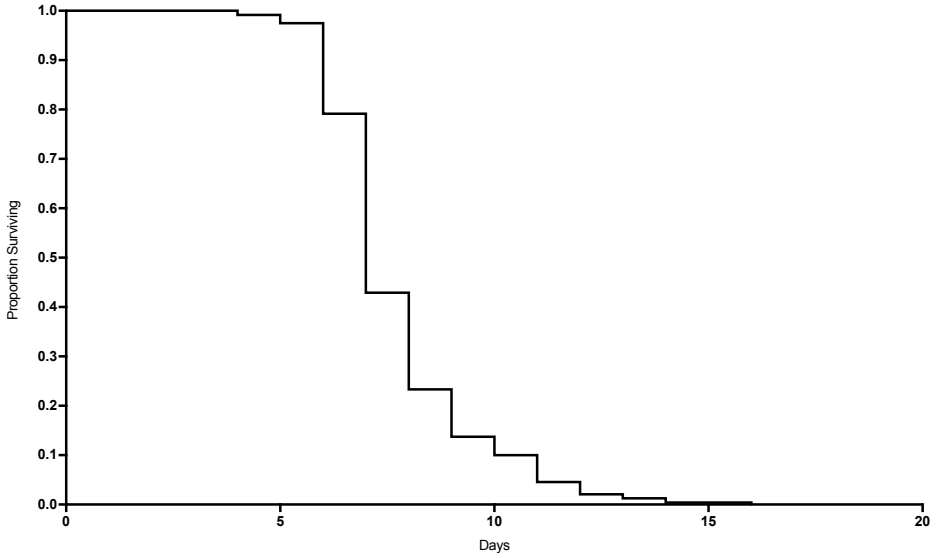


Fig 1. The completion time (CT) before successful hatchlings became 2nd-instar juveniles was calculated by days. See Table 4.

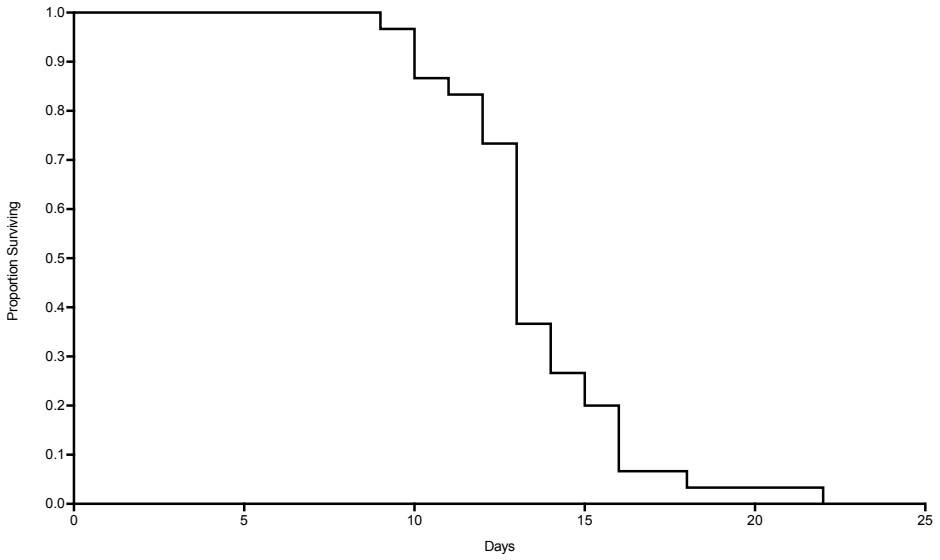
L1. Water-only control for prey & plants (C1)



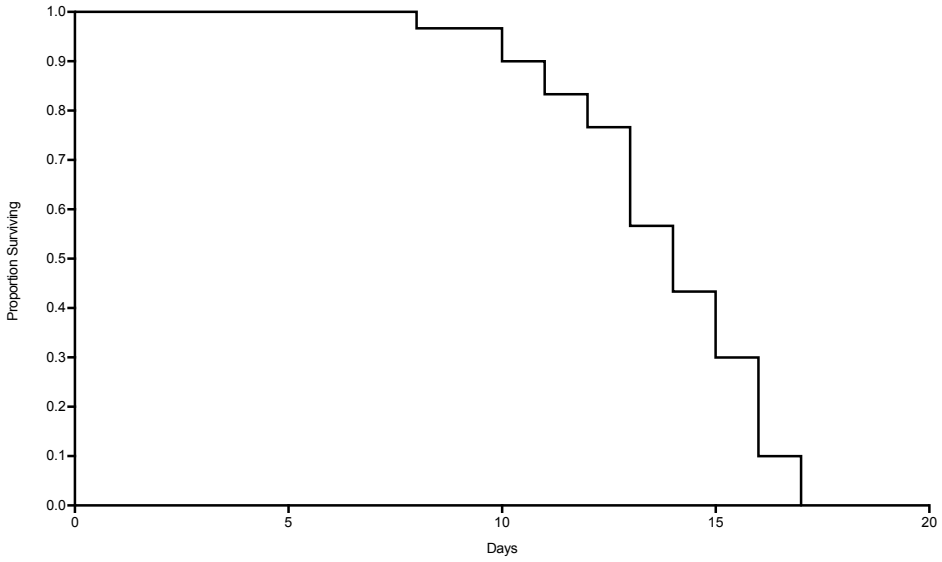
L2. Water-only control for artificial nectar (C2)



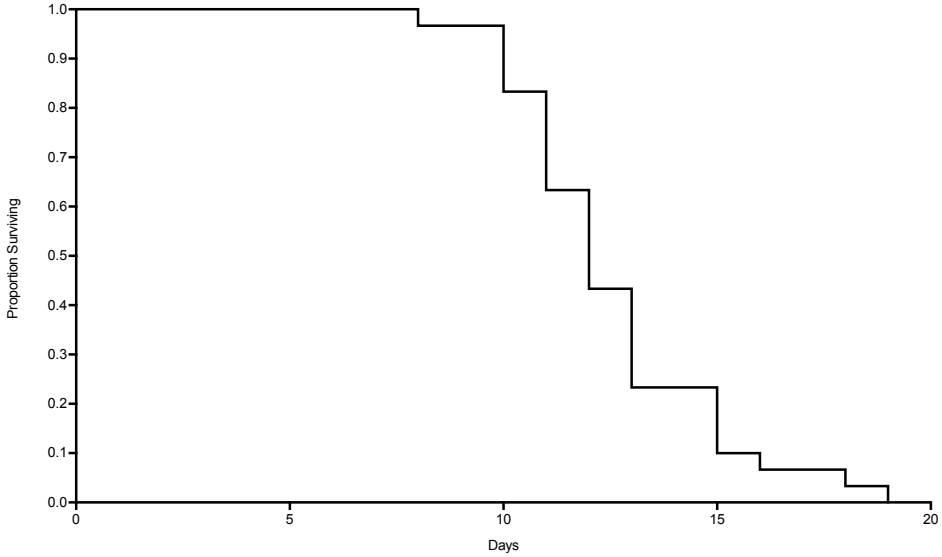
L3. No-blood female on Day 1



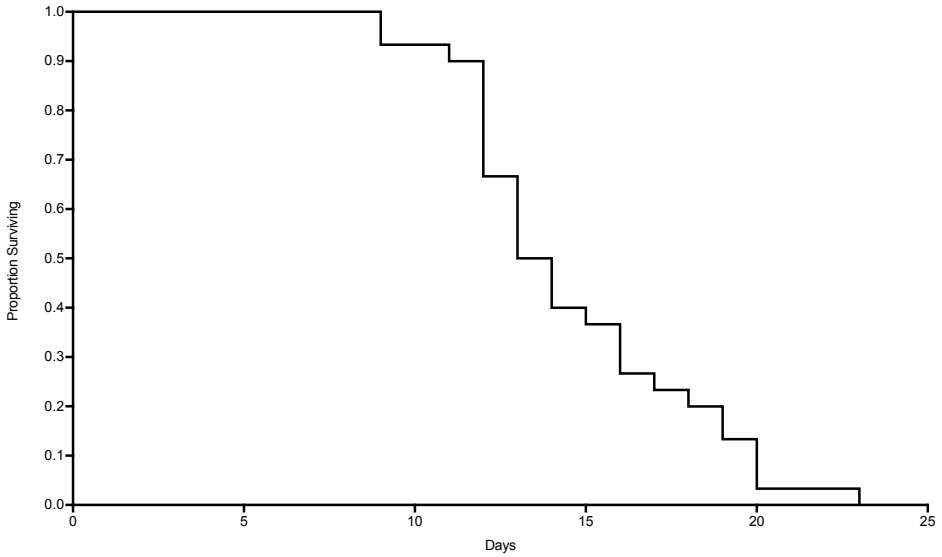
L4. Blood female on Day 1



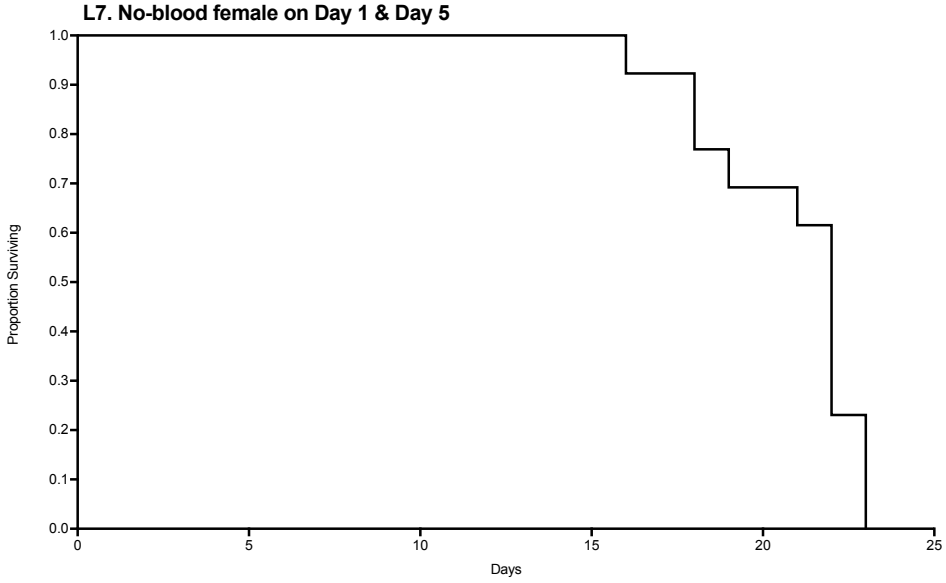
L5. Male on Day 1



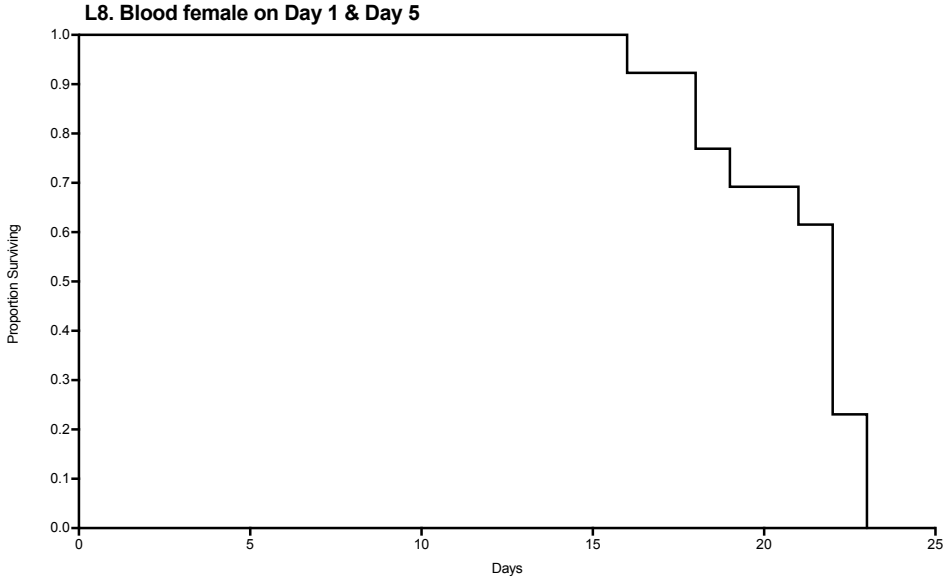
L6. No-blood female on Day 5



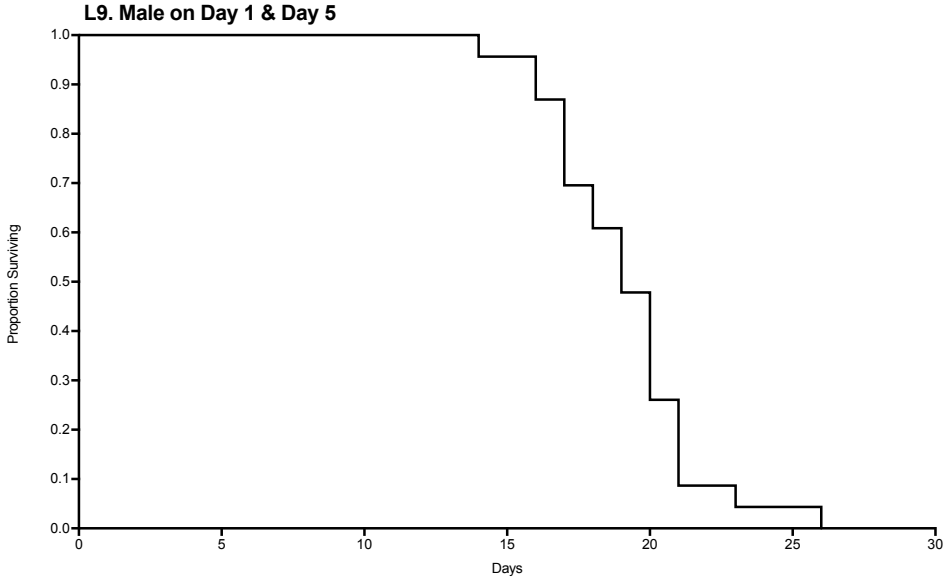
L7. No-blood female on Day 1 & Day 5



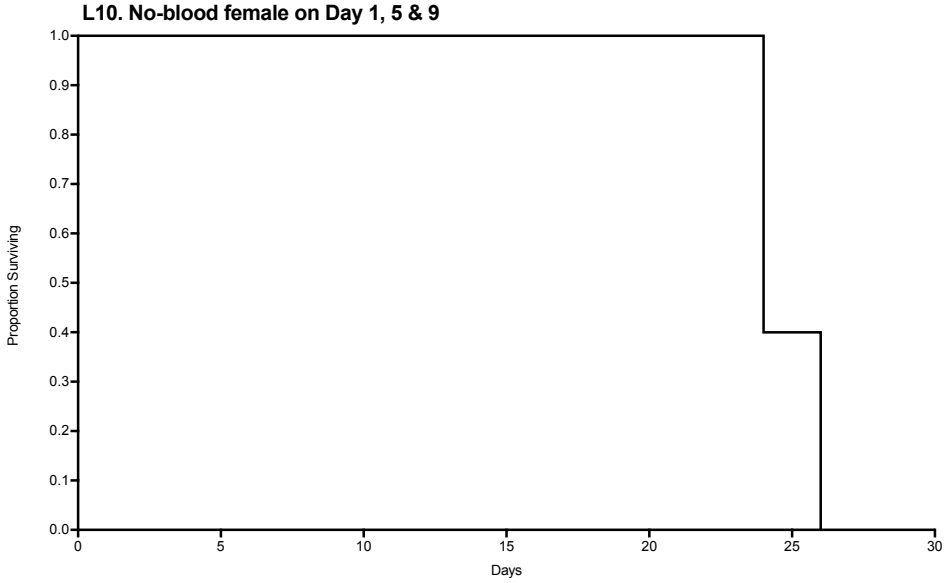
L8. Blood female on Day 1 & Day 5



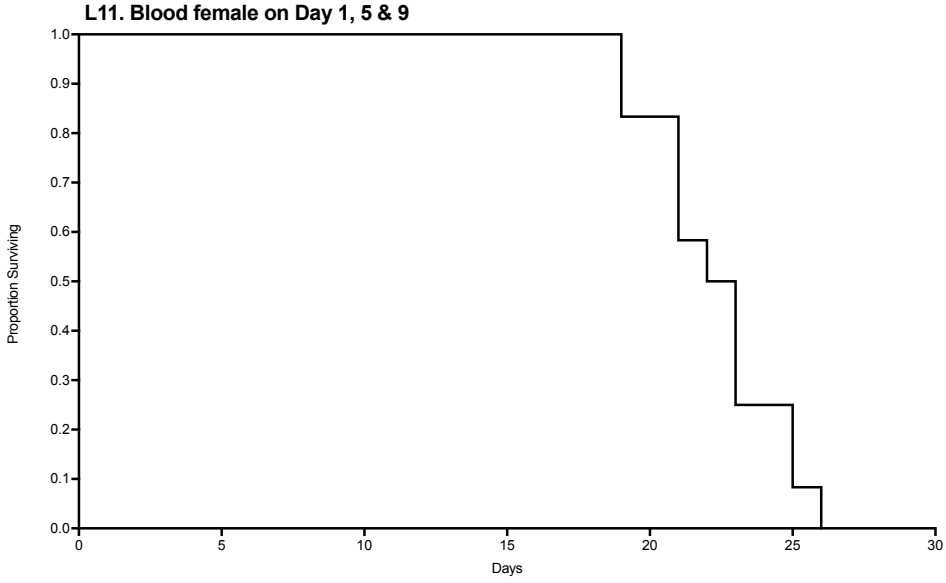
L9. Male on Day 1 & Day 5



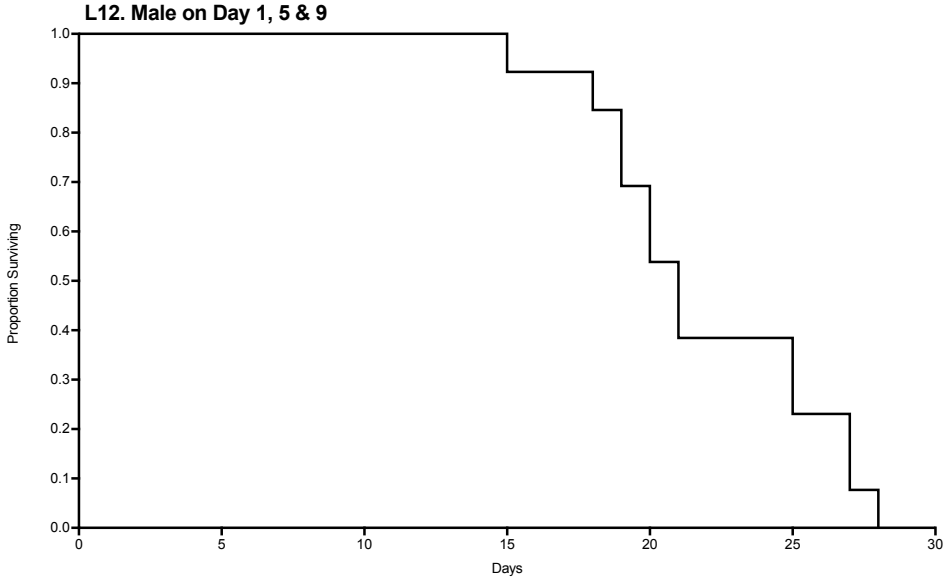
L10. No-blood female on Day 1, 5 & 9



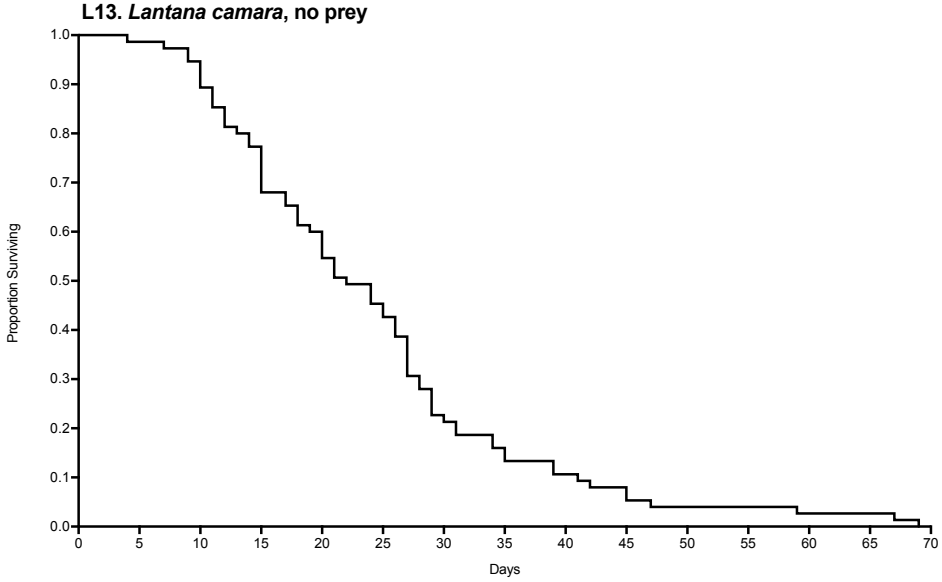
L11. Blood female on Day 1, 5 & 9



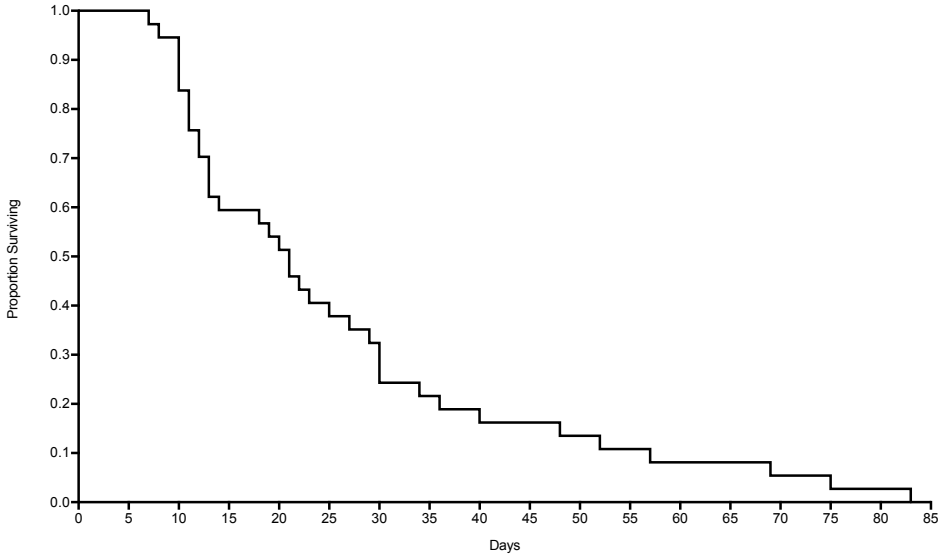
L12. Male on Day 1, 5 & 9



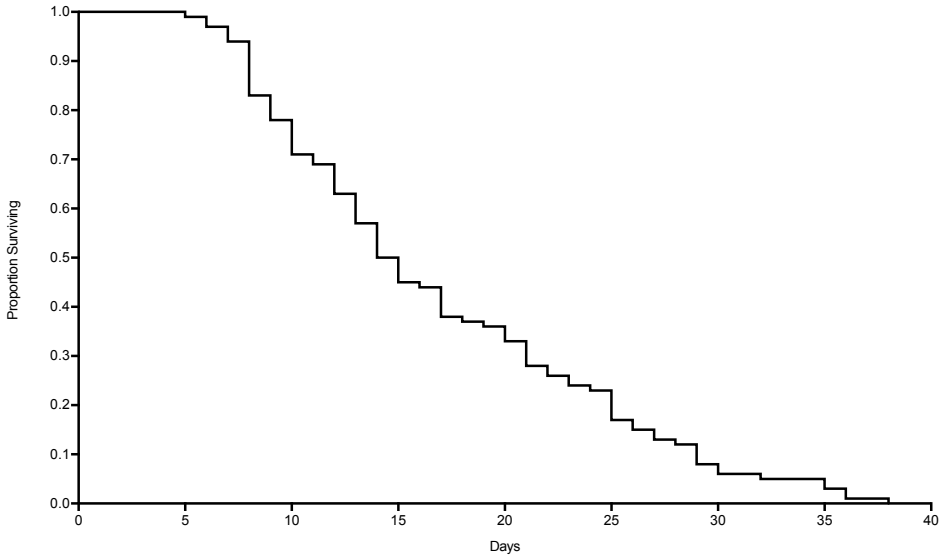
L13. *Lantana camara*, no prey



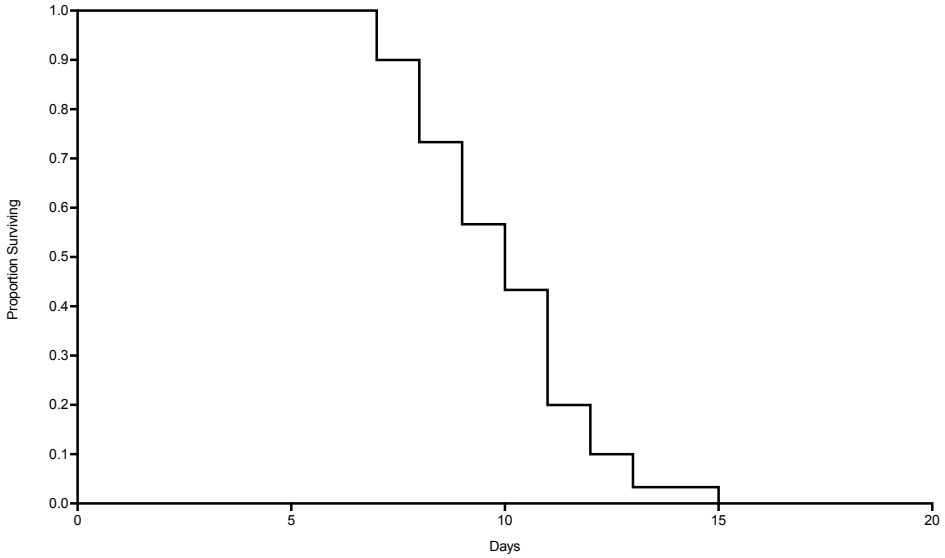
L14. *Lippia kituensis*, no prey



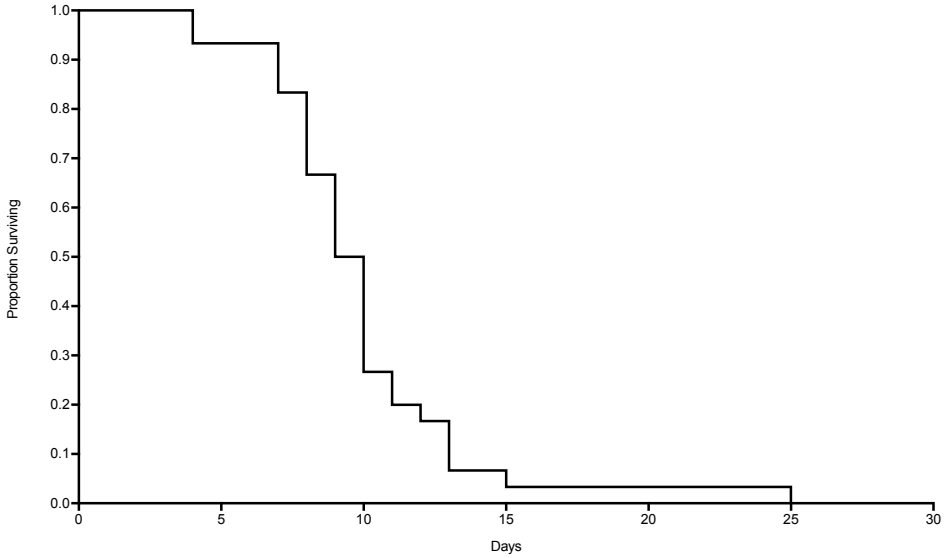
L15. *Parthenium hysterophorus*, no prey



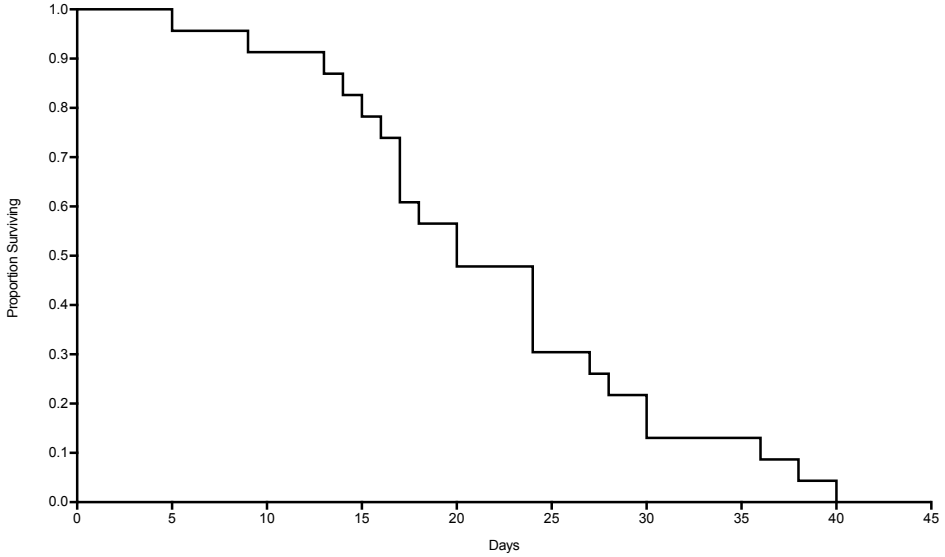
L16. *Ricinus communis*, no prey



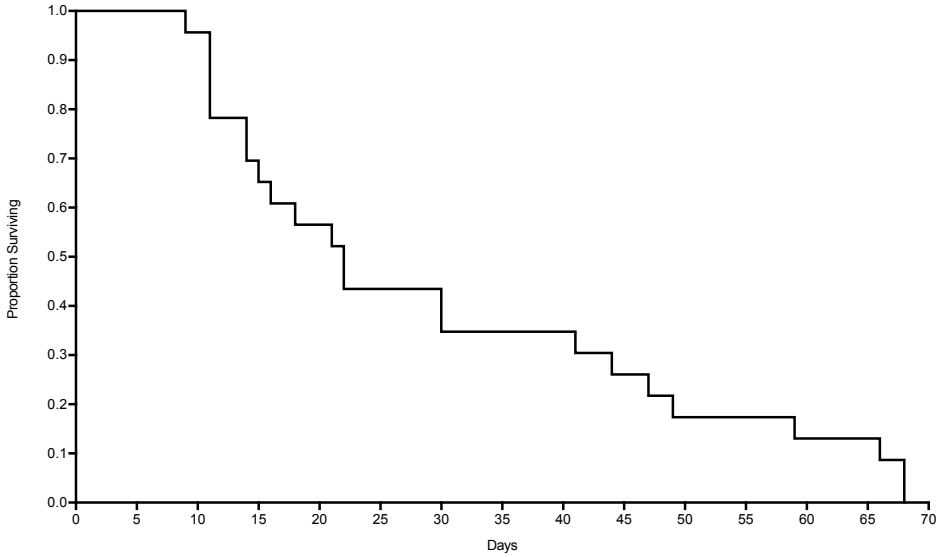
L17. *Hibiscus rosa-sinensis*, no prey



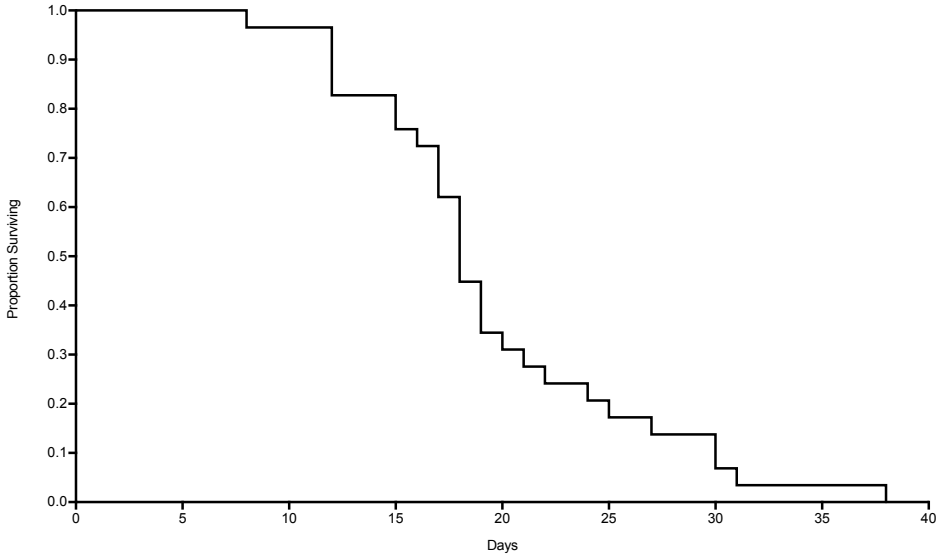
L18. *Lantana camara*, no-blood female on Day 1



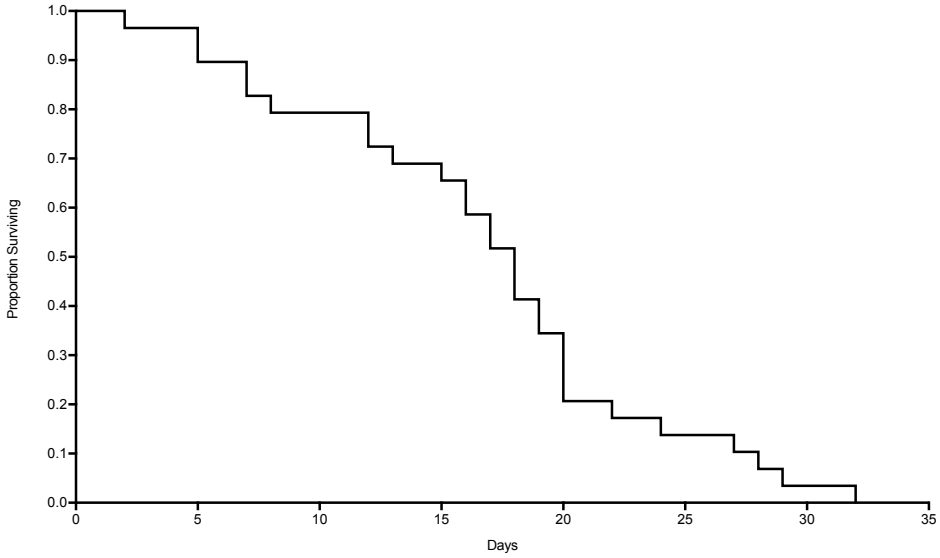
L19. *Lippia kituensis*, no-blood female on Day 1



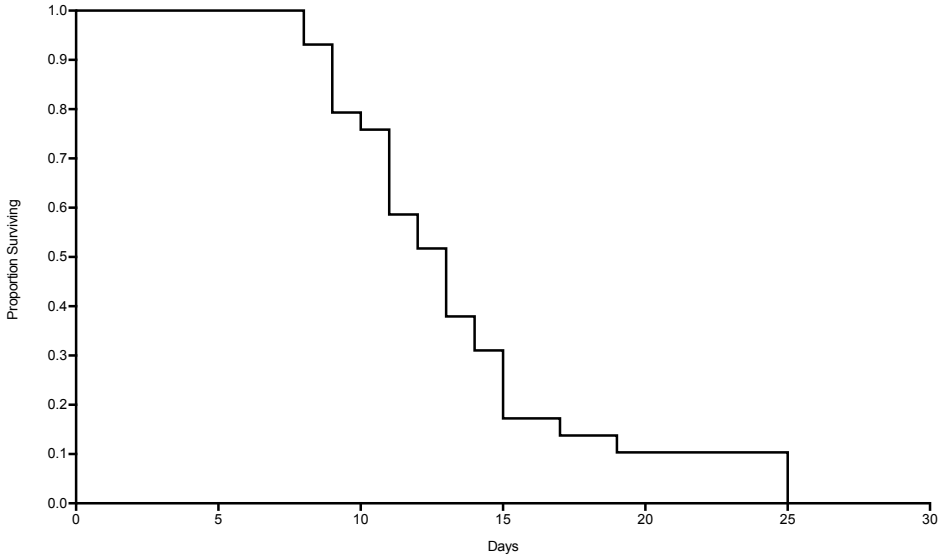
L20. *Parthenium hysterophorus*, no-blood female on Day 1



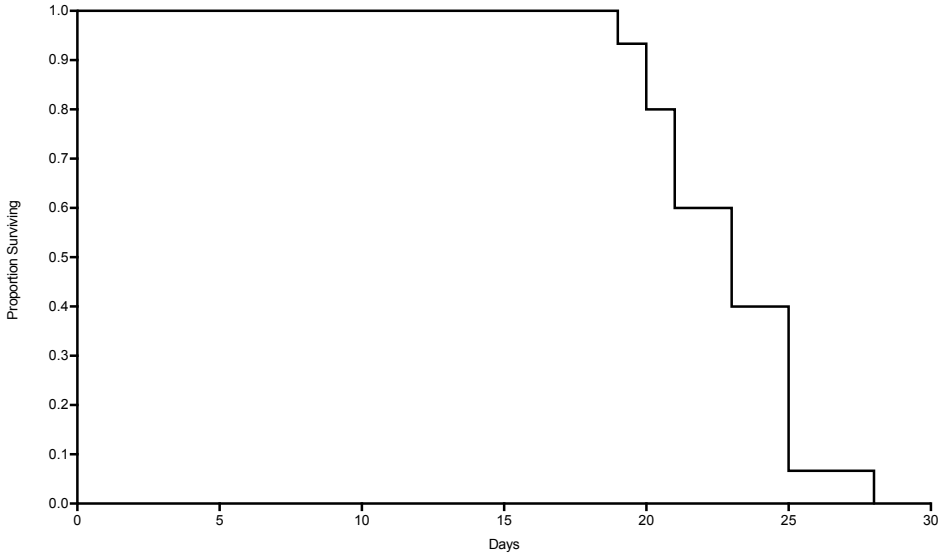
L21. *Ricinus communis*, no-blood female on Day 1



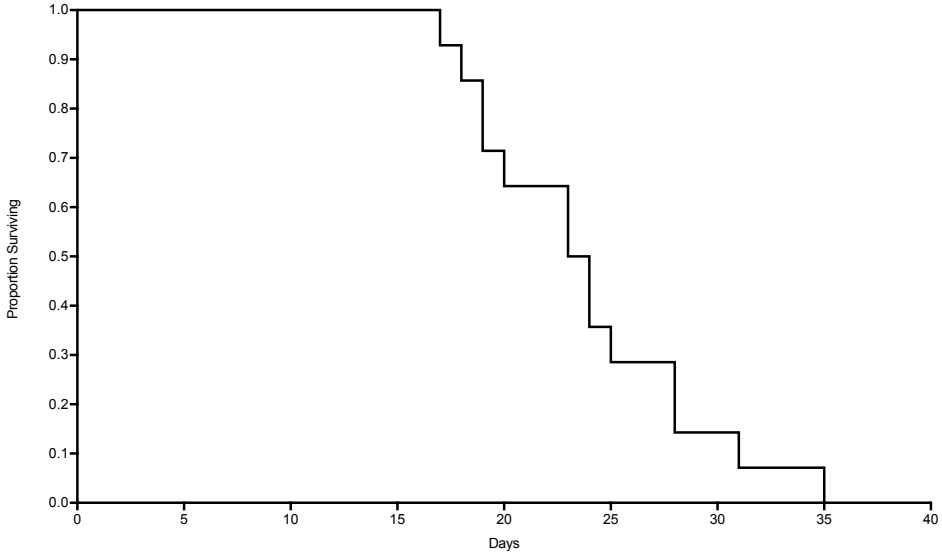
L22. *Hibiscus rosa-sinensis*, no-blood female on Day 1



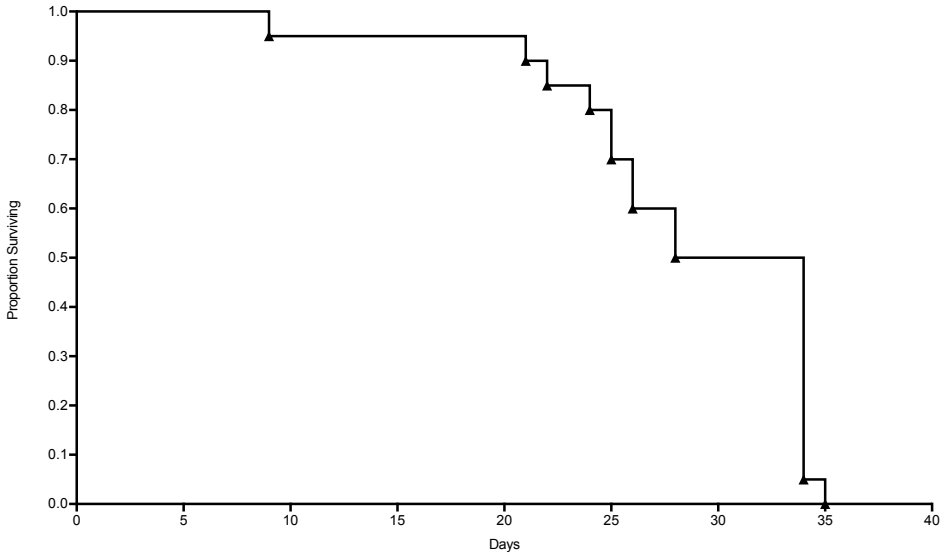
L27. *Parthenium hysterophorus*, no-blood female on Day 1 & 5



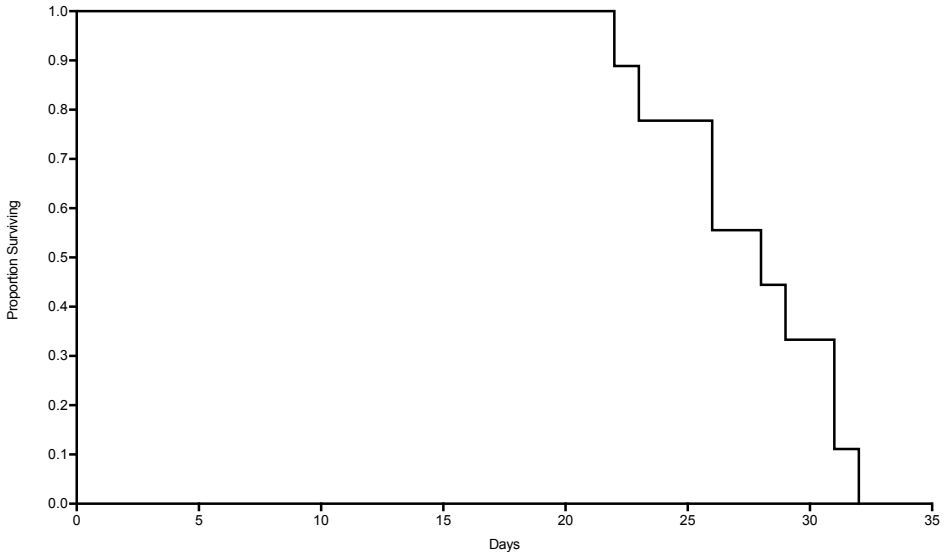
L28. *Parthenium hysterophorus*, blood female on Day 1 & 5



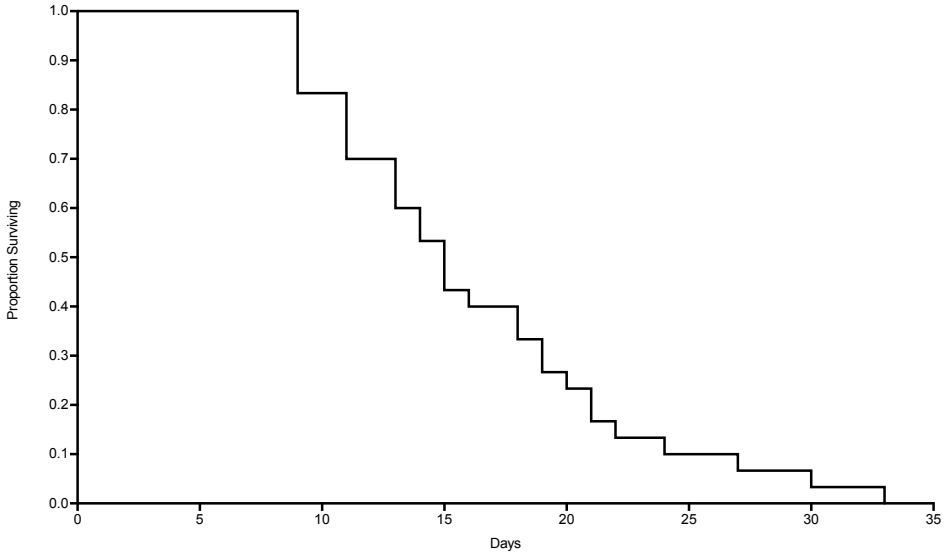
L29. *Ricinus communis*, no-blood female on Day 1 & 5



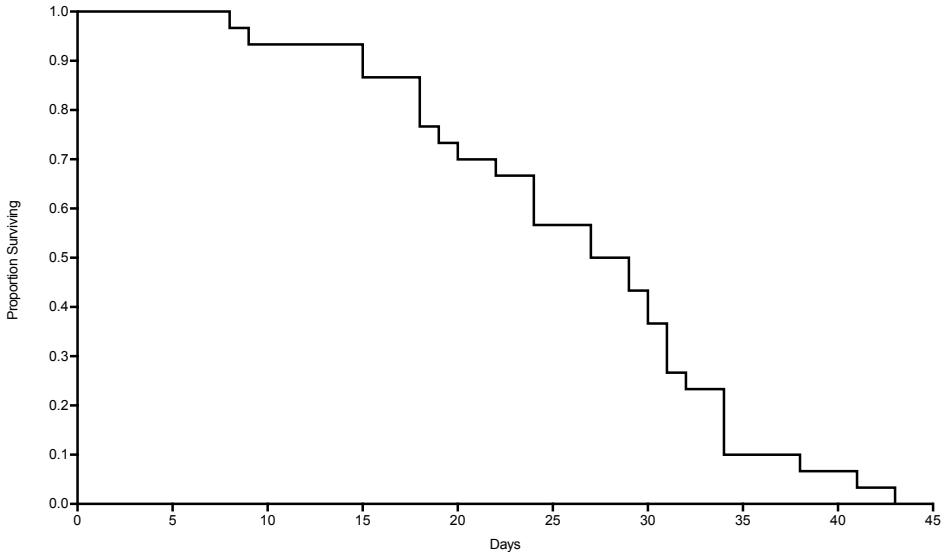
L30. *Ricinus communis*, blood female on Day 1 & 5



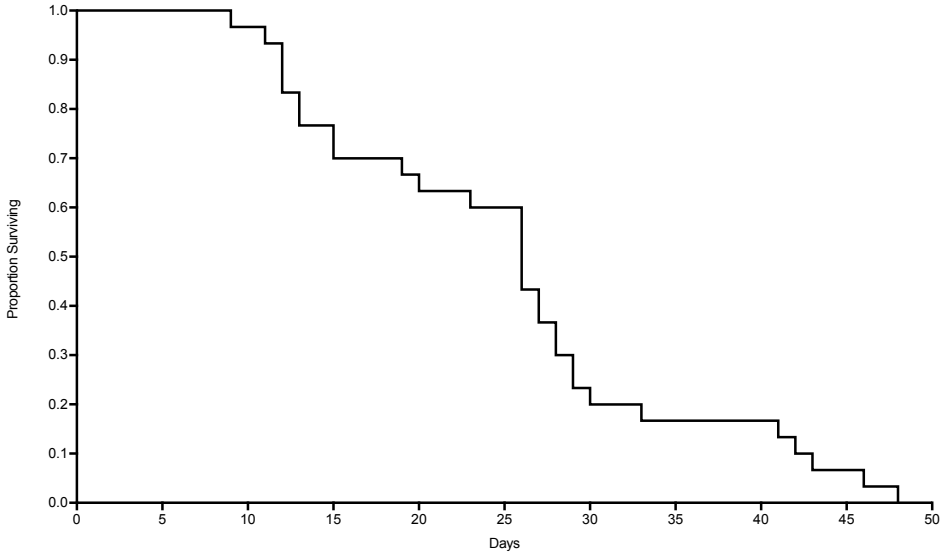
L31. 1% sucrose, no prey



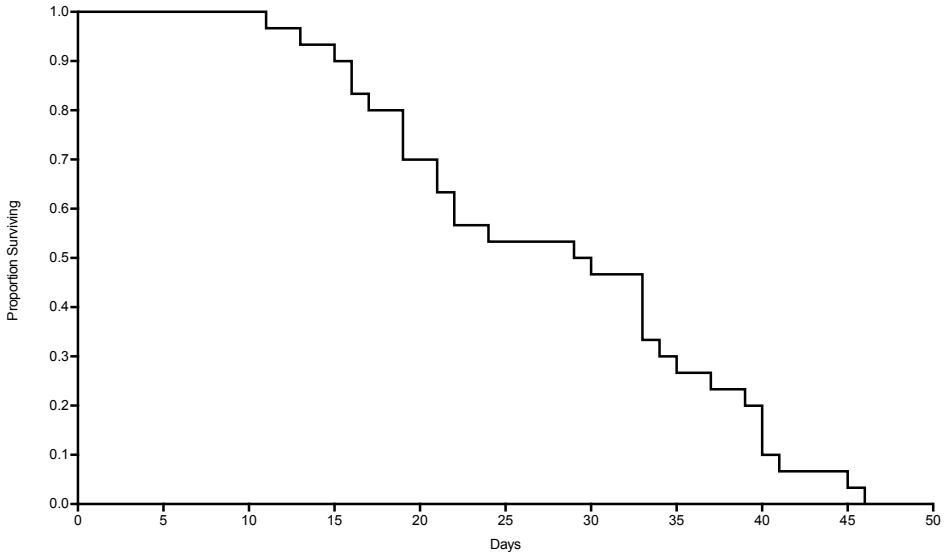
L32. 5% sucrose, no prey



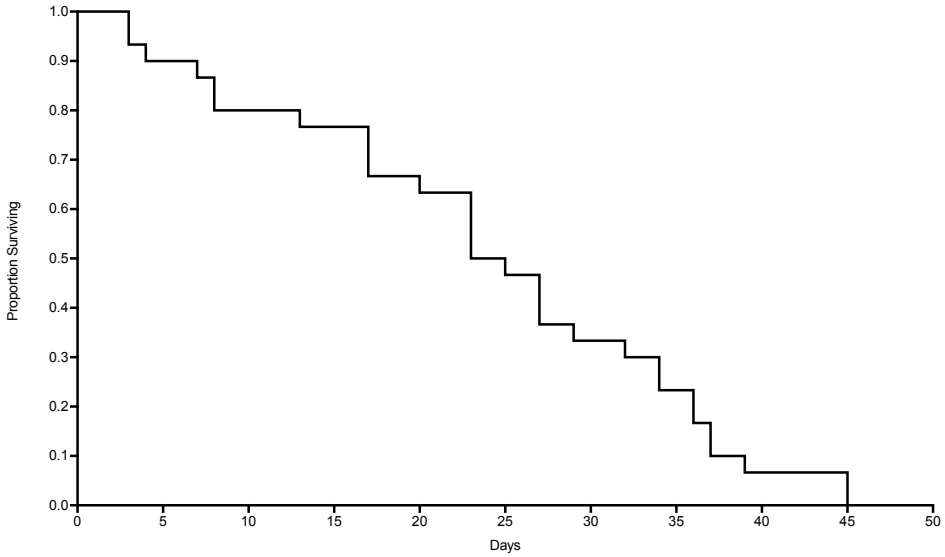
L33. 10% sucrose, no prey



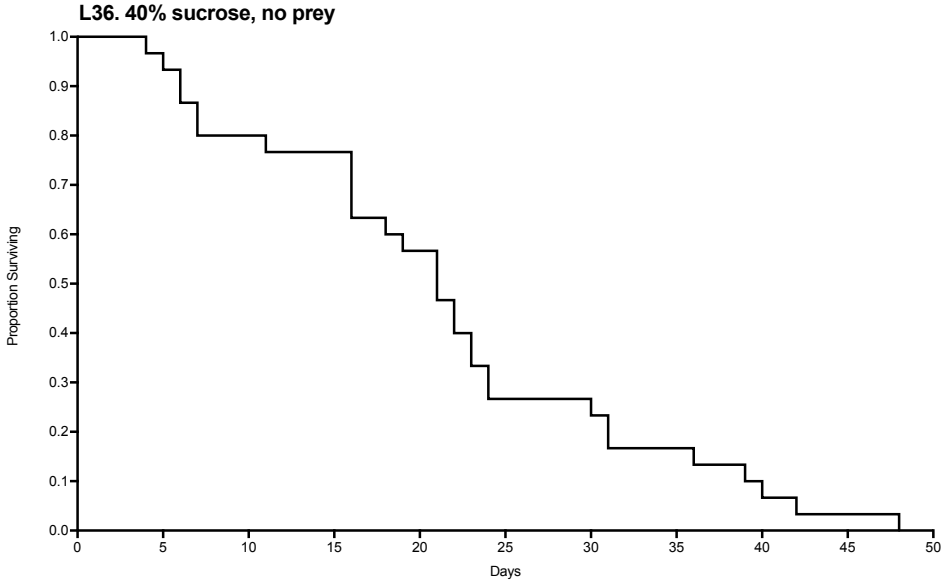
L34. 20% sucrose, no prey



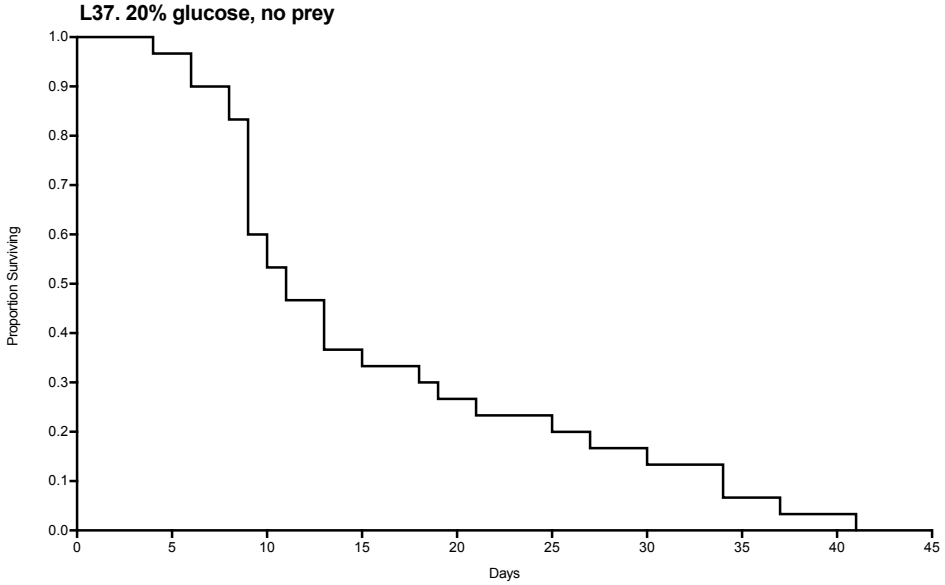
L35. 30% sucrose, no prey



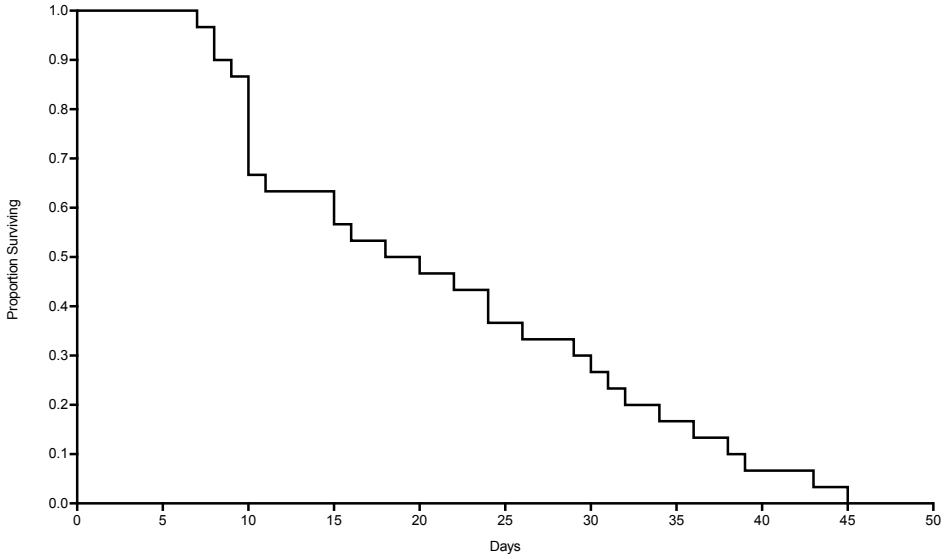
L36. 40% sucrose, no prey



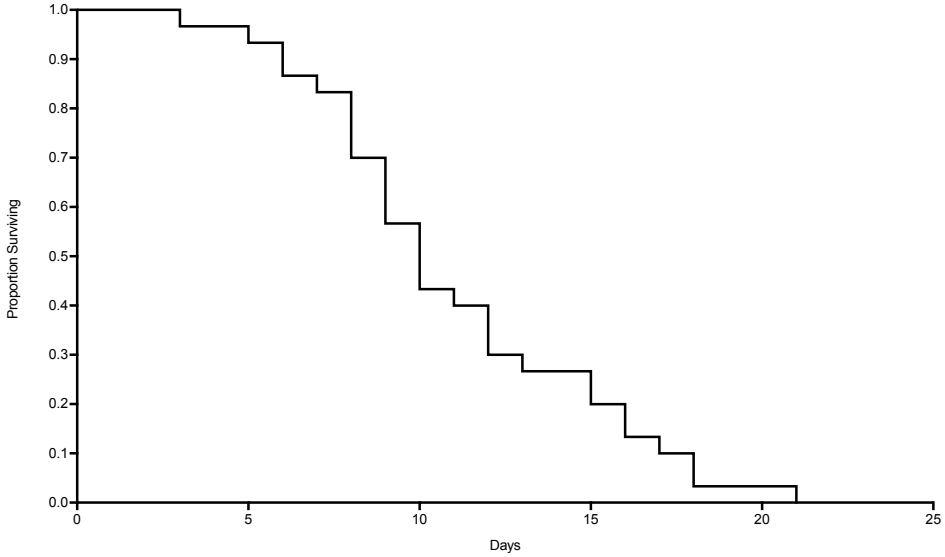
L37. 20% glucose, no prey



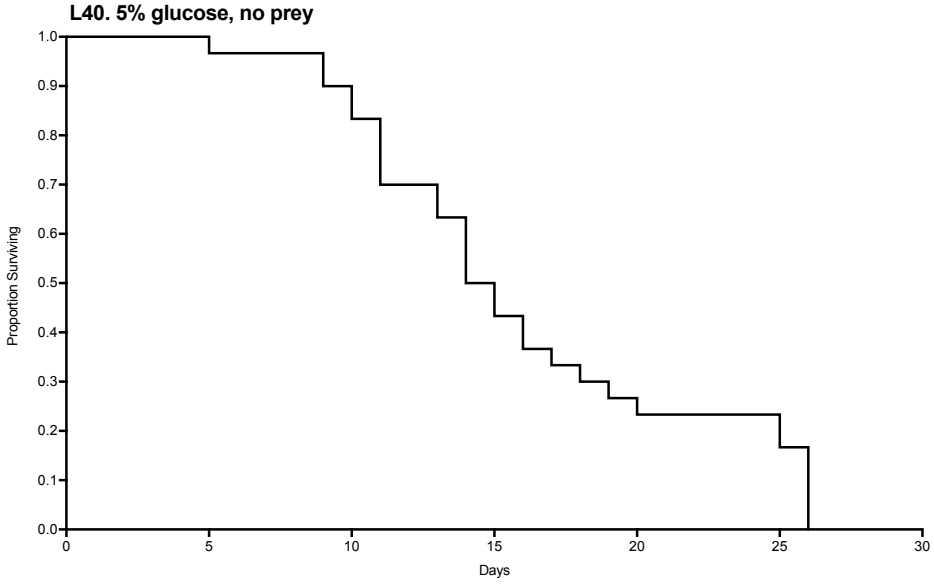
L38. 20% fructose, no prey



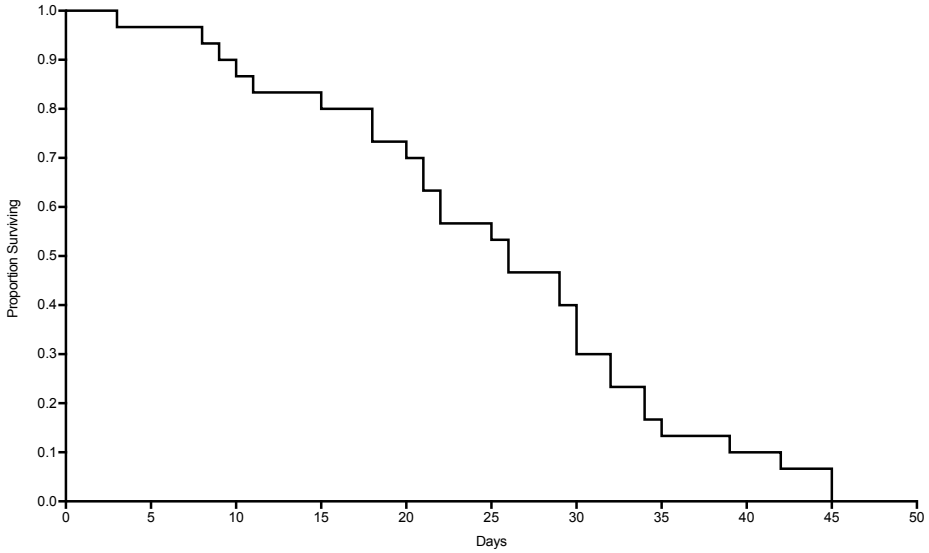
L39. 20% maltose, no prey



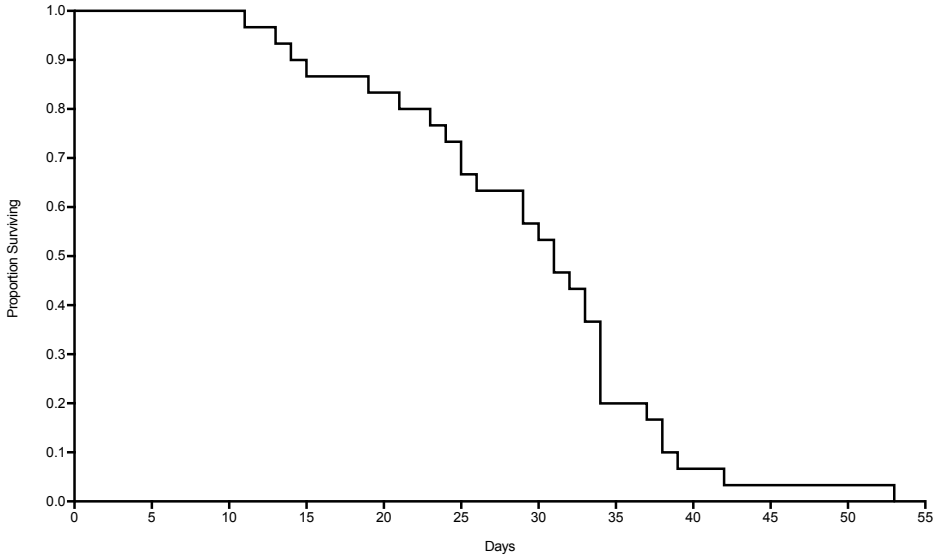
L40. 5% glucose, no prey



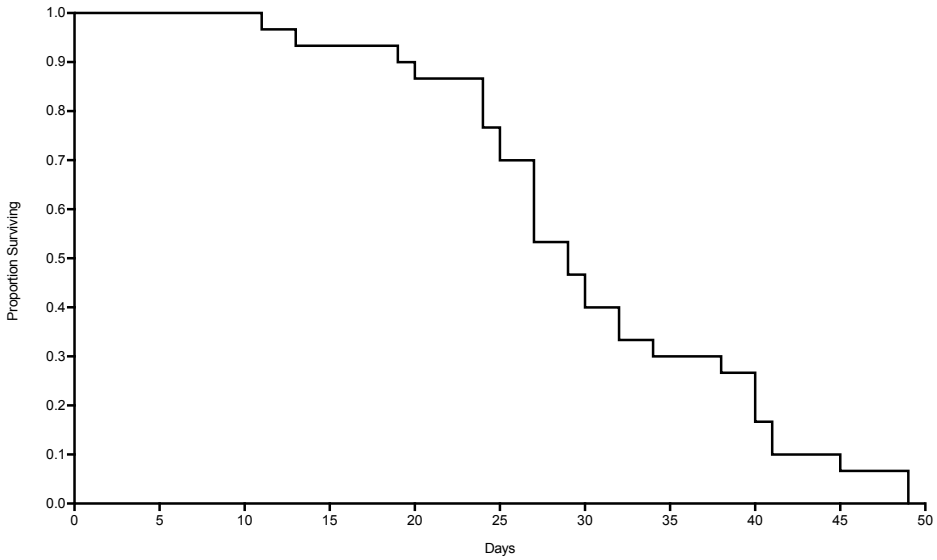
L41. 5% fructose, no prey



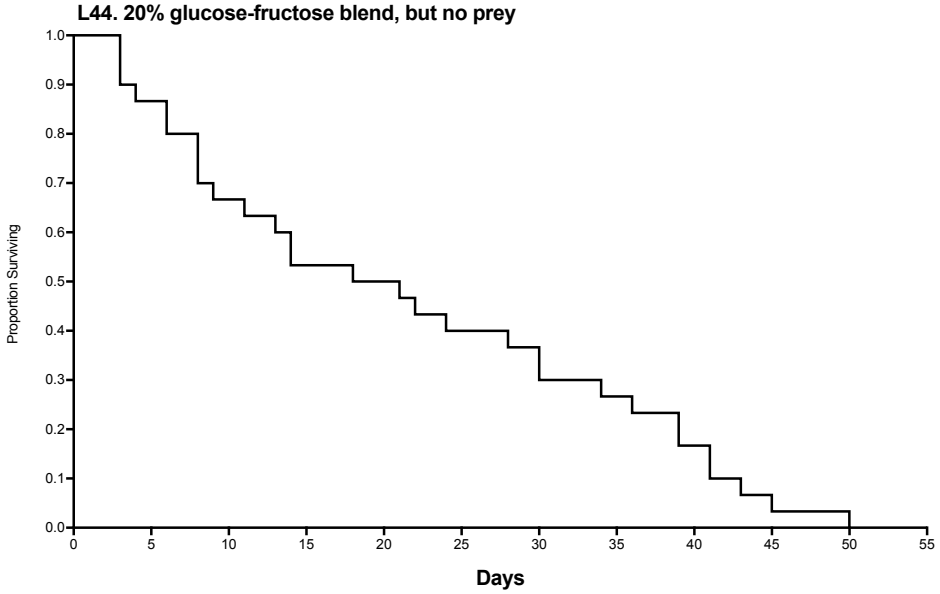
L42. 5% glucose-fructose blend, but no prey



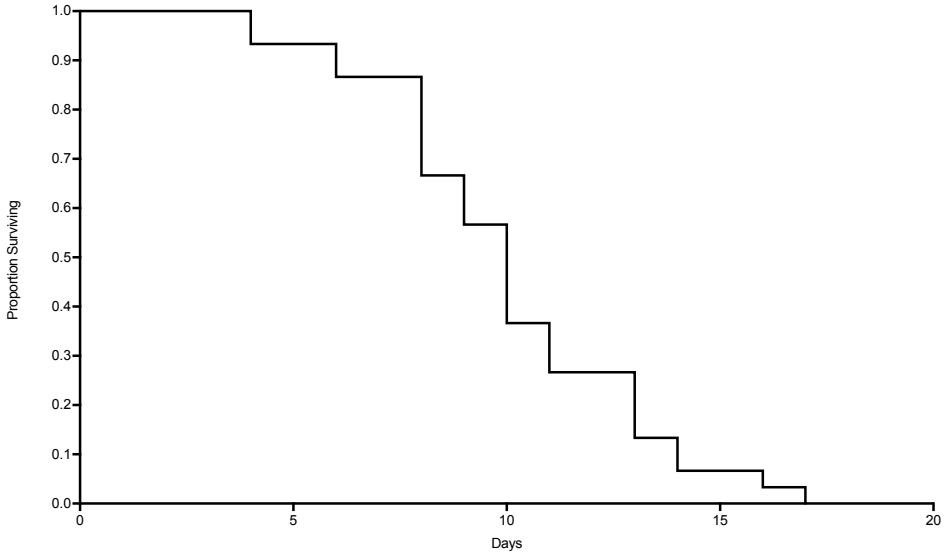
L43. 10% glucose-fructose blend, but no prey



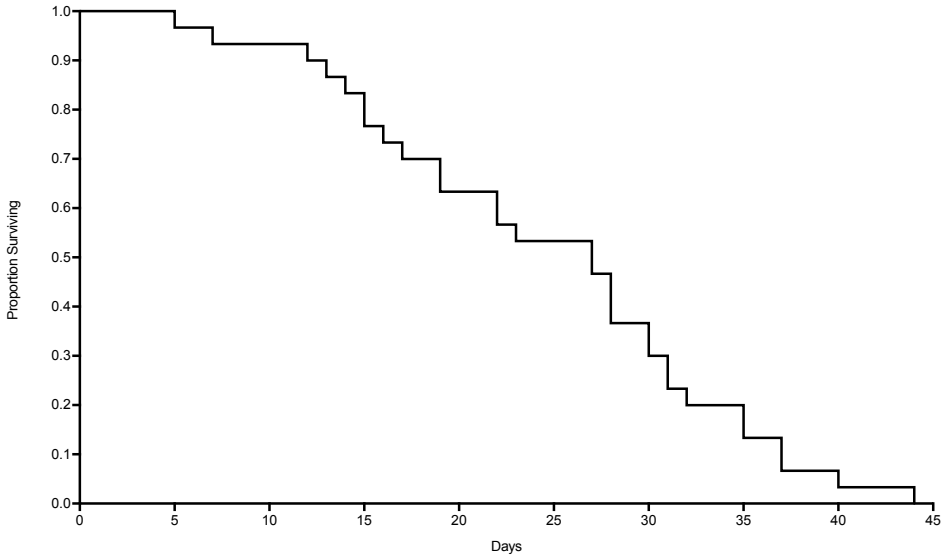
L44. 20% glucose-fructose blend, but no prey



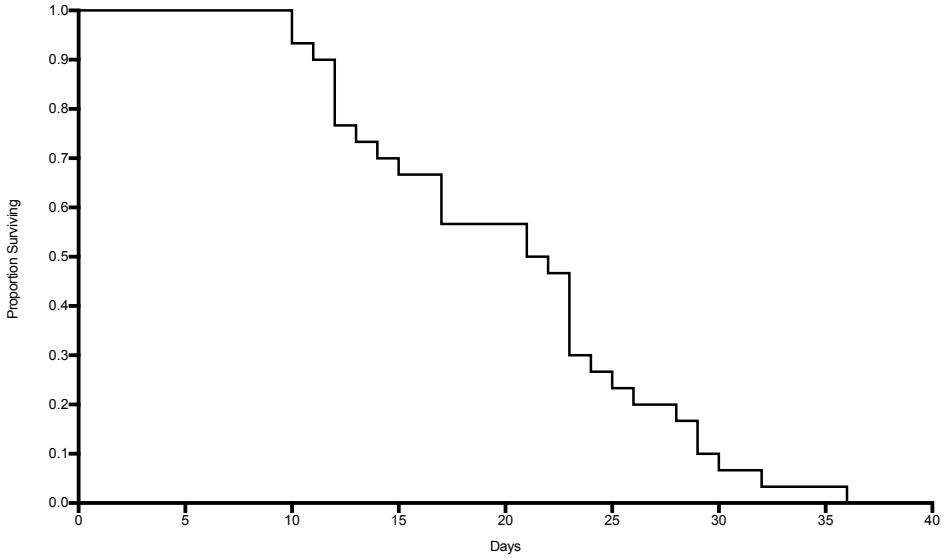
L45. 4% amino-acid blend (Glu, Gly, Pro, Ser) but no prey



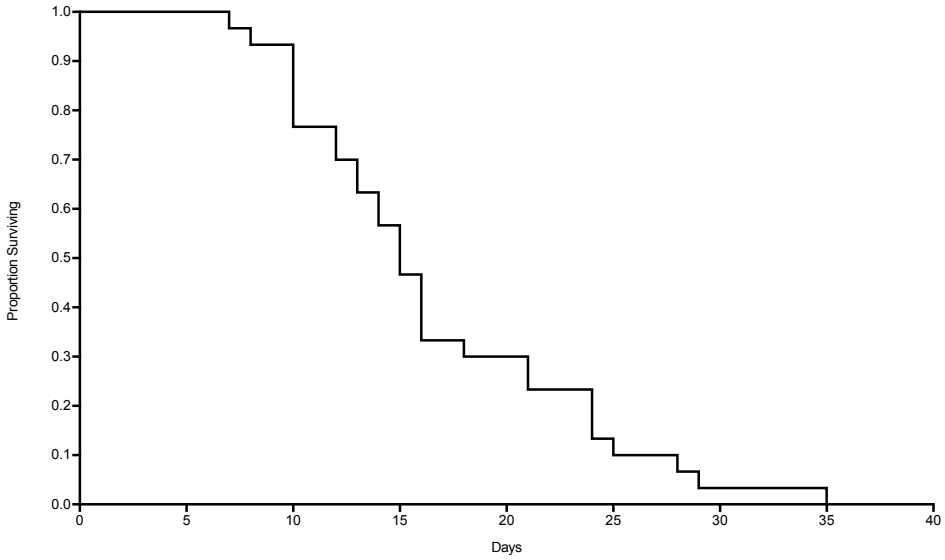
L46. 20% glucose+amino-acid blend, but no prey



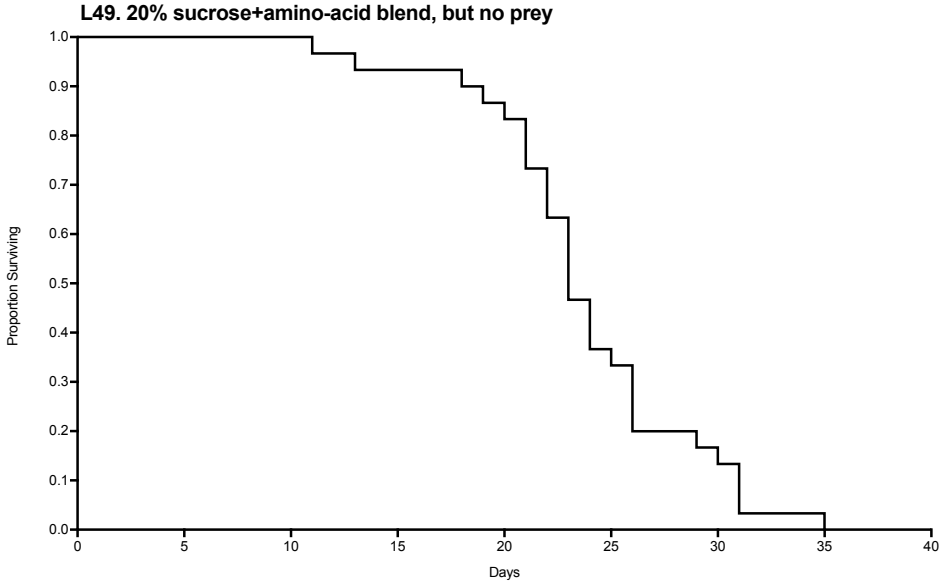
L47. 20% fructose+amino-acid blend, but no prey



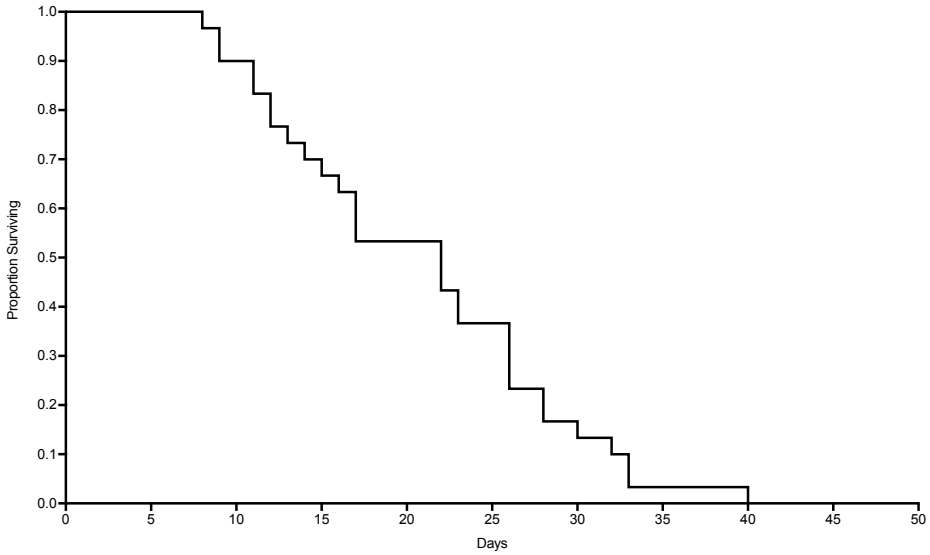
L48. 20% maltose+amino-acid blend, but no prey



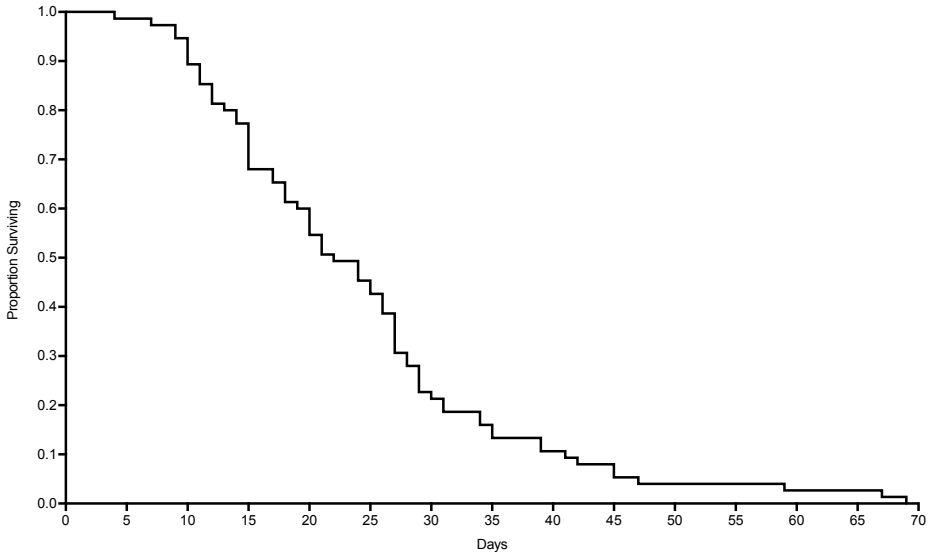
L49. 20% sucrose+amino-acid blend, but no prey



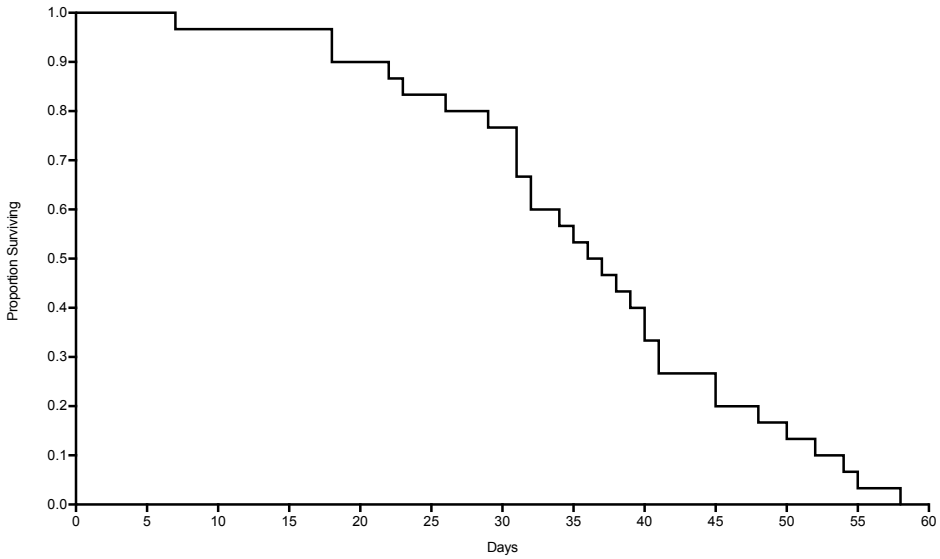
L50. Sugar-only *L. camara* blend, but no prey



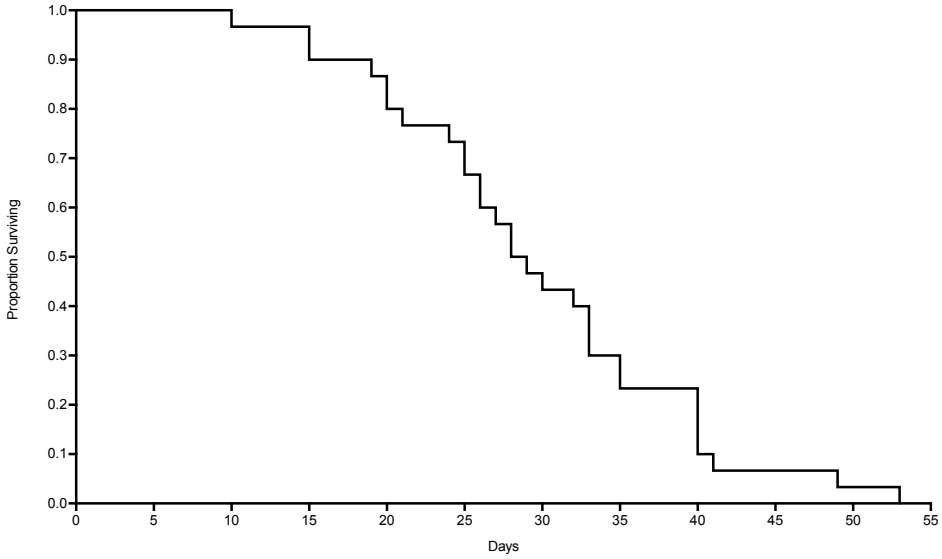
L51. Full *L. camara* blend, but no prey



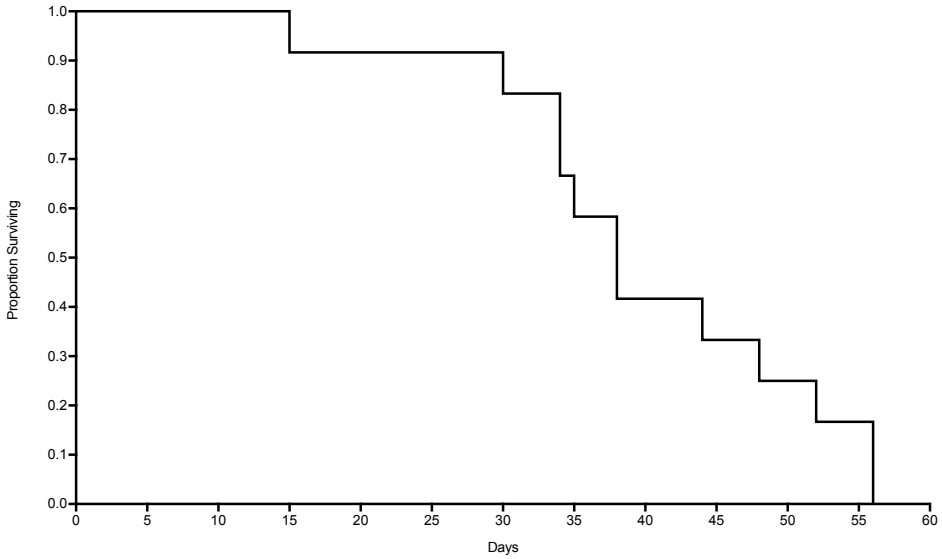
L52. Full blend & ate one no-blood female



L53. Full blend & ate one blood female



L54. Full blend & ate two no-blood females



L55. Full blend & ate two blood females

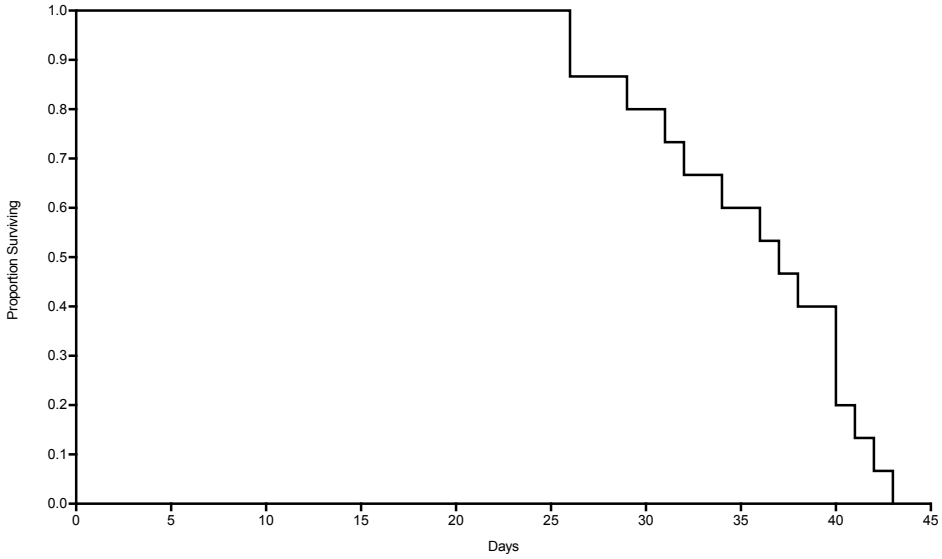


Fig. 2. The longevity (L) of hatchlings did not complete the first instar but died were calculated by days. See table 4.

CHAPTER FOUR: DISCUSSION

The potential relevance of *Evarcha culicivora* to efforts in the context of malaria

Even people who dislike spiders tend to like knowing there is a spider with an active preference for mosquitoes as prey. Mosquitoes can be a nuisance. They buzz, bite and keep us awake at night, but many of these insects are also notorious as vectors of some particularly serious human diseases, including filariasis, yellow fever, dengue, West-Nile fever and especially malaria (Becker et al. 2010). In human history, the impact of malaria has been enormous (Cox 2010), but malaria is still with us. It remains the vector-borne disease with the most severe impact on public health and regional economies (Collins & Paskewitz 1995; Kokwaro 2009). However, in the mid-20th century, the geographic range in which there is a high risk of malaria has become primarily tropical and subtropical regions (Packard 2007), and especially with Sub-Saharan Africa. The World Health Organization estimated that in 2015 about 88% of malaria cases and 90% of malaria deaths in the world were in sub-Saharan These descriptive statistics are only a first step toward getting a perspective on the significance of malaria (Koram & Molyneux 2007). For a person suffering from a bout of malaria, the symptoms would be the primary perspective. These include severe headaches and profuse sweating, along with vomiting and, in especially serious instances, convulsions. The signature symptom of malaria is a characteristic cycling between fever and chills. However, understanding the public-health impact of malaria requires going beyond the symptoms. Malaria tends to be intertwined with other diseases and with poverty, forming an entangled web where each can be envisaged as a cause and an effect of the other (Bonds et al. 2010; Chase 2012). This makes the impact of malaria difficult to specify and quantify (Spielman & D'Antonio 2001).

Malaria is not unique to people. It is a disease caused by *Plasmodium* and related genera of single-cell parasites from the phylum Apicomplexa (Garnham 1966). These parasites have a complex life cycle (Packard 2007), with multiple stages in a definitive host (the host in which it reproduces sexually as well as asexually) and multiple stages in an intermediate host (i.e., the host in which it reproduces only asexually). A wide variety of terrestrial vertebrates (reptiles, birds and mammals) serve as intermediate hosts for different malaria-parasite species (Collins & Paskcwitz 1995; Gu et al. 2011) and, even human malaria might more accurately be characterized as five diseases instead of only one (Perez-Tris et al. 2005). People are normal intermediate hosts for *Plasmodium falciparum*, *P. knowlesi*, *P. malariae*, P. *ovale* and *P. vivax*), with *P. vivax* being the most widespread and *P. falciparum* being the most lethal. *Plasmodium falciparum* is also the dominant human-malaria parasite in sub-Saharan Africa (Guerra et al. 2008).

When referring to *Plasmodium*, it is often convenient simply to say that the definitive hosts (also called the 'vectors') are mosquitoes. However, to be more precise, we should say female mosquitoes because males subsist primarily on nectar, not blood (Clements 1999). Even the rule that definitive hosts are female mosquitoes has exceptions when it is lizard malaria (Schall 1990, 2000).

The same basic pattern is followed in all *Plasmodium* life cycles (Fig. 1) (White et al. 2011). When a mosquito penetrates the skin of the intermediate host, a form of the parasite called sporozoites enters the host along with the mosquito's saliva. The sporozoites then move into the blood stream and, when they reach the liver, they invade hepatocytes (i.e., liver cells). While inside a hepatocyte, a sporozoite turns into a schizont, a schizont being basically a bag of yet another version of the parasite, namely the merozoites. After about a week, the merozoites

burst out of the schozont and hepatocyte, and then enter the blood stream where they enter red blood cells (erythrocytes).

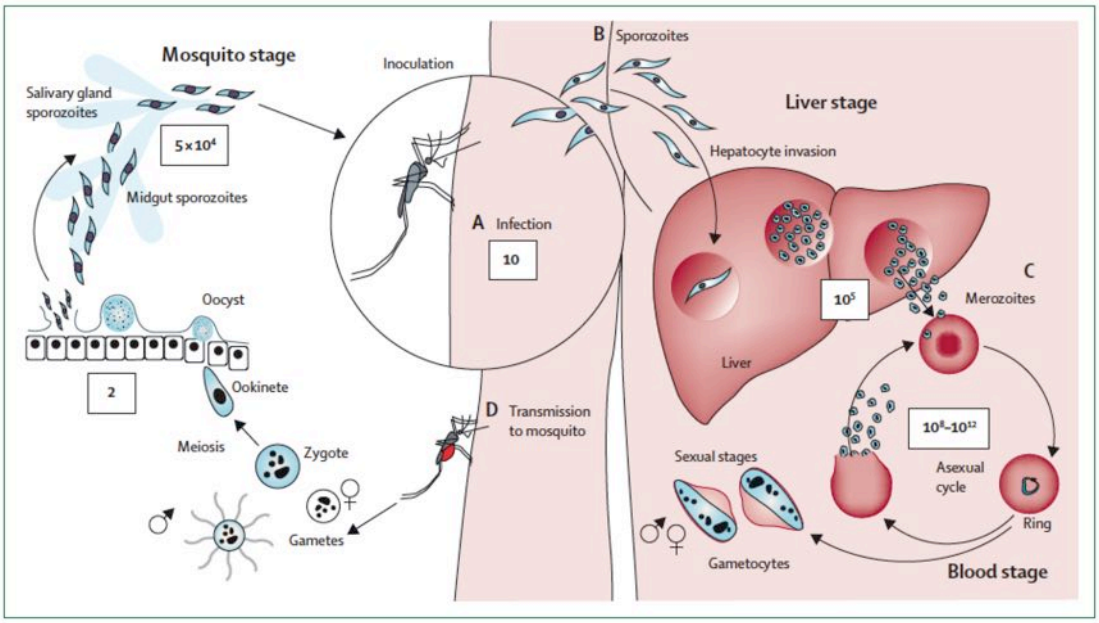


Fig 1. Life cycle of *Plasmodium falciparum* in the human body and the body of an anopheline mosquito. Drawing from White et al. (2014). The cycle begins with inoculation of motile sporozoites into the skin (A), after which the sporozoites travel to the liver (B). Each sporozoite, when it is in the liver, invades a hepatocyte and then multiplies inside a sack called a schizont.. After about a week, the schizont bursts, releasing into the bloodstream thousands of the next stage in the parasites life cycle, the merozoites. The merozoites move into the blood stream where they invade red blood cells and begin a cycle of asexual reproduction (C). Illness starts when total number of the asexual parasite in the circulation reaches roughly 100 million. Eventually some of the parasites in the blood stream develop into precursors of the gametes. These are called gametocytes. Gametocytes are taken with blood by a feeding anopheline mosquito (D) and then turn into gametes (eggs and sperm). Sexual reproduction occurs in the mosquito's digestive tract, with the zygote transforming into a form called an ookinete and then an oocyst in the mosquito gut. When the oocyst bursts, sporozoites are released and the sporozoites migrate to the mosquito's salivary glands. There they wait to be inoculated along with saliva into the intermediate host when the mosquito takes its next blood meal. The entire cycle can

take about a month. Estimated numbers of parasites are shown in boxes. A total burden of 10^{12} parasites corresponds to roughly 2% parasitaemia in an adult (White et al. 2014).

In the red blood cell, transformations continue. The merozoite turns into a trophozoite and then the trophozoite becomes a schizont (i.e., a bag of merozoites inside the red-blood cell). When the schizont and red blood cell rupture, hoards of merozoites enter the bloodstream and then infect more red blood cells. Cycles of merozoites synchronously destroying red blood cells and then infecting more red blood cells are responsible for the recurrent-fever cycle's characteristic of malaria. These cycles of asexual reproduction typically reach a level corresponding to illness when the number of asexual parasites in the bloodstream reaches about 100 million.

Eventually some of the merozoites develop into a different form of the parasite, the gametocyte and this is a step that connects the cycle back to the mosquito. There are male and female versions of gametocytes. Sexual reproduction occurs when gametocytes are in the blood ingested by a competent female mosquito. In the mosquito, gametocytes quickly become gametes (eggs from female gametocytes and sperm from male gametocytes) that form zygotes that turn into ookinetes. Ookinetes, being motile, penetrate the wall of the mosquito's midgut where they form oocysts. Over time, oocysts enlarge and then burst open to release sporozoites and the sporozoites migrate to the mosquito salivary gland. Now the mosquito is armed with the infectious form of the parasite. The next time the mosquito takes a blood meal, the sporozoites are transferred to the intermediate host and a new life cycle begins.

Historically, public health efforts to reduce malaria incidence have relied on targeting the vector more than on targeting the parasite, and this continues to be the case (Becker et al. 2010). The dominant means of vector control in sub-Sahara Africa is the use of insecticide-treated mosquito nets and practising indoor residual spraying with insecticides (Alonzo & Turner 2013). About 3.2 billion people are currently at

risk of contracting malaria (the World Malaria Report (2015) of the WHO Global Malaria Programme), which is nearly half of the world's population. Progress being made when compared with figures from 15 years ago (Fig. 2), as it indicates that the risk level has dropped by 37%, and mortality rate has dropped by 60%. Put another way, millions of malaria deaths were averted by a massive international effort at malaria prevention and malaria treatment.

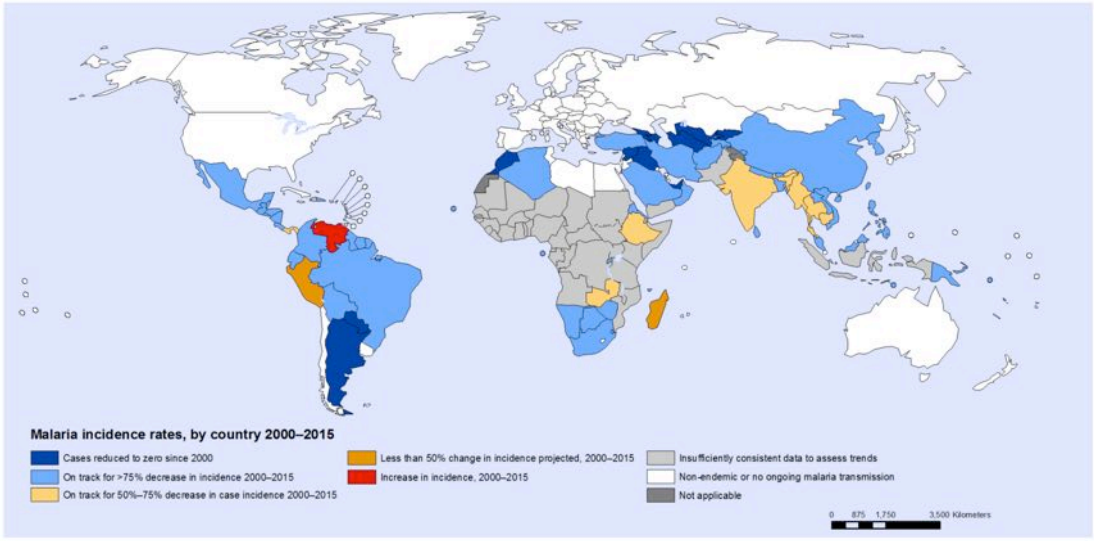


Fig 2. Projected changes in malaria incidence rates, by country, 2000-2015.

Date source: World Malaria Report 2015. From

http://gamapserver.who.int/mapLibrary/Files/Maps/WMR2015_ChangesInIncidence.png (accessed 08/03/2016).

It may be easy to let the effectiveness of these measures suggest that mosquito predators, including *E. culicivora*, will make little difference. In 2007, the Bill and Melinda Gates Foundation announced that the eradication of malaria should be the goal (Roberts & Enserink 2007, Grabowsky 2008), and there has been considerable enthusiasm about actually meeting this goal in the foreseeable future. However, if the goal is the eradication of malaria instead of just a reduction and control, then there is still a long way to go, and it will be expensive (Mill et al. 2008; Moonen et al. 2010). There cause for skepticism about whether the world would bear the cost of eradication if control is working well enough (Hommel 2008). This suggests that dismissing the relevance of predators is premature.

There is now considerable concern about 'residual malaria', this being an expression used for malaria transmission by mosquitoes that are not stopped by insecticide-treated mosquito nets and indoor spraying with insecticides (Govella & Ferguson 2010); Killeen 2013, 2014; Homan 2016). If eradication is the goal, then residual malaria cannot be ignored and, owing to natural selection, we should be prepared for what is now called 'residual malaria' becoming more prominent (Ferguson et al. 2010).

Other details about mosquitoes are important in the context of malaria. More than 3,500 mosquito species have been described, but only one genus, *Anopheles*, includes species that are competent vectors of human malaria (Clements 1999; Molina-Cruz et al. 2013). In this genus there are about 500 described species, but only about 70 species are known to be competent human-malaria vectors (Harbach 2004). *Anopheles gambiae* is currently the most notorious of these species (Spielman & D'Antonio 2001). However, instead of being a single species, *An. gambiae* is a species complex (Coetzee et al. 2000, 2013) in which constituent species tend to have

distinctly different behaviour and distinctly different relevance to human malaria, while at the same time being morphologically indistinguishable (Coluzzi et al. 1985). Molecular methods have become the standard for distinguishing between them (Fanello et al. 2002) and, when uncertain, the mosquito is referred to as *An. gambiae sensu lato*.

Anopheles gambiae sensu stricto has special characteristics that make it the most notorious vectors of *P. falciparum* (Sinka et al. 2010), with two of these characteristics being olfactory anthropophily (McBride 2016) and a strong bias toward anthropophagy (Takken & Verhultst 2013). Evidence for olfactory anthropophagy, which come from trapping with odour baits and from olfactometer experiments in the laboratory, implies that this mosquito is highly proficient at detecting and finding the source of human odour (Takken & Knols 1999; Carey et al. 2010). Strong anthropophagi means taking blood meals primarily from people and there have been many studies backing up this conclusion (White 1974; Githeko et al. 1996; Antonio et al. 2002; Mwanganqi et al. 2003; Dabire et al. 2008) and yet there are also reports suggesting that, in some instances, *An. gambiae s.s.* takes frequent blood meals from domestic livestock and other non-human animals (Diatta et al. 1998; Bøgh et al. 2001; Duchemin et al. 2001). These reports suggest that the level of zoophagy versus anthropophagy can be influenced by geographical variables, population density, host availability and other factors.

Before the use of bed nets became widespread in Africa, the standard characterization of *An. gambiae s.s* had been that of a highly anthropohilic, anthrophagic and endophilic mosquito that was predisposed to feed late at night, when people are sleeping instead of being alert and ready to defend themselves against the mosquito (Githeko et al. 1996; Wanji et al. 2003, Pates & Curtis 2005). All of these

are characteristics that contribute to this mosquito's effectiveness as a malaria vector, but there is more. Some blood-feeding mosquitoes are autogenous, meaning that they do not require a blood meal before producing their first batch of eggs, but *An. gambiae s.s.* is anautogenous, meaning it needs a blood meal for egg production, and *An. gambiae s.s.* also tends to take blood meals more than once in a single gonotrophic cycle (Scott & Takken 2012). Frequent blood meals correspond to frequent opportunities for infecting people with the malaria parasite. It is also important that *An. gambiae s.s.* has a lifespan long enough for the malaria parasite to undergo sexual reproduction and then load sporozoites into salivary glands in time for infecting people during a later feeding event (Spielman & D'Antonio 2001). When females lay their eggs, it tends to be in areas associated with human activity and the time for larval development tends to be short enough to support rapid arrival of new generations of blood-feeding adults (Betson et al. 2009; Minakawa et al. 2004).

When malaria in sub-Sahara Africa is discussed, there is an understandable emphasis on An. *gambiae s.s.*, but this does not mean that other malaria vectors are trivial. For example, the *An. gambiae s.l.* complex includes *An. arabiensis*, a species that is normally characterized as being primarily zoophagic (Pates et al. 2001). However, *An. arabiensis* is a highly competent vector of *P. falciparum* that also takes blood meals from people and tends to be common around human dwellings. In some localities, *An. arabiensis* is the primary vector of human malaria (e.g., Mwangangiel et al. 2013). *Anopheles funestus* is from a different species group and it, like *An. gambiae s.s.*, is strongly anthropophagic and highly competent as a vector of *P. falciparum* In many instances, *An. funestus* is the dominant malaria vector (Cooke et al. 2015). *Anopheles coustani*, *An. moucheti*, *An. nili*, *An. pharoensis*, *An. rivulorum*, *An. rufipes* and other African species, although known to be competent malaria

vectors (Gillies et al. 1968; Faye et al. 1997; Antonio-Nkondjio et al. 2002; Awolola et al. 2002; Kwada et al. 2012; Stevenson et al. 2012), have often been, until recently, ignored when discussing malaria, but they are now being viewed more carefully in the context of residual malaria. Along with *An*. *funestus*, these species are particularly interesting in the context of residual malaria because their blood-feeding times span into early in the evening and early in the morning when people tend to be away from their bed nets (Kitau et al. 2012; Russell et al. 2010; Yohannes & Boelee 2012; Gatton et al. 2013; Bayoh et al. 2014; Moiroux et al. 2014; Soufoufara et al. 2014; Ototo et al. 2015).

Mosquitoes begin their lives in water. The aquatic larvae hatch from the eggs and later turn into aquatic pupae from which the adults emerge as the flying insects that feed on blood if they are females and plant products if they are males (Clements 1999). Despite being better known for taking blood meals, female mosquitoes often also take non-blood meals (Foster 1995; Gu et al. 2011). In most instances, this means nectar, but there are exceptions (Gary & Foster 2004; Junila et al. 2010). There are even reports of female mosquitoes feeding on caterpillars (George et al. 2014).

Aquatic juveniles, flying adults, visiting animals for blood and visiting plants for sugar meals - this makes for many contexts in which predators might kill mosquitoes. However, when considering *E*. *culicivora*, we can probably ignore predation on the aquatic stages and we can probably ignore predation at night when the adult mosquito tends to feed from hosts and plants. This makes *E*. *culicivora* rather different from the predators that have been more often considered in the context of predation on mosquitoes.

The predators that have been more often considered are species that target the larvae (Sabatinelli et al. 1991; Howard et al. 2007; Fischer et al. 2013), not the adults,

of the mosquito. Although less common, research on predators that target adult mosquitoes (e.g., Gonsalves et al. 2013) could be of particular interest. After all, malaria vectors, as well as the vectors of dengue, yellow fever and other mosquito-borne diseases, are specifically adult mosquitoes – more specifically adult female mosquitoes, these being the individuals that take blood meals. Another consideration is that, by killing blood-carrying female mosquitoes of vector species, a predator is killing the individuals that might be or might soon become, infected with a disease agent and it is also killing the mosquitoes that might soon produce the eggs that will initiate the next generation of aquatic juveniles that mature to become more disease vectors.

When there is an interest in the predators of adult mosquitoes, spiders in particular warrant careful consideration. Spiders are found in virtually all of the terrestrial habitats where adult mosquitoes occur and there are a lot of spider species to consider: over 46,000 species belonging to 114 families have been named and described (World Spider Catalog (http://www.wsc.nmbe.ch, accessed 12/03/2016). The common name 'spider' refers to the chelicerate species in the order Araneae and class Arachnida, and this is the seventh most diversified order of animals in general (Codington & Levi 1991). Among spiders, the Salticidae, with almost 6,000 described species is the largest family (Maddison 2015). Although there are salticids and other spiders that target aquatic prey, terrestrial prey is the norm (Jackson & Pollard 1996).

Many spiders probably eat mosquitoes and some might eat especially many mosquitoes (Jackson & Cross 2015). However, *E. culicivora*, being a mosquito-specialist, is different. Currently, *E. culicivora* and one other salticid species are the only predators for which there is substantial evidence of specialization on mosquitoes as prey. The other salticid is *Paracyrba wanlessi*, a species from Peninsular Malaysia

(Żabka & Kovac 1996), but it would be misleading to characterize *P. wanlessi* as being a spider that specializes on mosquitoes in Asia in the same way *E. culicivora* specializes on mosquitoes in Africa.

For example, although both species express a strong, active preference for mosquitoes as prey (Jackson et al. 2014), these two species live in rather different habitats. Instead of being found primarily on or near human dwellings, *P. wanlessi* is most often found in the hollow internodes (culms) of bamboo (Kovac & Streit 1996; Żabka & Kovac 1996). Like *E. culicivora*, *P. wanlessi* is a terrestrial predator that targets adult mosquitoes with specialized prey capture behaviour, feature-detection mechanisms and prey-choice behaviour; however, unlike *E. culicivora*, *P. wanlessi* is also an aquatic predator that targets juvenile mosquitoes (i.e., larvae and pupae) with different specialized prey capture behaviour, feature-detection mechanisms and prey-choice behaviour. It is important to appreciate what is going on here. Depending on what it encounters, a single individual of *P. wanlessi* can switch rapidly from expressing specialization on one type of prey (larvae and pupae in water) to expressing specialization on another very different type of prey (adult mosquitoes away from water). This is an example of a particular type of polyspecialization (West-Eberhard 2003) in which a predator adopts a conditional strategy. Also known as 'predatory versatility' (Curio 1976), this type of polyspecialization illustrates rather emphatically that specialization on a particular type of prey is qualitatively different from evidence of limitation to a particular type of prey.

Setting aside specialized predation by *P. wanlessi* on aquatic juvenile mosquitoes, we might say that specialized predation on adult mosquitoes is a characteristic *P. wanlessi* shares with *E. culicivora*, but even this is misleading. It is too simplistic. The prey-choice and prey-capture behaviour of *E. culicivora*

demonstrates that this is a predator for which 'adult mosquito' is not a single prey category (Jackson & Cross 2015). For *E. culicivora*, the distinction that matters includes female mosquitoes vs. male mosquitoes, blood-carrying female mosquitoes vs. no-blood female mosquitoes, mosquitoes from the genus *Anopheles* vs. mosquitoes from other genera. However, there is no evidence suggesting that these distinctions are relevant to *P. wanlessi*.

This illustrates why, for understanding predatory specialization, it is important to consider the predator's own prey-classification system instead of formal scientific taxonomy (Nelson & Jackson 2011). Scientific taxonomy of a predator's prey is important for understanding food webs, trophic niches and related topics in community ecology (Futuyma & Moreno 1988; Stouffer et al. 2007; Thompson et al. 2012; Pekar & Toft 2105), but this is different from advancing our understanding of precisely how a predator might be specialized. The predator's own perspective matters for this because specialization pertains to specific ways in which a predator has become well adapted at targeting particular types of prey. *Evarcha culicivora* and *P. wanlessi* are two predators that express specialization on prey that, on the basis of scientific taxonomy, are lumped together as a category (family Culicidae, common name 'mosquito'). As far as it goes, we can call both of them 'mosquito specialists', but just saying that does not actually get us very far.

In this thesis, my goal was to investigate the expression of specialization by *E. culicivora* more broadly.

As a sideline, I participated in some research on preferences and natural diet (Appendix 1). Preferences as revealed by prey-choice behaviour are not data pertaining to a predator's natural diet. Natural diet is something different and must be determined by other means (field data). It is of interest to determine how closely diet

converges with preferences, but it is rarely possible to determine this accurately. There was an unusual opportunity to do this for *E. culicivora* and the findings show, with a large sample size, that there is a remarkable convergence (Appendix 1). The prey-choice data also illustrate how just stating a preferred prey type is not adequate when characterizing *E. culicivora*. Instead, it needs to be understood that this predator has a preference profile, with hierarchies of preference.

Specialization, however, pertains to more than prey-choice behaviour and my goal in this thesis was to investigate specialization by *E. culicivora* in another two contexts.

In Chapter 2, my hypothesis was that *E. culicivora* has an innate specialized predisposition to time predatory activity to occur in the morning when its preferred prey, blood-carrying anthropophilic anopheline mosquitoes, are resting while digesting night-time blood meals. My results show that, under natural conditions, *E. culicivora* tends to be active as a predator primarily in the early morning when it can find resting female anthropophilic mosquitoes that fed on blood the previous night. I also found that *E. culicivora* is innately predisposed to express stronger preference in the morning than in the afternoon for blood-carrying mosquitoes. This is the first study to show specialized adaptive timing of predation by a mosquito-specialist predator.

In Chapter 3, I investigated specialization in the context of the nutritional ecology of *E. culicivora* hatchlings, having decided to focus on hatchlings because this is the first active, feeding stage in the spider's life cycle and, therefore, a stage when nutritional needs can be expected to be especially pronounced. I determined how different prey-only feeding regimes affected completion success, completion

time and longevity. I also investigated how access to plant-derived nutrients either alone or in conjunction with prey affected the hatchling's performance.

That *E. culicivora* appears to be a mosquito-eating specialist and also a plant-feeding specialist is of particular interest. Feeding on plant-derived nutrients is probably not what most people think about when and if they think about spiders. The tradition has been to characterize spiders as strictly predators. Yet there is a growing awareness that feeding on plant products may be common among spiders (Nyffeler 2016).

Evarcha culicivora is known to be attracted to the odour of two plant species *Lantana camara* and *Ricinus communis* in olfactometer experiments (Cross & Jackson 2009), and there is unpublished evidence of attraction to a wider range of plant species, including especially *Lippia kituensis* (R.R. Jackson, personal communication). We also know that *E. culicivora* juveniles in particular frequently ingest fructose (plant sugar) in the field and the laboratory (Kuja et al. 2012). However, Chapter 3 is the first report from research in which *E. culicivora* was reared on diets that included plants or artificial nectar, with or without prey also being available.

I found effects from plants and artificial nectar when provided alone and when provided in addition to prey. I also found that the plant species mattered, with *Lantana* and *Lippia* being the most beneficial. It appears to be accurate to characterize *E. culicivora* as a specialist at using these plants, as well as being a specialist at preying on mosquitoes. It was specifically metabolic specialization that was of interest in Chapter 3, but attraction to particular plant species in olfactometer experiments and frequent finding of *E. culicivora* on particular plant species in the

field suggests that, as with specialization on particular kinds of prey, *E*. *culicivora*'s mode of specialization on plants is broader than just metabolic.

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APPENDIX ONE: CONVERGENCE BETWEEN A MOSQUITO-EATING PREDATOR'S NATURAL DIET AND ITS PREY-CHOICE BEHAVIOR

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Short title: Mosquito predator's natural diet and preferences

Abstract

On the basis of 1,115 records of *Evarcha culicivora* feeding in the field, we can characterize this East African jumping spider (Salticidae) as being distinctively stenophagic. We can also, on the basis of laboratory prey-choice experiments, characterize *E. culicivora* as having a specialized prey-classification system and a hierarchy of innate preferences for various categories of mosquitoes and other arthropods. Prey from the field belonged to 11 arthropod orders, but 94.5% of the prey records were dipterans. Mosquitoes were the dominant prey (80.2% of the records), with the majority (82.9 %) of the mosquitoes being females, and thereafter midges were the most common prey (9.2% of the records). Preference profiles that were determined from experiments showed strong convergence with natural diet in some, but not all, instances. In experiments, *E. culicivora* adults appeared to distinguish between six prey categories and juveniles between seven, with blood-carrying anopheline female mosquitoes being ranked highest in preference. For adults, this was followed by blood-carrying culicine female mosquitoes and then anopheline female mosquitoes not carrying blood, but these two preferences were reversed for juveniles. Moreover, for juveniles, but not for adults, anopheline male mosquitoes seem to be a distinct prey category ranked in preference after blood-carrying culicine females and, for both adults and juveniles, preference for midges is evident when the alternatives are not mosquitoes. These findings illustrate the importance of going beyond simply specifying preferred prey categories when characterizing predators as 'specialized' and a need to make clear conceptual distinctions between a predator's natural diet, the prey categories that are relevant to the predator, and the predator's prey-choice behaviour.

Keywords: Specialization, preference, stenophagy, spider, Salticidae, *Evarcha culicivora*, *Anopheles gambiae*

1.0. Introduction

Making a clear distinction between preference and natural diet is important when discussing predatory specialization [1] because, although natural diet is simply what a predator eats in the field, preference is an inherent product of a predator's perceptual processes, decision-making capacities and motivation. A combination of laboratory experiments and field sampling is necessary for determining the extent to which preferences and natural diet converge but, in research on many predators, this combination is often unrealistic. However, *Evarcha culicivora*, the predator we consider here, is an exception because a long-term research programme on this jumping spider (family Salticidae) from East Africa has allowed for large data sets from the field pertaining to natural diet and large data sets from laboratory prey-choice experiments pertaining to preferences.

Spiders are usually characterized as being 'generalist predators' (e.g. [2–4]) but often, when reading the literature on spiders and other predators, it is difficult to discern whether 'generalist' refers to euryphagy (i.e. inclusion of a wide range of prey in the predator's natural diet), indiscriminate feeding (i.e. the absence of pronounced prey-choice behaviour) or some combination of the two (see [1]). It is particularly misleading when the expression 'generalist predator' is used for characterizing the prey-choice behaviour of salticid spiders. The majority of well-designed experimental studies on salticids have revealed distinct preferences, the most notable examples coming from salticids that target ants as preferred prey [5] and from other salticids that target spiders as preferred prey [6]. It is also premature to use 'generalist' as a synonym for 'euryphagy', and then apply it to salticids as a group because tabulated

data pertaining to natural diet are scarce for this large spider family of almost 6,000 described species [7].

It is relevant to our research that *E. culicivora* is a salticid because salticids can be unusually cooperative subjects in prey-choice experiments owing to their unique, complex eyes and their ability to see prey in remarkably fine detail [8,9]. For example, even when distant from its prey, a salticid can initiate distinctive predatory behaviour which a scientist may record and use as evidence of a salticid's decisions [10]. Moreover, in research on salticids, prey-choice experiments can be designed to avoid the risk of experimental outcome being influenced by uncontrolled prey behaviour. Many salticids [10], including *E. culicivora* (table 1), are known to respond to lures (dead prey mounted in life-like posture on cork discs) or even to virtual prey generated by computer animation software (e.g. [14]), with these responses corresponding well to how they respond to living, active prey.

Evarcha culicivora's predatory strategy is strikingly unusual. This spider feeds indirectly on vertebrate blood by actively choosing blood-carrying female mosquitoes as its preferred prey [11] and, by actively choosing *Anopheles* as its preferred mosquitoes [15], it singles out the particular mosquito genus to which all human malaria vectors belong [16]. Vision is not the only sensory modality that matters to *E. culicivora*, with olfaction in particular having various [1], and sometimes surprising (e.g. [17]), roles in this species' biology. However, vision-based prey-choice behaviour by *E. culicivora* has been the most thoroughly investigated and, regardless of whether living prey, lures or virtual prey were used, all earlier studies have confirmed this salticid's distinctive preference for blood-carrying mosquitoes (table 1).

In the most comprehensive study of *E. culicivora*'s preferences to date [12], lures were made from a non-biting midge species, *Clinotanypus claripennis* (Chironomidae), and from both sexes of two mosquito species, *Anopheles gambiae s.s.* and *Culex quiquefasciatus*. For both mosquito species, a distinction was made between females that carried blood and females that did not carry blood. For ascertaining the strengths of *E. culicivora*'s different preferences, three testing protocols (simultaneous, alternate-day and alternative-prey) were used (table 2) and, for each protocol, individuals were subjected to pre-trial fasts of different durations. In simultaneous testing, *E. culicivora* was given the choice between two lures at the same time, with each lure being made from a different kind of prey. In alternate-day testing, *E. culicivora* was shown a single lure of one type on one day and a single lure of another type on the next day, with only those test pairs in which *E. culicivora* chose one prey, but not the other, being used as data for determining preference. In alternative-prey testing, *E. culicivora* was again shown a lure of one type on one day and a lure of another type on the next day. However, in these tests, the spider was shown a lure whilst feeding on the other prey type, with a 'choice' being recorded only if the spider dropped the prey it was eating while approaching the lure. The prey type that the spider ate on the first day was used as a lure on the second day.

Irrespective of whether test spiders were adults or juveniles, Nelson and Jackson [12] found a primary preference for blood-carrying female mosquitoes, but preference for blood meals was expressed more strongly by adults than by juveniles. In addition, adults and juveniles chose female mosquitoes in preference to male mosquitoes (also see [18]) and anopheline mosquitoes in preference to culicine mosquitoes (also see [14,15,19]), but juveniles expressed a stronger preference than adults for anophelines (table 2). These findings illustrate that, when characterizing

predators as ‘specialized’, it is important to consider preference profiles instead of simply specifying a preferred prey category.

Yet our understanding of *E. culicivora*’s preference profile has remained incomplete. In previous research, only a limited number of non-mosquito prey species were used and at least one of the prey individuals was always a mosquito (tables 1 and 2). For example, the only non-mosquito prey species used by Nelson and Jackson [12] and by Jackson and Nelson [13] was *C. claripennis*, a chironomid midge, whereas a different chironomid midge, *Nilodorum brevibucca*, was the only non-mosquito prey species that Nelson and Jackson [15] used for making lures. Experiments using virtual prey by Nelson and Jackson [15] and by Dolev and Nelson [14,19] have been based primarily on the way the resting postures of anophelines and culicines differ [20], although Dolev and Nelson [14,19] also used virtual house flies (*Musca domestica*). Jackson et al. [11] used a wider range of non-mosquito prey (aphids, caterpillars, fruit flies and spiders, as well as six midge species) but the lures were always stationary and presented simultaneously and, in that study, the pre-trial fast was always 7 days (table 1).

One of our goals has been to considerably extend previous research on the prey-choice behaviour of *E. culicivora* by using a wider range of non-mosquito species and also, for the first time, including experiments in which non-mosquito species are paired with other non-mosquito species. Being interested in specifically innate preferences, we endeavoured to standardize test spiders’ prior experience with prey. Another goal has been to link our prey-choice experiments more closely than has been the case in the past to an understanding of this predator’s natural diet. When selecting the prey to use in our experiments, we were guided by having more than 1,000 records of prey eaten by *E. culicivora* in the field. This large sample of prey

from the field, combined with a wide range of experiments designed specifically for gaining insight into how *E. culicivora* innately categorizes prey, has given us an unprecedented opportunity to determine the extent to which a predator's natural diet corresponds with its innate preferences.

2.0. Material and methods

2.1. Prey records from the field

Our field site was the town of Mbita Point in western Kenya, including the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology (elevation 1200 m above sea level, latitude 0◦25'S–0◦30'S, longitude 34◦10'E). We accumulated records of prey in the field by adopting a simple, informal method. When we and other personnel from our Mbita Point laboratory saw an individual of *E. culicivora* (the 'predator') in the field that was the act of feeding, we put this predator, along with its prey, in a plastic vial and then we separated the predator from its prey by shaking the vial or, by using a soft brush, prodding the predator until it released the prey. In the laboratory we identified the prey and also recorded whether the predator was an adult female, an adult male or a juvenile. We used forceps to press on the abdomen of any female mosquito taken from *E. culicivora* and, whenever red or reddish brown fluid was noticeable, we recorded that the female mosquito was carrying blood. Besides using our new prey records from 2002–2015, we include the 202 records from work in 1994, 1995, 1997, 1998 and 2001 [21] which were collected using the same procedure.

2.2. Laboratory procedures

On the whole, our apparatus, experimental procedures and laboratory rearing protocol corresponded to the methods used by Nelson and Jackson [12], except that the specific prey pairings used by Nelson and Jackson [12] differed from the prey pairings we used here. Although some of the particular pairings of prey types had been used previously [11], the apparatus and testing procedures used here (and by Nelson and Jackson [12]) were considerably different (see table 1).

Our test spiders were adult females (4.5–5.0 mm in body length) as well as mid-size juveniles (3.0 mm). We decided not to use adult males because, although no male-female differences in *E. culicivora*'s preference were detected in the previous research (table 1), there is a tendency for salticid males to be less responsive to prey than salticid females [22]. We adopted 3.0 mm as the standard body length for juvenile test spiders because, in earlier experiments ([12]; table 2), juvenile-adult divergence in preference was evident only when juveniles were 3.5 mm or less in body length. We decided not to use juveniles that were smaller than 3.0 mm because we wanted to avoid a large juvenile-adult size disparity. All test spiders came from laboratory cultures and the body length of each test spider was accurate to the nearest 0.5 mm.

The standard rearing diet in the laboratory was blood-carrying female mosquitoes (*Anopheles gambiae sensu stricto*) and midges (*Nilodorum brevibucca*), with both prey types being provided *ad libitum* every Monday, Wednesday and Friday. As it was our goal to detect *E. culicivora*'s innate preference profile, we used test spiders that had been on this standard diet in most of the prey-choice experiments. The only exception pertained to determining whether experience with a non-preferred prey might alter test-spider preference. For this, some of the test spiders in one subset

of prey-choice experiments in the 'complete series' (see below) were on an alternative rearing diet ('spider-only') in which the only food they received was oecobiid spiders. Findings from other experiments in our present study showed no evidence of test spiders on the standard diet expressing any preference for oecobiid spiders. To minimize rearing time, we only used juveniles as test spiders for the spider-only diet.

Lures

In all experiments, we used lures instead of living prey (table 3), with the body length of each lure being accurate to the nearest 0.5 mm. The mosquitoes that we used for making lures were *Anopheles gambiae sensu stricto* and *Culex quinquefasciatus* (henceforth referred to simply as *Anopheles* and *Culex*). For both mosquito species, there were three types (males, blood female and no-blood female), making a total of six mosquito types used in experiments. 'Blood females' received a blood meal 4 h before being fed to the spiders that were maintained on the standard diet or before being used for making lures. 'No-blood females' received no blood meals, but all mosquito types had unrestricted access to glucose (6% solution).

Besides the six mosquito types, we used 29 non-mosquito prey species as lures (table 3), making a total of 35 prey types. Using these lures, we carried out three series of experiments ('complete', 'mosquito' and 'non-mosquito') and, for all experiments, we adopted a simultaneous-presentation testing protocol (see [12]). In the complete series, we used nine of the non-mosquito prey species as well as the six mosquito types, with each of the nine-non-mosquito species being paired with each other and also with each mosquito type. Data from pairing most of the different mosquito types were already available from an earlier study [12] and, after presenting our new findings from the complete series, we added these earlier findings to derive a

fuller profile of *E. culicivora*'s preferences. For this, we only used the data in Nelson and Jackson [12] from simultaneous-presentation testing. However, we provide data here for two important cells (see table 2) that were missing from the earlier study (blood *Anopheles* females paired with *Culex* males and blood *Culex* females paired with *Anopheles* males).

In the mosquito series, we used blood and no-blood female *Anopheles*, with both mosquito types being paired with the 20 non-mosquito prey species that were not used in the complete series and had not been used in any of the previous studies (table 1). In the non-mosquito series, where we used six midge species and four spider species, we paired each midge species with each other midge species and paired each spider species with each other spider species. The six midge species had been paired with mosquitoes in previous experiments [11], but not with each other, and they were used as stationary lures in that study but as moving lures here.

Each individual used for making a lure had been preserved in 80% ethanol. To make lures, we positioned the prey item in lifelike posture on the centre of a cork disc (thickness 2 mm, diameter slightly more than the body length of the prey). For preservation, the prey item and the cork disc were then sprayed with a transparent plastic adhesive (Crystal Clear Lacquer, Atsco Australia Pty). Further information about making lures can be found elsewhere [1,11].

2.3. Experimental procedures

As our experimental apparatus (figure 1) and basic testing procedures were as described by Nelson and Jackson [12], only essential details will be summarized here. An arena (140 mm × 115 mm, 35 mm high) made from transparent glass sat centred on top of a plastic stand (190 mm × 165 mm, 150 mm high). There was an

‘introduction hole’ (diameter: 8 mm) in the arena floor and a matching hole in the plastic stand. A rubber bung in the introduction hole could be removed temporarily to let the test spider enter the arena. The introduction hole was situated with its closer side 10 mm from one of the narrow ends of the arena and, outside the opposite narrow end of the arena, there was a ‘left lure hole’ and a ‘right lure hole’ (diameter of each, 5 mm). A lure of one type was centred on top of the right hole and a lure of another type was centred on the top of the left hole, with the side for each lure being decided at random for every trial. The lure stayed in place because the diameter of the hole in the stand was less than the diameter of cork disc that held the prey item. With this arrangement, a test spider inside the arena could see, but not contact or smell, the lures.

A metal prong attached to a camera cable-release cord was connected to the underside of each of the two cork discs. When we pressed the cable-release, the two lures moved in unison 5 mm upward and then, when we released the cable 0.5 s later, the lures moved back to the floor. As soon as the test spider entered the arena, we began a procedure of using the cable-release device for moving the pair of lures up and down once every 30 s.

There were two circles made from thin copper wire (diameter, 36 mm), with the lure hole on the left being at the centre of one circle and with the lure hole on the right being at the centre of the other circle. Part of each wire circle extended under the arena, with this part being defined as the ‘choice area’ for the particular lure inside that circle. Owing to the glass being transparent, the choice areas were visible to the experimenter when the arena was viewed from above. A trial began after the test spider entered the arena, after which it was allowed 15 min to choose a lure by entering one of the two choice areas.

As in earlier experiments [12], one of our criteria for recording a 'choice' was that we saw the test spider enter the choice area with its gaze fixated on a lure. We used the term 'fixation' for instances in which the corneal lenses of a salticid's large forward-facing principal eyes (see [9]) were held oriented toward a lure (see [8]). There were rare occasions when the 15-min test period ended with the test spider still outside the choice area, but with its gaze fixated on a lure and, on these occasions, we extended the test period until the test spider either made a choice or turned away.

As a step toward being more confident that test spiders were actively choosing between the two lures in our experiments, we adopted another two criteria which had not been used in previous experiments (table 1). One of these criteria was that the test spider had to fixate its gaze at least once on each lure and then maintain continuous fixation on this lure for at least 10 s. The other new criterion was that, while at least 20 mm away from the nearest perimeter of the corresponding choice area, the test spider had to fixate its gaze on the lure that it chose and then maintain continuous fixation on this lure until it had walked inside that choice area.

As another prerequisite for a successful trial, we confirmed that the test spider had probably been motivated to capture prey when it chose a lure. Immediately after making its choice, the test spider was transferred to a cylindrical plastic rearing cage (diameter 55 mm, height 100 mm); 30 min later, three midges (*N. brevibucca*) were put in the cage with the spider. Whenever a test spider failed to capture one of the midges during a 60-min interval, we ignored the data from the trial in which this test spider had been used and we did not use this test spider again.

Nelson and Jackson [15] had adopted a similar, but stricter, criterion for accepting trials as successful. The day after an experimental trial using two lures or two virtual prey, a live prey trial was initiated using the same two prey types. The data

for an experimental trial were only accepted if the test spider had made the same choice when tested with living prey as it had with lures or virtual prey. The variety of prey we used in the present study meant that adopting this stricter criterion would have been prohibitively difficult.

All testing was carried out between 0800 and 1400 hours (laboratory photoperiod 12L:12D, lights on at 0700 hours) and no test spider or lure was used more than once. In addition to ambient lighting from fluorescent ceiling lamps, the apparatus was lit from 400 mm overhead by a 100-W incandescent lamp. Between trials, the apparatus was washed with 80% ethanol followed by distilled water and then dried.

In the present study, where our objective was to detect variation in preference strength across a much wider range of prey types than had been considered in the past, it was not realistic to adopt all of the experimental protocols and pre-trial fasting durations used by Nelson and Jackson [12]. As a compromise, we used only simultaneous-presentation testing, this being the testing protocol that, in the earlier study, appeared to be the most effective for detecting preferences and, as each trial was on a single day instead of two days, it was the simplest method to use. As another compromise, only two fasting durations (7-day and 1-day) were used, as these appeared to be, in the previous study [12], the most effective for discriminating between preference strengths.

For each pair of two prey types in the complete series, we first used test spiders that had fasted for 7 days and then, whenever findings were not significant after a 7-day fast, we carried out another experiment using the same pair of prey types, but this time with test spiders that had fasted for only 1 day. We used only 7-day fasting for the mosquito series, where the mosquitoes were always *Anopheles*

females. The rationale for this decision was that, based on findings from the complete series and from earlier research (table 1), we expected to find a strong preference for female *Anopheles* mosquitoes. We used only 1-day fasting in the non-mosquito series because, for this series, our objective was only to detect whether a preference was present (i.e. as our objective here did not include measuring the strengths of preferences, we used the fasting duration that was most effective at detecting even a weak preference). For each experiment, we continued testing until we had a specified number of successful trials (30 for each experiment in the complete series; 25 for each experiment in the mosquito and non-mosquito series). Data were then analyzed using chi-square tests of goodness of fit (null hypothesis: equal likelihood of choosing each prey type).

We use the expression 'strong preference' when, for a given prey pair in the complete series, significantly more test spiders chose one instead of the other after a 7-day fast. We use the expression 'weak preference' when, for a given pair in the complete series, significantly more test spiders chose one instead of the other after a 1-day fast, but not after the 7-day fast. We use the expression 'no preference' for instances in which there was no significant tendency to choose one prey type instead of the other after the 7-day or after the 1-day fast.

Wanting a sharp distinction between weak and strong preference, we decided to set alpha at 0.01, with this decision also being influenced by there being especially many prey-choice experiments in this study. However, instances of an experimental outcome being NS with alpha set at 0.01 when it would have been significant if alpha had instead been 0.05 were rare and, when it did happen, the consequences of alpha being 0.01 instead of 0.05 were only that preference strength was recorded as weak

instead of strong (i.e. it never entailed a change from preference present to nil preference).

3.0. Results

3.1. Prey records from the field

We accumulated 1,115 records of *E. culicivora* feeding on prey in the field (tables 4 and 5) and there was no striking variation related to whether the *E. culicivora* individuals were adult females, adult males or juveniles. Mosquitoes accounted for 80.2% of the 1,115 records, with midges being the second most common prey type (9.2%). After midges, the next most common prey type (5.0% of the 1,115 records) was 'other dipterans' (i.e. non-mosquito and non-midge species from the order Diptera).

Prey belonged to 11 arthropod orders (tables 4 and 5). The prey from the order Hemiptera included aphids and leafhoppers, these being insects that used to be assigned to the order Homoptera. The prey from the order Hymenoptera were two winged ants. Ranked by prevalence in the records, the total prey were Diptera (94.5%), Araneae (2.1%), Ephemeroptera (1.0%), Hemiptera (including Homoptera; 1.0%), Lepidoptera (0.4%), Mantodea (0.3%), Blattodea (0.2%), Hymenoptera (0.2%), Orthoptera (0.2%) and Psocoptera (0.2%). The majority of the 103 midges were chironomids (76.7%). About half (52.2%) of the 23 spiders in the prey records were salticids and seven of the 12 salticids were conspecific individuals (5 juveniles being eaten by adults and 2 adults being eaten by opposite-sex conspecific adults).

The majority of the 895 mosquitoes in the field records (table 5) were adult females (82.9%). Of the 895 mosquitoes, 33.1% were anophelines (genus *Anopheles*), 35.3% were culicines and 31.6% could not be identified to subfamily. Of the 316 culicines, 54.4% were *Culex* and 45.6% were *Aedes*. There was a remarkable

consistency in the percentages of mosquitoes that were females: 86.8% of 296 *Anopheles*, 84.3% of 172 *Culex*, 74.3% of 144 *Aedes* and 82.3% of the 283 mosquitoes that could not be identified to subfamily. We confirmed that 13.5% of the 742 female mosquitoes were carrying blood. The percentage of females for which blood was detected was: 11.3% of 257 *Anopheles* females, 25.5% of 145 *Culex* females, 14.0% of 107 *Aedes* females and 8.2% of 233 female mosquitoes that could not be identified to subfamily.

All dipterans were adults and all lepidopterans were larvae. The mayflies and barklice were adults. All of the aphids were probably adults, but the other hemipterans were juveniles. All mantises, cockroaches and crickets were juveniles. Oecobiid spiders were a mixture of adults and juveniles, two conspecific individuals were adults, and all other spiders were juveniles.

3.2. Choice between mosquito and non-mosquito prey

Adult female and juvenile test spiders from the complete series expressed a consistent strong preference for blood female mosquitoes (*Anopheles* and *Culex*) regardless of which of the nine non-mosquito prey species was used (tables 6 and 7). Juvenile test spiders also expressed a consistent strong preference for no-blood *Anopheles* females regardless of which of the nine alternative prey species was used. Adults differed from juveniles by expressing only a weak preference for no-blood *Anopheles* females when the alternative was a midge, but resembled juveniles by expressing a strong preference when the alternative was any other non-mosquito species.

Findings from the complete series were more complex when other mosquitoes were paired with non-mosquito prey (tables 6 and 7), where ‘other mosquitoes’ refers to no-blood *Culex* females, *Culex* males and *Anopheles* males. Adults and juveniles

did not express a preference when no-blood *Culex* females or *Culex* males were paired with midges (tables 6 and 7). However, there was a juvenile-adult difference when an *Anopheles* male was paired with a midge: adult test spiders expressed no preference for the mosquito but juveniles expressed a weak preference for the mosquito. Juveniles and adults expressed weak preferences for a no-blood *Culex* female, a *Culex* male or an *Anopheles* male when the alternative was a vinegar fly or a mayfly and they expressed strong preferences when the alternative was a fruit fly, cricket, caterpillar, aphid or oecobiid spider.

In the mosquito series (table 8), we paired an *Anopheles* blood or no-blood female mosquito with one of 15 non-mosquito prey species that had not been used in the complete series (table 7) or in any previously published experiments (table 1). For this series, we found that the number of adult and juvenile test spiders that chose the mosquito was always significantly larger than the number that chose the non-mosquito prey. As the pre-trial fast duration was always 7 days, our findings from the mosquito series corresponded to the definition that we used in the complete series for 'strong preference'.

3.3. Choice between non-mosquito species

Adult and juvenile test spiders expressed no preference when different midge species were paired with each other in the complete series (tables 6 and 7) and in the non-mosquito series (table 9). However, adult and juvenile test spiders expressed a weak preference in the complete series for midges when the alternative was a vinegar fly or mayfly and a strong preference for midges when the alternative was a fruit fly, cricket, caterpillar, aphid or oecobiid spider.

Adult and juvenile test spiders in the complete series (tables 6 and 7) expressed no preference when vinegar flies were paired with mayflies or fruit flies but they expressed a strong preference when vinegar flies were paired with crickets, caterpillars, aphids or oecobiid spiders. Adult and juvenile test spiders also expressed no preference when mayflies were paired with fruit flies, but they expressed a weak preference for mayflies when the alternative was a cricket and they expressed a strong preference for mayflies when the alternative was a caterpillar, aphid or oecobiid spider. Adult and juvenile test spiders also expressed a strong preference for fruit flies when the alternative was a caterpillar, aphid or oecobiid spider; however, adults expressed a strong preference, and juveniles only a weak preference, for fruit flies when the alternative was a cricket. When crickets, caterpillars, aphids and oecobiid spiders were paired with each other, no preferences were expressed by adult or juvenile test spiders. Moreover, in the non-mosquito series, adult and juvenile test spiders expressed no preferences when the different spider species were paired with each other (table 9).

3.4. Effect of rearing diet on prey-choice behaviour

When juvenile test spiders in the complete series were reared on a spider-only diet (table 6), their preferences were never significantly different (chi-square tests of independence) from the preferences of juvenile spiders that had been on the standard diet: blood *Anopheles* females paired with ghost midges ($\chi^2 = 1.02$, $p = 0.313$), blood *Anopheles* females paired with oecobiid spiders ($\chi^2 = 2.07$, $p = 0.150$), no-blood *Anopheles* females paired with ghost midges ($\chi^2 = 0.13$, $p = 0.718$), no-blood *Anopheles* females paired with oecobiid spiders ($\chi^2 = 2.96$, $p = 0.085$), *Anopheles* males paired with ghost midges (7-day fast: $\chi^2 = 0.27$, $p = 0.605$; 1-day fast: $\chi^2 = 0.22$,

$p = 0.640$), *Anopheles* males paired with oecobiid spiders ($\chi^2 = 1.07$, $p = 0.301$), ghost midges paired with oecobiid spiders ($\chi^2 = 0.16$, $p = 0.688$), vinegar flies paired with oecobiid spiders ($\chi^2 = 4.04$, $p = 0.044$) or caterpillars paired with oecobiid spiders (7-day fast: $\chi^2 = 0.07$, $p = 0.795$; 1-day fast: $\chi^2 = 0$, $p = 1$).

3.5. Preference indexes

Using our new data (table 7) combined with the data in Nelson and Jackson [12] from pairing mosquito types, we calculated a preference index (table 10) for each of the 15 prey types from the complete series. Our new data include blood *Anopheles* females paired with *Culex* males and blood *Culex* females paired with *Anopheles* males (table 6), these being the cells missing from Nelson and Jackson [12]. The resulting indexes ranged from 0 for prey that was never preferred to another prey and 28 for a prey that was strongly preferred to all other prey.

Irrespective of whether test spiders were juveniles or adults, and irrespective of diet (table 10), the preference indexes of crickets, caterpillars, aphids and oecobiid spiders were 0, the indexes of vinegar flies, mayflies and fruit flies were clustered at 7 and 8, and the indexes of no-blood *Culex* females, *Culex* males and midges were clustered at 12. Blood *Anopheles* females had the highest preference indexes (27 for juveniles and 28 for adults).

There were, however, distinct juvenile-adult differences when the prey was a no-blood *Anopheles* female, an *Anopheles* male or a blood *Culex* female. *Anopheles* males had a preference index of 17 when test spiders were juveniles but only 12 when test spiders were adults. No-blood *Anopheles* females had a preference index of 25 when test spiders were juveniles, but only 19 when test spiders were adults. The

preference index of blood *Culex* females was 26 when test spiders were adults, but only 22 when test spiders were juveniles.

4.0. Discussion

4.1. Evarcha culicivora's natural diet

Determining a spider's natural diet can be a daunting task, but perhaps less so when the spider lives in a web because identifiable prey can often be found in the web, with some web-building spiders wrapping their prey in silk and then leaving it in the web as a larder to feed from at a later time ([24–27]; for a cautionary note, see [28]). Salticids are less accommodating because, barring a few exceptions [29], these are predators that find, capture and eat their prey without using a web [30]. In the field, prey can be collected and identified whenever a salticid is encountered in the act of feeding, but determining natural diet in this way is a slow, laborious process.

Understandably, the sample sizes in salticid prey records may often be too small for robust conclusions about natural diet, but it can be hard to say how small is too small. However, even when we made an admittedly arbitrary decision to accept 20 clearly specified prey records as a minimum, we found only a few published records for salticids other than *E. culicivora* (table 11). The prey records for *E. culicivora* (1,115) are more than 10 times larger than records for any of these other salticid species, but records for *E. culicivora* were accumulated over a span of 19 years in the course of doing intensive research on this particular species. Moreover, this species is from a field site almost on the equator where all life-cycle stages are active and abundant year round. For most salticid species, it would seem unrealistic to expect prey records of comparable size.

From our prey records, we can safely conclude that *E. culicivora* lives up to its species' name. It certainly eats mosquitoes. It also appears to have a narrow natural

diet which suggests that, for this predator, ‘stenophagy’ is an appropriate expression. However, it might be prudent to consider what we achieve and what we imply when we characterize *E. culicivora*’s natural diet in this way.

The taxonomic resolution we adopt will influence our use of the terms ‘monophagy’, ‘stenophagy’ and ‘euryphagy’. For example, if we use phyla as our level of taxonomic resolution, then we would conclude that *E. culicivora*’s natural diet is an instance of monophagy because we would then say this spider eats a single type of prey, in this case arthropods. However, if we use orders or families as our level of taxonomic resolution, then characterizing *E. culicivora*’s natural diet as being a distinctive example of stenophagy becomes more interesting. Almost 95% of the prey in our records came from one insect order (Diptera) and 80% came from a single dipteran family, the Culicidae, these being the insects people call ‘mosquitoes’. A natural diet biased strongly toward a single insect family may be unusual for spiders or for predators in general, but this is conjecture, not simply a fact. Moreover, stenophagy is not a synonym for specialization and natural diet is conceptually distinct from preference [10].

4.2. Alignment between Evarcha culicivora’s natural diet and preferences

Although preference cannot be determined on the basis of natural diet alone, we found striking instances of alignment between the two for *E. culicivora*. In particular, female mosquitoes dominated the prey records from the field (66.5% of the 1,115 records) and, in prey-choice experiments, the highest preference indexes derived from the complete series were for female mosquitoes (tables 5 and 7). Male mosquitoes (13.7% of 1,115) and midges (9.2%) were the next most prevalent prey types in the prey

records and, in experiments, the preference indexes for these prey types were higher than the preference indexes for non-mosquito or non-midge prey (table 10).

All non-mosquito and non-midge prey types were scarce in our field records. Only 3.4% of the prey were non-dipteran insects and spiders accounted for only 2.1% of the prey records (table 5). Insects other than dipterans and other than mayflies (i.e. the next most common prey type) accounted for only 2.4% of the records. Data on prey choice from the complete series revealed no evidence of crickets, caterpillars, aphids or oecobiid spiders being preferred to any other prey. As there was also no expression of preference in the non-mosquito series when different spider species were paired with each other (table 9), we propose that *E. culicivora* expresses no preference for spiders in general. However, there were instances of preference being expressed for fruit flies and for vinegar flies, these being prey we chose as proxies for 'other dipterans' in the complete series. There were also instances in the complete series of *E. culicivora* expressing preference for mayflies. Yet mayflies and 'other dipterans' were scarce in the field records.

In our non-mosquito series of prey-choice experiments (table 9), as well as in the complete series (table 7), there was no evidence to suggest that *E. culicivora* distinguishes between different kinds of midges, but there was evidence that *E. culicivora* distinguishes between different kinds of mosquitoes. Previous research has revealed that *E. culicivora* has a preference for anophelines [12,15] when the alternatives are culicines, as well as for blood female mosquitoes when the alternatives are no-blood female mosquitoes [11–13]. However, anophelines were not markedly more common than culicines in our field prey records. Moreover, blood females were less, not more, common than no-blood female mosquitoes in our prey records (table 5).

Although there were instances of 'preference' not being in close alignment to natural diet, misalignment would not surprise us. The definition of 'preference' does not somehow demand alignment. However, the extent to which *E. culicivora*'s preferences for particular mosquitoes really are misaligned with prey eaten in the field is uncertain because we were limited in our capacity to identify prey that we removed from feeding individuals, especially when the prey was a soft-bodied mosquito that the spider had already begun to crush with its chelicerae. This meant there often were times when we could not determine whether a mosquito was an anopheline or a culicine (table 5) and, owing to the mosquito and the predator actively digesting blood that might have been present, our methods (i.e. simply pressing on the mosquito's abdomen and recording whether there were signs of liquid blood) may have seriously underestimated the numbers of female mosquitoes that had been carrying blood when captured.

4.3. Prey categorization by Evarcha culicivora

In the complete series, we used 15 prey types chosen on the basis of findings from earlier prey-choice experiments (table 1) as well as on the basis of our records of *E. culicivora*'s prey in the field. Besides giving us evidence of preferences, these data can be used for proposing which types of prey were treated by *E. culicivora* as being members of different categories. The rationale for these hypotheses is that the expression of preference is evidence that *E. culicivora* has the capacity and motivation to discriminate between the two types of prey being considered in an experiment. In all instances, we simply looked for instances of a preference, leaving aside the distinction between strong and weak preference. There was no comparable evidence of capacity and motivation when test spiders failed to choose one prey type

significantly more often than another (i.e. when we recorded experimental outcomes as instances of 'nil preference'). Moreover, whenever a preference was expressed for a member of a particular category, and whenever this member was paired with any member of any other category, the direction of preference (i.e. which of the two prey types was chosen significantly more often) was consistent in each instance. We also found no evidence of a preference whenever a member of one category was paired with another member of the same category.

Using this procedure, we identified seven categories for juveniles and six for adults (table 12), with blood *Anopheles* females, blood *Culex* females and no-blood *Anopheles* females being distinct categories for all our test spiders. For juveniles, *Anopheles* males were also a distinct category, but adults treated *Anopheles* males as members of a group that also included midges and *Culex* males. Another three categories for juveniles and adult females included more than one constituent prey type and these were the categories with the lowest preference indexes (tables 10 and 12).

The categories we determined from the complete series were remarkably coherent. Moreover, we discerned these categories regardless of whether test spiders had been on a standard diet or on a 'spider-only' rearing diet. On this basis, we can characterize these categories as being 'innate', but 'innate' does not mean 'inflexible'. Although beyond the scope of our present study, operant conditioning and other environmental shaping would be expected to influence prey categorization by *E. culicivora*. Furthermore, we have not addressed questions about the origins and adaptive significance of the way *E. culicivora* categorizes prey, as this was also beyond the scope of our study.

4.4. Evarcha culicivora's own prey-classification system

When categorizing prey, it may be tempting to rely on formal scientific taxonomy, which is appropriate when considering food webs and other topics in community ecology [46–49]. It is also common practice to use scientific taxonomy when sampling the relative availability of different kinds of prey in a predator's habitat and when comparing these samples with records of the prey actually eaten by the predator. Significant disparities, when found, indicate that the predator's natural diet is biased toward a subset of available potential prey, and 'ecological selectivity' is a convenient expression for these disparities.

Although determining ecological selectivity was not an objective in our study, we are confident that sampling for potential prey in *E. culicivora*'s habitat would reveal that midges, known locally as 'lake flies', vastly outnumber mosquitoes along the shores of Lake Victoria [50,51]. Yet it is important to emphasize that, when we conclude that *E. culicivora* has a strong preference for mosquitoes as prey, it is not on the basis of ecological selectivity. Understanding predatory specialization has been our primary goal, and an understanding of predatory specialization depends on considering the different ways in which a predator experiences and classifies its prey [1,10].

Unlike scientists, non-human predators do not rely on Latin names from formal taxonomy when they classify their prey. It is the predator's own prey-classification system, and not evidence from ecological selectivity, which highlight one of the most interesting discoveries about *E. culicivora*'s categorization of prey.

This is a predator that does a lot of classifying. The insects that scientists assign to the family Culicidae are not being experienced by this spider as a single prey category. Instead, the distinctions that matter to *E. culicivora* include whether the

mosquito is an anopheline or a culicine, whether it is a male or a female and whether it is a female that is or is not carrying blood. Moreover, the juveniles of *E. culicivora* adopt an *Anopheles*-specific prey-capture method ([52]; see also [1]) and stronger preferences have been expressed by juveniles than by adults for anophelines ([12]; table 12).

Paracyrba wanlessi is another salticid that specializes at preying on mosquitoes, but it does not classify its prey in the same way as *E. culicivora* [1]. For *P. wanlessi*, the distinctions that matter are whether a mosquito is an adult or juvenile and whether the prey is in or away from water [53], but there is no evidence of these categories being relevant to *E. culicivora*. Although *E. culicivora* and *P. wanlessi* can both be said to be salticid species that "prefer mosquitoes" as prey, this simplistic statement hides major differences in how these two predators classify their prey.

Furthermore, there is no evidence that19 other salticid species, also from East Africa, resemble *E. culicivora* by discriminating between blood meals (blood-carrying *An. gambiae s.s.* females) and non-blood meals (lake flies or *An. gambiae s.s.* males) [13]. Yet we need to be open to the logical possibility of finding pronounced ecological selectivity toward mosquitoes by predators that express no preference for mosquitoes. Many insectivorous predators, including many salticids, may experience mosquitoes not as a distinct prey category and instead as just another "bug" (see [8,54,55]). It is at least a logical possibility that some of these predators eat disproportionately more anopheline than culicine mosquitoes, female than male mosquitoes or blood-carrying than bloodless mosquitoes, without any of these being distinct categories within a prey-classification system adopted by these predators.

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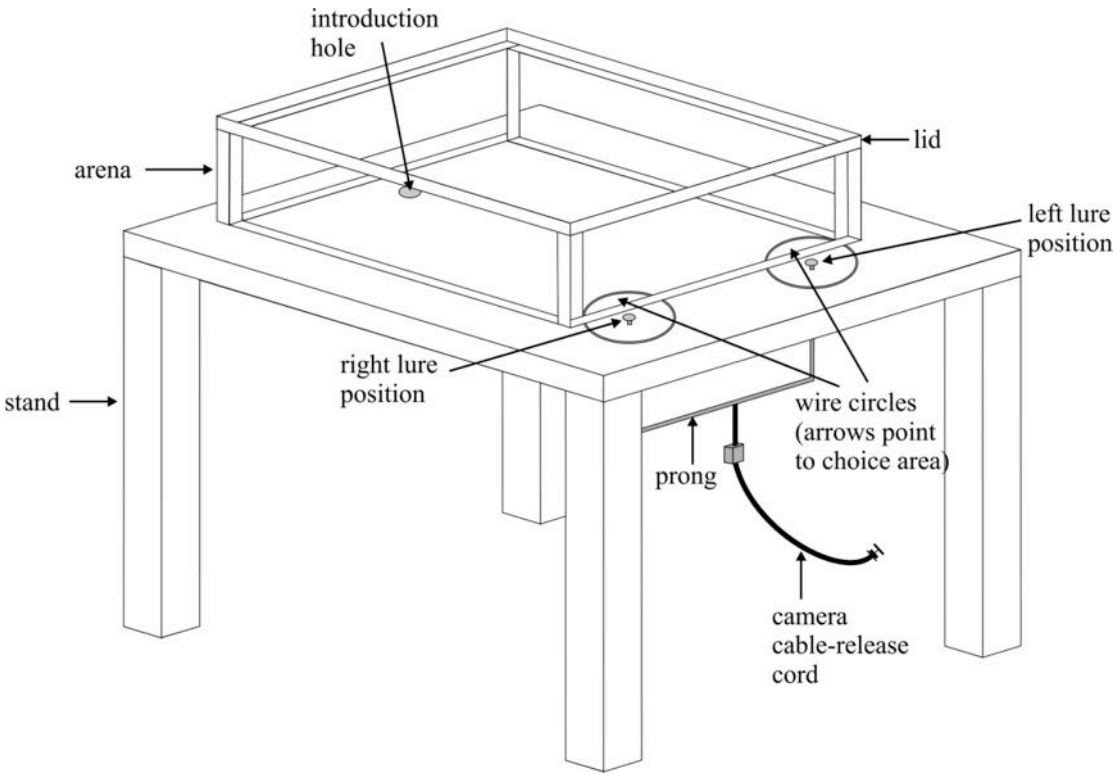

Figure 1. Prey-choice apparatus used for determining the preference profile of *Evarcha culicivora*. Rectangular glass arena with glass lid, sitting on top of Plexiglas stand. Test spider entered arena through the introduction hole. Two lures presented simultaneously, with one being in the left lure position and the other in the right lure position. Movement of lures controlled by using a camera release cord and metal prong. A wire circle surrounded each lure and extended underneath the arena, with the 'choice area' being the semicircular region within the arena.

Table 1. Summary of experiments demonstrating that *Evarcha culicivora* expresses preference for indirect blood meals. Unless otherwise stated, 'adult' test spiders were males and females. For each pair of prey, significantly more test spiders chose prey 1 (blood-carrying female mosquito) instead of prey 2 (not carrying blood). *Ae*.: *Aedes*. *An*.: *Anopheles*. *Cx*.: *Culex*.

prey 1 (blood)	prey 2 (no blood)	test spiders	stimuli	study
Ae. *aegypti*	*Ae*. *aegypti* female	adults and juveniles	stationary lures	[11]
Ae. *aegypti*	*Ae*. *aegypti* male	adults and juveniles	stationary lures	[11]
Cx. *quinquefasciatus*	*Cx*. *quinquefasciatus* female	adults and juveniles	stationary lures	[11]
Cx. *quinquefasciatus*	*Cx*. *quinquefasciatus* male	adults and juveniles	stationary lures	[11]
An. *funestus*	*An*. *funestus* male	adults and juveniles	stationary lures	[11]
An. *gambiae*	*An*. *gambiae* female	adults and juveniles	stationary lures	[11]
An. *gambiae*	*An*. *gambiae* male	adults and juveniles	stationary lures	[11]
An. *gambiae*	*An*. *gambiae* male	adult females	moving lures	[12]
An. *gambiae*	*An*. *gambiae* male	adult females	living prey	[13]
An. *gambiae*	ghost midge: *Chaoborus* sp.	adults	stationary lures	[11]
An. *gambiae*	ghost midge: *Chaoborus* sp.	adult females and juveniles	moving lures	present study
An. *gambiae*	chironomid midge: *Ablabesmyia nilotica*	adults	stationary lures	[11]
An. *gambiae*	chironomid midge: *Chironomus imicola*	adults	stationary lures	[11]
An. *gambiae*	chironomid midge: *Clinotanypus claripennis*	adults	stationary lures	[11]
An. *gambiae*	chironomid midge: *Clinotanypus claripennis*	adult females and juveniles	moving lures	[12]
An. *gambiae*	chironomid midge: *Clinotanypus claripennis*	adult females	living prey	[13]
An. *gambiae*	chironomid midge: *Conochironomus acutistus*	adults	stationary lures	[11]
An. *gambiae*	chironomid midge: *Nilodorum brevibucca*	adults and juveniles	stationary lures	[11]
An. *gambiae*	chironomid midge: *Nilodorum brevibucca*	adult females and juveniles	moving lures	present study
An. *gambiae*	aphid: *Brevicoryne brassicae*	adults and juveniles	stationary lures	[11]
An. *gambiae*	aphid: *Brevicoryne brassicae*	adult females and juveniles	moving lures	present study
An. *gambiae*	caterpillar *Chilo parttelus*	adults	stationary lures	[11]
An. *gambiae*	caterpillar: *Chilo parttelus*	adult females and juveniles	moving lures	present study
An. *gambiae*	fruit fly: *Ceratitis capitata*	adults	stationary lures	[11]

An. gambiae	fruit fly: *Ceratitis capitata*	adult females and juveniles	moving lures	present study
An. gambiae	nephilid spider: *Nephilengys*	adults and juveniles	stationary lures	[11]
An. gambiae	nephilid spider: *Nephilengys*	adult females and juveniles	moving lures	present study
An. gambiae	oecobiid spider:*Oecobius amboseli*	adults	stationary lures	[11]
An. gambiae	oecobiid spider: *Oecobius amboseli*	adult females and juveniles	moving lures	present study
An. gambiae	assassin bug: *Nagusta*	adult females and juveniles	moving lures	present study
An. gambiae	barklouse; *Ectopsocus californicus*	adult females and juveniles	moving lures	present study
An. gambiae	brown rice hopper: *Nilaparvuta lugens*	adult females and juveniles	moving lures	present study
An. gambiae	cricket	adult females and juveniles	moving lures	present study
An. gambiae	cockroach	adult females and juveniles	moving lures	present study
An. gambiae	green leaf hopper: *Nephotettix nigropictus*	adult females and juveniles	moving lures	present study
An. gambiae	house fly: *Musca domestica*	adult females and juveniles	moving lures	present study
An. gambiae	mantis	adult females and juveniles	moving lures	present study
An. gambiae	mayfly	adult females and juveniles	moving lures	present study
An. gambiae	moth fly	adult females and juveniles	moving lures	present study
An. gambiae	vinegar fly: *Drosophila melanogaster*	adult females and juveniles	moving lures	present study
An. gambiae	whitefly	adult females and juveniles	moving lures	present study
An. gambiae	clubionid spider: *Clubiona*	adult females and juveniles	moving lures	present study
An. gambiae	hersiliid spider: *Hersilia caudata* Audouin	adult females and juveniles	moving lures	present study
An. gambiae	jumping spider: *Natta horizontalis*	adult females and juveniles	moving lures	present study
An. gambiae	wolf spider: *Pardosa messingerae*	adult females and juveniles	moving lures	present study

Table 2. Preference strengths of *Evarcha culicivora* with respect to specific pairs of prey. Determined from data of Nelson and Jackson [12]. Mosquitoes used: *Anopheles gambiae s.s*. and *Culex quinquefasciatus* (shortened to *Anopheles* and *Culex*). Female (f) and male (m) mosquitoes used. Midge (Chironomidae): *Clinotanypus claripenni*. Strong preference for prey 1: significantly more test spiders chose prey 1 than chose prey 2 after a 14-day pre-trial fast. Medium preference for prey 1: significantly more test spiders chose prey 1 than chose prey 2 after a 7-day fast, but not after longer fast. Weak preference for prey 1: no significant choice after longer fasts, but significantly more test spiders chose prey 1 than chose prey 2 after a 1-day fast. One exception, indicated by minus sign in front of w: after 1-day fast, significantly more test spiders chose prey 2 than chose prey 1. Nil preference: no significant choice detected after 1-day, 7-day or 14-day fasts.

		adult test spider			juvenile test spider		
prey 1	prey 2	simultaneous presentation	alternate day	alternate prey	simultaneous presentation	alternate day	alternate prey
blood *Anopheles*	blood *Culex* (f)	weak[1]	weak	nil	medium[1]	weak	nil
(f)	no-blood *Anopheles* (f)	strong	medium	weak	medium	medium	nil
	no-blood *Culex* (f)	strong	medium	weak	medium	medium	nil
	Anopheles (m)	strong	medium	weak	medium	medium	nil
	Culex (m)	-	-	-	-	-	-
	midge	strong	medium	nil	medium	medium	nil
blood *Culex*	no-blood *Anopheles* (f)	strong	medium	weak	-weak[2]	nil	nil
(f)	no-blood *Culex* (f)	strong	medium	weak	medium	weak	nil
	Anopheles (m)	-	-	-	-	-	-
	Culex (m)	strong	medium	weak	medium	weak	nil
	midge	strong	medium	weak	medium	weak	nil
no-blood *Anopheles*	no-blood *Culex* (f)	weak[3]	weak	nil	medium[3]	medium	nil
(f)	*Anopheles* (m)	weak	weak	nil	medium	weak	nil
	Culex (m)	weak	weak	nil	medium	weak	nil
	midge	weak[3]	weak	nil	medium[3]	weak	nil
no-blood *Culex*	*Anopheles* (m)	nil	nil	nil	nil	nil	nil
(f)	*Culex* (m)	nil	nil	nil	nil	nil	nil

	midge	nil	nil	nil	nil	nil	nil
Anopheles (m)	*Culex* (m)	nil	nil	nil	medium	weak	nil
	midge	nil	nil	nil	weak	nil	nil
Culex (m)	midge	nil	nil	nil	nil	nil	nil

[1]corresponds to preference found by Nelson and Jackson [15] when using lures and when using virtual prey
[2]in this instance, preference for prey 2 (i.e., juvenile's preference for *Anopheles* over-rides preference for blood)
[3]corresponds to preference found by Nelson and Jackson [15] when using lures

Table 3. Arthropods used as lures in present study.

common name	genus, species	family	order	body length
assassin bug[2,4]	*Nagusta*	Reduviidae	Hemiptera	4.0 mm
aphid[2,3]	*Brevicoryne brassicae*	Aphidae	Homoptera	3.0 mm
barklouse[2,4]	unidentified	unidentified	Psocoptera	3.0 mm
brown rice hopper[1,4]	*Nilaparvuta lugens*	Delphacidae	Homoptera	3.0 mm
caterpillar[1,3]	*Chilo parttelus*	Crambidae	Lepidoptera	4.5 mm
chironomid midge[2,5]	*Ablabesmyia nilotica*	Chironomidae	Diptera	4.0 mm
chironomid midge[2,5]	*Chironomus imicola*	Chironomidae	Diptera	5.0 mm
chironomid midge[2,5]	*Clinotanypus claripennis*	Chironomidae	Diptera	5.0 mm
chironomid midge[2,5]	*Conochironomus acutistus*	Chironomidae	Diptera	5.0 mm
chironomid midge[2,3,5]	*Nilodorum brevibucca*	Chironomidae	Diptera	4.5 mm
clubionid spider[2,4]	*Clubiona*	Culbionidae	Araneae	4.0 mm
cockroach[2,4]	unidentified	Blatellidae	Blattodea	4.0 mm
cricket[1,3]	*Acheta domesticus*	Gryllidae	Orthoptera	4.0 mm
fruit fly[1,3]	*Ceratitis capitata*	Tephritidae	Diptera	4.5 mm
ghost midge[2,3,5]	*Chaoborus* sp.	Chaoboridae	Diptera	4.5 mm
green leaf hopper[1,4]	*Nephotettix nigropictus*	Cicadellidae	Homoptera	4.0 mm
hersiliid spider[2,4]	*Hersilia caudata*	Hersiliidae	Araneae	3.0 mm
house fly[1,4]	*Musca domestica*	Muscidae	Diptera	6.0 mm
jumping spider[2,4,5]	*Natta horizontalis*	Salticidae	Araneae	3.0 mm
long-legged fly[2,4]	unidentified	Dolichopodidae	Diptera	5.0 mm
mantis[2,4]	unidentified	Mantidae	Mantodea	4.5 mm
mayfly[2,3]	unidentified	Baetidae	Ephemeroptera	4.5 mm
moth fly[2,4]	unidentified	Psychodidae	Diptera	3.0 mm
mosquito[1,3,4]	*Anopheles gambiae s.s.*	Culicidae	Diptera	4.5 mm
mosquito[1,3]	*Culex quinquefasciatus*	Culicidae	Diptera	4.5 mm
nephilid spider[1,4,5]	*Nephilengys*	Nephilididae	Araneae	4.0 mm

oecobiid spider[2,3,5]	*Oecobius amboseli*	Oecobidae	Araneae	3.0 mm
vinegar fly[1,3]	*Drosophila melanogaster*	Drosophilidae	Diptera	3.0 mm
whitefly[2,4]	unidentified	Aleyrodidae	Homoptera	2.0 mm
wolf spider[2,4,5]	*Pardosa messingerae*	Lycosidae	Araneae	3.0 mm

[1]from stock cultures (see [11,23])
[2]collected as needed from Mbita Point field site
[3]used in complete series
[4]used in mosquito series
[5]used in non-mosquito series

Table 4. Records of prey on which adult females, adult males and juveniles of *Evarcha culicivora* were found feeding in the field. Each prey type listed, when possible, by common name, genus, species, order and family. Sex of mosquitoes and whether female mosquitoes were carrying blood indicated. Blood female: there was evidence that these mosquitoes were carrying blood. No-blood female: there was no evidence that these mosquitoes were carrying blood.

order	description	genus, species	family	records for adult females[1]	records for adult males[2]	records for juveniles[3]	all records[4]
Diptera	blood female anopheline mosquito	*Anopheles*	Culicidae	12 (2.7%)	9 (2.6%)	8 (2.4%)	29 (2.6%)
Diptera	blood female culicine mosquito	*Culex*	Culicidae	19 (4.3%)	13 (3.8%)	5 (1.5%)	37 (3.3%)
Diptera	blood female culicine mosquito	*Aedes*	Culicidae	6 (1.3%)	5 (1.5%)	4 (1.2%)	15 (1.3%)
Diptera	unidentified blood female mosquito	unidentified	Culicidae	4 (0.9%)	7 (2.0%)	8 (2.4%)	19 (1.7%)
Diptera	no-blood female anopheline mosquito	*Anopheles*	Culicidae	91 (20.4%)	61 (17.8%)	76 (23.2%)	228 (20.4%)
Diptera	no-blood female culicine mosquito	*Culex*	Culicidae	49 (11.0%)	31 (9.1%)	28 (8.5%)	108 (9.7%)
Diptera	no-blood female culicine mosquito	*Aedes*	Culicidae	46 (10.3%)	17 (5.0%)	29 (8.8%)	92 (8.3%)
Diptera	unidentified no-blood female mosquito	unidentified	Culicidae	78 (17.5%)	68 (19.9%)	68 (20.7%)	214 (19.2%)
Diptera	male anopheline mosquito	*Anopheles*	Culicidae	22 (4.9%)	9 (2.6%)	8 (2.4%)	39 (3.5%)
Diptera	male culicine mosquito	*Culex*	Culicidae	11 (2.5%)	4 (1.2%)	12 (3.7%)	27 (2.4%)
Diptera	male culicine mosquito	*Aedes*	Culicidae	10 (2.2%)	17 (5.0%)	10 (3.0%)	37 (3.3%)
Diptera	unidentified male mosquito	unidentified	Culicidae	23 (5.2%)	14 (4.1%)	13 (4.0%)	50 (4.5%)
Diptera	ghost midge	*Chaoborus*	Chaoboridae	13 (2.9%)	8 (2.3%)	3 (0.9%)	24 (2.2%)
Diptera	chironomid midge	unidentified	Chironomidae	22 (4.9%)	33 (9.6%)	24 (7.3%)	79 (7.1%)
Diptera	moth fly	unidentified	Psychodidae	3 (0.7%)	4 (1.2%)	5 (1.5%)	12 (1.1%)
Diptera	long-legged fly	unidentified	Dolichopodidae	2 (0.4%)	1 (0.3%)	1 (0.3%)	4 (0.4%)
Diptera	unidentified fly	unidentified	unidentified	8 (1.8%)	22 (6.4%)	10 (3.0%)	40 (3.6%)
Araneae	conspecific juvenile	*Evarcha culicivora*	Salticidae	2 (0.4%)	3 (0.9%)	0	5 (0.4%)
Araneae	opposite-sex conspecific adult	*Evarcha culicivora*	Salticidae	1 (0.2%)	1 (0.3%)	0	2 (0.2%)
Araneae	jumping spider	*Natta*	Salticidae	1 (0.2%)	0	1 (0.3%)	2 (0.2%)
Araneae	unidentified jumping spider	unidentified	Salticidae	2 (0.4%)	1 (0.3%)	0	3 (0.3%)

Araneae	oecobiid spider	*Oecobius amboseli*	Oecobiidae	0	1 (0.3%)	3 (0.9%)	4 (0.4%)
Araneae	wolf spider	unidentified	Lycosidae	1 (0.2%)	2 (0.6%)	1 (0.3%)	4 (0.4%)
Araneae	unidentified non-salticid spider	unidentified	unidentified	1 (0.2%)	1 (0.3%)	1 (0.3%)	3 (0.3%)
Ephemeroptera	mayfly	unidentified	Baetidae	5 (1.1%)	4 (1.2%)	2 (0.6%)	11 (1.0%)
Hemiptera	leafhopper	unidentified	Cicadellidae	2 (0.4%)	1 (0.3%)	1 (0.3%)	4 (0.4%)
Hemiptera	big-eyed bug	*Geocoris*	Geocoridae	1 (0.2%)	0	1 (0.3%)	2 (0.2%)
Hemiptera	mirid bug	unidentified	Miridae	1 (0.2%)	0	1 (0.3%)	2 (0.2%)
Hemiptera	aphid	unidentified	Aphidae	2 (0.4%)	1 (0.3%)	0	3 (0.3%)
Lepidoptera	caterpillar	unidentified	unidentified	1 (0.2%)	0	4 (1.2%)	5 (0.4%)
Mantodea	mantis	unidentified	Mantidae	1 (0.2%)	2 (0.6%)	0	3 (0.3%)
Blattodea	cockroach	unidentified	Blatellidae	1 (0.2%)	0	1 (0.3%)	2 (0.2%)
Hymenoptera	winged ant	unidentified	Formicidae	1 (0.2%)	1 (0.3%)	0	2 (0.2%)
Orthoptera	cricket	unidentified	Gryllidae	2 (0.4%)	0	0	2 (0.2%)
Psocoptera	barklouse	unidentified	unidentified	1 (0.2%)	1 (0.3%)	0	2 (0.2%)

[1]Total 445
[2]Total 342
[3]Total 328
[4]Total 1,115

Table 5. Analysis of field records (see table 4) of prey on which *Evarcha culicivora* was found feeding.

prey	records for adult females	records for adult males	records for juveniles	total for all *E. culicivora*
mosquitoes	371 (83.4%)	255 (74.6%)	269 (82.0%)	895 (80.3%)
female mosquito	305 (68.5%)	211 (61.7%)	226 (68.9%)	742 (66.5%)
blood female mosquito	41 (9.2%)	34 (9.9%)	25 (7.6%)	100 (9.0%)
no-blood female mosquito	264 (59.3%)	177 (51.8%)	201 (61.3%)	642 (57.6%)
male mosquitoes	66 (14.8%)	44 (12.9%)	43 (13.1 %)	153 (13.7%)
Anopheles	125 (28.1%)	79 (23.1%)	92 (28.0%)	296 (26.5%)
Culex	79 (17.8%)	48 (14.0%)	45 (13.7%)	172 (15.4%)
Aedes	62 (13.9%)	39 (11.4%)	43 (13.1%)	144 (12.9%)
culicine mosquitoes	141 (31.7%)	87 (25.4%)	88 (26.8%)	316 (28.3%)
unidentified mosquitoes	105 (23.6%)	89 (26.0%)	89 (27.1%)	283 (25.4%)
Anopheles females	103 (23.1%)	70 (20.5%)	84 (25.6%)	257 (23.0%)
Culex females	68 (15.3%)	44 (12.9%)	33 (10.1%)	145 (13.0%)
Aedes females	52 (11.7%)	22 (6.4%)	33 (10.1%)	107 (9.6%)
culicine females	120 (27.0%)	66 (19.3%)	66 (20.1%)	252 (22.6%)
unidentified female mosquitoes	82 (18.4%)	75 (21.9%)	76 (23.2%)	233 (20.9%)
midges	35 (7.9%)	41 (12.0%)	27 (8.2%)	103 (9.2%)
non-mosquito dipterans	48 (10.8%)	68 (19.9%)	43 (13.1%)	159 (14.3%)
Diptera	419 (94.2%)	323 (94.4%)	312 (95.1%)	1054 (94.5%)
non-mosquito and non-midge Diptera	13 (2.9%)	27 (7.9%)	16 (4.9%)	56 (5.0%)
non-mosquito and non-midge Diptera + mayflies	18 (4.0%)	31 (9.1/%)	18 (5.5%)	67 (6.0%)
insects	437 (98.2%)	333 (97.4%)	322 (98.2%)	1092 (97.9%)
non-dipteran insects	18 (4.0%)	10 (2.9%)	10 (3.0%)	38 (3.4%)
non-dipteran insects and non-mayfly insects	13 (2.9%)	6 (1.8%)	8 (2.4%)	27 (2.4%)
spiders	8 (1.8%)	9 (2.6%)	6 (1.8%)	23 (2.1%)
non-mosquito and non-midge prey	39 (8.8%)	46 (13.5%)	32 (9.8%)	117 (10.5%)
total number of records	445 (39.9%)	342 (30.7%)	328 (29.4%)	1,115

Table 6. Findings for adult females and juveniles of *Evarcha culicivora* ('test spiders') in the complete series of prey-choice experiments (see text). For each experiment (row), simultaneous presentation testing was used. $n = 30$ test spiders for each row. See table 3 for details pertaining to prey and text for methods. Chironomid midge: *Nilodorum brevibucca*. Experiments with 1-day pre-trial fasts carried out only when findings were NS after 7-day fast. Data analysis: test of goodness of fit (null hypothesis: as likely to choose prey 2 as to choose prey 1).

prey 1	prey 2	diet	fast	adult female test spiders		juvenile test spiders	
				chose prey 1	test of goodness of fit	chose prey 1	test of goodness of fit
blood *Anopheles* female	*Culex* male	standard	7-day	30	χ^{2}= 30.00, p<0.001	28	χ^{2}= 22.53, p<0.001
blood *Anopheles* female	ghost midge	standard	7-day	28	χ^{2}= 22.53, p<0.001	29	χ^{2}= 26.13, p<0.001
blood *Anopheles* female	ghost midge	spider-only	7-day	-	-	30	χ^{2}= 30.00, p<0.001
blood *Anopheles* female	chironomid midge	standard	7-day	24	χ^{2}= 10.80, p = 0.001	27	χ^{2}= 19.20, p<0.001
blood *Anopheles* female	vinegar fly	standard	7-day	29	χ^{2}= 26.13, p<0.001	24	χ^{2}= 10.80, p = 0.001
blood *Anopheles* female	mayfly	standard	7-day	25	χ^{2}= 13.33, p<0.001	27	χ^{2}= 19.20, p<0.001
blood *Anopheles* female	fruit fly	standard	7-day	28	χ^{2}= 22.53, p<0.001	29	χ^{2}= 26.13, p<0.001
blood *Anopheles* female	cricket	standard	7-day	27	χ^{2}= 19.20, p<0.001	30	χ^{2}= 30.00, p<0.001
blood *Anopheles* female	caterpillar	standard	7-day	25	χ^{2}= 13.33, p<0.001	28	χ^{2}= 22.53, p<0.001
blood *Anopheles* female	aphid	standard	7-day	27	χ^{2}= 19.20, p<0.001	27	χ^{2}= 19.20, p<0.001
blood *Anopheles* female	oecobiid spider	standard	7-day	29	χ^{2}= 26.13, p<0.001	28	χ^{2}= 22.53, p<0.001
blood *Anopheles* female	oecobiid spider	spider-only	7-day	-	-	30	χ^{2}= 30.00, p<0.001
blood *Culex* female	*Anopheles* male	standard	7-day	25	χ^{2}= 13.33, p<0.001	12	χ^{2}= 1.20, p = 0.273
blood *Culex* female	*Anopheles* male	standard	1-day	-	-	7	χ^{2}= 8.53[1], p = 0.003
blood *Culex* female	ghost midge	standard	7-day	29	χ^{2}= 26.13, p<0.001	30	χ^{2}= 30.00, p<0.001
blood *Culex* female	chironomid midge	standard	7-day	26	χ^{2}= 16.13, p<0.001	30	χ^{2}= 30.00, p<0.001
blood *Culex* female	vinegar fly	standard	7-day	27	χ^{2}= 19.20, p<0.001	28	χ^{2}= 22.53, p<0.001
blood *Culex* female	mayfly	standard	7-day	30	χ^{2}= 30.00, p<0.001	29	χ^{2}= 26.13, p<0.001
blood *Culex* female	fruit fly	standard	7-day	27	χ^{2}= 19.20, p<0.001	30	χ^{2}= 30.00, p<0.001
blood *Culex* female	cricket	standard	7-day	30	χ^{2}= 30.00, p<0.001	26	χ^{2}= 16.13, p<0.001
blood *Culex* female	caterpillar	standard	7-day	25	χ^{2}= 13.33, p<0.001	26	χ^{2}= 16.13, p<0.001
blood *Culex* female	aphid	standard	7-day	30	χ^{2}= 30.00, p<0.001	30	χ^{2}= 30.00, p<0.001

blood *Culex* female	oecobiid spider	standard	7-day	28	$\chi^2 = 22.53$, $p<0.001$	25	$\chi^2 = 13.33$, $p<0.001$
no-blood *Anopheles* female	ghost midge	standard	7-day	21	$\chi^2 = 4.80$, $p = 0.028$	25	$\chi^2 = 13.33$, $p<0.001$
no-blood *Anopheles* female	ghost midge	standard	1-day	27	$\chi^2 = 19.20$, $p<0.001$	-	-
no-blood *Anopheles* female	ghost midge	spider-only	7-day	-	-	26	$\chi^2 = 16.13$, $p<0.001$
no-blood *Anopheles* female	chironomid midge	standard	7-day	17	$\chi^2 = 0.53$, $p = 0.465$	24	$\chi^2 = 10.80$, $p = 0.001$
no-blood *Anopheles* female	chironomid midge	standard	1-day	26	$\chi^2 = 16.13$, $p<0.001$	-	-
no-blood *Anopheles* female	vinegar fly	standard	7-day	25	$\chi^2 = 13.33$, $p<0.001$	28	$\chi^2 = 22.53$, $p<0.001$
no-blood *Anopheles* female	mayfly	standard	7-day	26	$\chi^2 = 16.13$, $p<0.001$	26	$\chi^2 = 16.13$, $p<0.001$
no-blood *Anopheles* female	fruit fly	standard	7-day	26	$\chi^2 = 16.13$, $p<0.001$	29	$\chi^2 = 26.13$, $p<0.001$
no-blood *Anopheles* female	cricket	standard	7-day	30	$\chi^2 = 30.00$, $p<0.001$	27	$\chi^2 = 19.20$, $p<0.001$
no-blood *Anopheles* female	caterpillar	standard	7-day	30	$\chi^2 = 30.00$, $p<0.001$	26	$\chi^2 = 16.13$, $p<0.001$
no-blood *Anopheles* female	aphid	standard	7-day	27	$\chi^2 = 19.20$, $p<0.001$	28	$\chi^2 = 22.53$, $p<0.001$
no-blood *Anopheles* female	oecobiid spider	standard	7-day	25	$\chi^2 = 13.33$, $p<0.001$	29	$\chi^2 = 26.13$, $p<0.001$
no-blood *Anopheles* female	oecobiid spider	spider-only	7-day	-	-	25	$\chi^2 = 13.33$, $p<0.001$
no-blood *Culex* female	ghost midge	standard	7-day	15	$\chi^2 = 0.00$, $p = 1$	16	$\chi^2 = 0.13$, $p = 0.715$
no-blood *Culex* female	ghost midge	standard	1-day	17	$\chi^2 = 0.53$, $p = 0.465$	14	$\chi^2 = 0.13$, $p = 0.715$
no-blood *Culex* female	chironomid midge	standard	7-day	14	$\chi^2 = 0.13$, $p = 0.715$	15	$\chi^2 = 0.00$, $p = 1$
no-blood *Culex* female	chironomid midge	standard	1-day	12	$\chi^2 = 1.20$, $p = 0.273$	15	$\chi^2 = 0.00$, $p = 1$
no-blood *Culex* female	vinegar fly	standard	7-day	15	$\chi^2 = 0.00$, $p = 1$	16	$\chi^2 = 0.13$, $p = 0.715$
no-blood *Culex* female	vinegar fly	standard	1-day	26	$\chi^2 = 16.13$, $p<0.001$	29	$\chi^2 = 26.13$, $p<0.001$
no-blood *Culex* female	mayfly	standard	7-day	22	$\chi^2 = 6.53$, $p = 0.011$	12	$\chi^2 = 1.20$, $p = 0.273$
no-blood *Culex* female	mayfly	standard	1-day	25	$\chi^2 = 13.33$, $p<0.001$	28	$\chi^2 = 22.53$, $p<0.001$
no-blood *Culex* female	fruit fly	standard	7-day	30	$\chi^2 = 30.00$, $p<0.001$	29	$\chi^2 = 26.13$, $p<0.001$
no-blood *Culex* female	cricket	standard	7-day	25	$\chi^2 = 13.33$, $p<0.001$	24	$\chi^2 = 10.80$, $p = 0.001$
no-blood *Culex* female	caterpillar	standard	7-day	30	$\chi^2 = 30.00$, $p<0.001$	25	$\chi^2 = 13.33$, $p<0.001$
no-blood *Culex* female	aphid	standard	7-day	24	$\chi^2 = 10.80$, $p = 0.001$	28	$\chi^2 = 22.53$, $p<0.001$
no-blood *Culex* female	oecobiid spider	standard	7-day	30	$\chi^2 = 30.00$, $p<0.001$	30	$\chi^2 = 30.00$, $p<0.001$
Anopheles male	ghost midge	standard	7-day	19	$\chi^2 = 2.13$, $p = 0.144$	15	$\chi^2 = 0.00$, $p = 1$
Anopheles male	ghost midge	standard	1-day	10	$\chi^2 = 3.33$, $p = 0.068$	27	$\chi^2 = 19.20$, $p<0.001$
Anopheles male	ghost midge	spider-only	7-day	-	-	13	$\chi^2 = 0.53$, $p = 0.465$

Anopheles male	ghost midge	spider-only	1-day	-	-	28	$\chi^2 = 22.53$, $p<0.001$
Anopheles male	chironomid midge	standard	7-day	13	$\chi^2 = 0.53$, $p = 0.465$	21	$\chi^2 = 4.80$, $p = 0.028$
Anopheles male	chironomid midge	standard	1-day	11	$\chi^2 = 2.13$, $p = 0.144$	29	$\chi^2 = 26.13$, $p<0.001$
Anopheles male	vinegar fly	standard	7-day	14	$\chi^2 = 0.13$, $p = 0.715$	16	$\chi^2 = 0.13$, $p = 0.715$
Anopheles male	vinegar fly	standard	1-day	24	$\chi^2 = 10.80$, $p = 0.001$	26	$\chi^2 = 16.13$, $p<0.001$
Anopheles male	mayfly	standard	7-day	17	$\chi^2 = 0.53$, $p = 0.465$	15	$\chi^2 = 0.00$, $p = 1$
Anopheles male	mayfly	standard	1-day	28	$\chi^2 = 22.53$, $p<0.001$	28	$\chi^2 = 22.53$, $p<0.001$
Anopheles male	fruit fly	standard	7-day	23	$\chi^2 = 8.53$, $p = 0.003$	27	$\chi^2 = 19.20$, $p<0.001$
Anopheles male	cricket	standard	7-day	26	$\chi^2 = 16.13$, $p<0.001$	26	$\chi^2 = 16.13$, $p<0.001$
Anopheles male	caterpillar	standard	7-day	25	$\chi^2 = 13.33$, $p<0.001$	28	$\chi^2 = 22.53$, $p<0.001$
Anopheles male	aphid	standard	7-day	30	$\chi^2 = 30.00$, $p<0.001$	25	$\chi^2 = 13.33$, $p<0.001$
Anopheles male	oecobiid spider	standard	7-day	25	$\chi^2 = 13.33$, $p<0.001$	29	$\chi^2 = 26.13$, $p<0.001$
Anopheles male	oecobiid spider	spider-only	7-day	-	-	27	$\chi^2 = 19.20$, $p<0.001$
Culex male	ghost midge	standard	7-day	20	$\chi^2 = 3.33$, $p = 0.068$	15	$\chi^2 = 0.00$, $p = 1$
Culex male	ghost midge	standard	1-day	12	$\chi^2 = 1.20$, $p = 0.273$	15	$\chi^2 = 0.00$, $p = 1$
Culex male	chironomid midge	standard	7-day	15	$\chi^2 = 0.00$, $p = 1$	11	$\chi^2 = 2.13$, $p = 0.144$
Culex male	chironomid midge	standard	1-day	16	$\chi^2 = 0.13$, $p = 0.715$	18	$\chi^2 = 1.20$, $p = 0.273$
Culex male	vinegar fly	standard	7-day	15	$\chi^2 = 0.00$, $p = 1$	22	$\chi^2 = 6.53$, $p = 0.011$
Culex male	vinegar fly	standard	1-day	24	$\chi^2 = 10.80$, $p = 0.001$	15	$\chi^2 = 0.00$, $p = 1$
Culex male	mayfly	standard	7-day	14	$\chi^2 = 0.13$, $p = 0.715$	21	$\chi^2 = 4.80$, $p = 0.028$
Culex male	mayfly	standard	1-day	28	$\chi^2 = 22.53$, $p<0.001$	24	$\chi^2 = 10.80$, $p = 0.001$
Culex male	fruit fly	standard	7-day	24	$\chi^2 = 10.80$, $p = 0.001$	29	$\chi^2 = 26.13$, $p<0.001$
Culex male	cricket	standard	7 day	27	$\chi^2 = 19.20$, $p<0.001$	24	$\chi^2 = 10.80$, $p = 0.001$
Culex male	caterpillar	standard	7-day	29	$\chi^2 = 26.13$, $p<0.001$	28	$\chi^2 = 22.53$, $p<0.001$
Culex male	aphid	standard	7-day	24	$\chi^2 = 10.80$, $p = 0.001$	25	$\chi^2 = 13.33$, $p<0.001$
Culex male	oecobiid spider	standard	7-day	30	$\chi^2 = 30.00$, $p<0.001$	24	$\chi^2 = 10.80$, $p = 0.001$
ghost midge	chironomid midge	standard	7-day	16	$\chi^2 = 0.13$, $p = 0.715$	16	$\chi^2 = 0.13$, $p = 0.715$
ghost midge	chironomid midge	standard	1 day	12	$\chi^2 = 1.20$, $p = 0.273$	26	$\chi^2 = 16.13$, $p<0.001$
ghost midge	vinegar fly	standard	7-day	12	$\chi^2 = 1.20$, $p = 0.273$	16	$\chi^2 = 0.13$, $p = 0.715$
ghost midge	vinegar fly	standard	1-day	24	$\chi^2 = 10.80$, $p = 0.001$	27	$\chi^2 = 19.20$, $p<0.001$

ghost midge	mayfly	standard	7-day	14	$\chi^2 = 0.13$, $p = 0.715$	12	$\chi^2 = 1.20$, $p = 0.273$
ghost midge	mayfly	standard	1-day	29	$\chi^2 = 26.13$, $p<0.001$	29	$\chi^2 = 26.13$, $p<0.001$
ghost midge	fruit fly	standard	7-day	23	$\chi^2 = 8.53$, $p = 0.003$	27	$\chi^2 = 19.20$, $p<0.001$
ghost midge	cricket	standard	7 day	28	$\chi^2 = 22.53$, $p<0.001$	26	$\chi^2 = 16.13$, $p<0.001$
ghost midge	caterpillar	standard	7 day	27	$\chi^2 = 19.20$, $p<0.001$	27	$\chi^2 = 19.20$, $p<0.001$
ghost midge	aphid	standard	7 day	30	$\chi^2 = 30.00$, $p<0.001$	29	$\chi^2 = 26.13$, $p<0.001$
ghost midge	oecobiid spider	standard	7-day	28	$\chi^2 = 22.53$, $p<0.001$	26	$\chi^2 = 16.13$, $p<0.001$
ghost midge	oecobiid spider	spider-only	7-day	-	-	27	$\chi^2 = 19.20$, $p<0.001$
chironomid midge	vinegar fly	standard	7-day	13	$\chi^2 = 0.53$, $p = 0.465$	13	$\chi^2 = 0.53$, $p = 0.465$
chironomid midge	vinegar fly	standard	1-day	23	$\chi^2 = 8.53$, $p = 0.003$	27	$\chi^2 = 19.20$, $p<0.001$
chironomid midge	mayfly	standard	7-day	15	$\chi^2 = 0.00$, $p = 1$	12	$\chi^2 = 1.20$, $p = 0.273$
chironomid midge	mayfly	standard	1-day	25	$\chi^2 = 13.33$, $p<0.001$	26	$\chi^2 = 16.13$, $p<0.001$
chironomid midge	fruit fly	standard	7-day	27	$\chi^2 = 19.20$, $p<0.001$	27	$\chi^2 = 19.20$, $p<0.001$
chironomid midge	cricket	standard	7-day	26	$\chi^2 = 16.13$, $p<0.001$	29	$\chi^2 = 26.13$, $p<0.001$
chironomid midge	caterpillar	standard	7-day	28	$\chi^2 = 22.53$, $p<0.001$	30	$\chi^2 = 30.00$, $p<0.001$
chironomid midge	aphid	standard	7-day	29	$\chi^2 = 26.13$, $p<0.001$	26	$\chi^2 = 16.13$, $p<0.001$
chironomid midge	oecobiid spider	standard	7-day	27	$\chi^2 = 19.20$, $p<0.001$	28	$\chi^2 = 22.53$, $p<0.001$
vinegar fly	mayfly	standard	7-day	12	$\chi^2 = 1.20$, $p = 0.273$	16	$\chi^2 = 0.13$, $p = 0.715$
vinegar fly	mayfly	standard	1-day	13	$\chi^2 = 0.53$, $p = 0.465$	17	$\chi^2 = 0.53$, $p = 0.465$
vinegar fly	fruit fly	standard	7-day	18	$\chi^2 = 1.20$, $p = 0.273$	16	$\chi^2 = 0.13$, $p = 0.715$
vinegar fly	fruit fly	standard	1-day	15	$\chi^2 = 0.00$, $p = 1$	13	$\chi^2 = 0.53$, $p = 0.465$
vinegar fly	cricket	standard	7-day	29	$\chi^2 = 26.13$, $p<0.001$	27	$\chi^2 = 19.20$, $p<0.001$
vinegar fly	caterpillar	standard	7-day	26	$\chi^2 = 16.13$, $p<0.001$	26	$\chi^2 = 16.13$, $p<0.001$
vinegar fly	aphid	standard	7-day	25	$\chi^2 = 13.33$, $p<0.001$	26	$\chi^2 = 16.13$, $p<0.001$
vinegar fly	oecobiid spider	standard	7-day	23	$\chi^2 = 8.53$, $p = 0.003$	29	$\chi^2 = 26.13$, $p<0.001$
vinegar fly	oecobiid spider	spider-only	7-day	-	-	24	$\chi^2 = 10.80$, $p = 0.001$
mayfly	fruit fly	standard	7-day	15	$\chi^2 = 0.00$, $p = 1$	13	$\chi^2 = 0.53$, $p = 0.465$
mayfly	fruit fly	standard	1-day	10	$\chi^2 = 3.33$, $p = 0.068$	18	$\chi^2 = 1.20$, $p = 0.273$
mayfly	cricket	standard	7-day	15	$\chi^2 = 0.00$, $p = 1$	20	$\chi^2 = 3.33$, $p = 0.068$
mayfly	cricket	standard	1-day	29	$\chi^2 = 26.13$, $p<0.001$	30	$\chi^2 = 30.00$, $p<0.001$

mayfly	caterpillar	standard	7-day	30	$\chi^2 = 30.00$, $p<0.001$	28	$\chi^2 = 22.53$, $p<0.001$
mayfly	aphid	standard	7-day	27	$\chi^2 = 19.20$, $p<0.001$	27	$\chi^2 = 19.20$, $p<0.001$
mayfly	oecobiid spider	standard	7-day	28	$\chi^2 = 22.53$, $p<0.001$	25	$\chi^2 = 13.33$, $p<0.001$
fruit fly	cricket	standard	7-day	25	$\chi^2 = 13.33$, $p<0.001$	17	$\chi^2 = 0.53$, $p = 0.465$
fruit fly	cricket	standard	1-day	-	-	25	$\chi^2 = 13.33$, $p<0.001$
fruit fly	caterpillar	standard	7-day	27	$\chi^2 = 19.20$, $p<0.001$	28	$\chi^2 = 22.53$, $p<0.001$
fruit fly	aphid	standard	7-day	26	$\chi^2 = 16.13$, $p<0.001$	29	$\chi^2 = 26.13$, $p<0.001$
fruit fly	oecobiid spider	standard	7-day	29	$\chi^2 = 26.13$, $p<0.001$	26	$\chi^2 = 16.13$, $p<0.001$
cricket	caterpillar	standard	7-day	21	$\chi^2 = 4.80$, $p = 0.028$	15	$\chi^2 = 0.00$, $p = 1$
cricket	caterpillar	standard	1-day	14	$\chi^2 = 0.13$, $p = 0.715$	17	$\chi^2 = 0.53$, $p = 0.465$
cricket	aphid	standard	7-day	14	$\chi^2 = 0.13$, $p = 0.715$	16	$\chi^2 = 0.13$, $p = 0.715$
cricket	aphid	standard	1-day	15	$\chi^2 = 0.00$, $p = 1$	12	$\chi^2 = 1.20$, $p = 0.273$
cricket	oecobiid spider	standard	7-day	13	$\chi^2 = 0.53$, $p = 0.465$	10	$\chi^2 = 3.33$, $p = 0.068$
cricket	oecobiid spider	standard	1-day	15	$\chi^2 = 0.00$, $p = 1$	16	$\chi^2 = 0.13$, $p = 0.715$
caterpillar	aphid	standard	7-day	16	$\chi^2 = 0.13$, $p = 0.715$	14	$\chi^2 = 0.13$, $p = 0.715$
caterpillar	aphid	standard	1-day	15	$\chi^2 = 0.00$, $p = 1$	14	$\chi^2 = 0.13$, $p = 0.715$
caterpillar	oecobiid spider	standard	7-day	11	$\chi^2 = 2.13$, $p = 0.144$	17	$\chi^2 = 0.53$, $p = 0.465$
caterpillar	oecobiid spider	standard	1-day	12	$\chi^2 = 1.20$, $p = 0.273$	12	$\chi^2 = 1.20$, $p = 0.273$
caterpillar	oecobiid spider	spider-only	7-day	-	-	16	$\chi^2 = 0.13$, $p = 0.715$
caterpillar	oecobiid spider	spider-only	1-day	-	-	12	$\chi^2 = 1.20$, $p = 0.273$
aphid	oecobiid spider	standard	7-day	14	$\chi^2 = 0.13$, $p = 0.715$	15	$\chi^2 = 0.00$, $p = 1$
aphid	oecobiid spider	standard	1-day	16	$\chi^2 = 0.13$, $p = 0.715$	16	$\chi^2 = 0.13$, $p = 0.715$

[1]number that chose prey 2 (*Anopheles* male) significantly more than number that chose prey 1 (blood *Culex* female)

Table 7. Preference strengths of *Evarcha culicivora* determined from complete series of simultaneous-presentation prey-choice experiments (table 6). Column 1: prey 1. Headings for each other column: prey 2. Strong preference for prey 1 (s): significantly more test spiders chose prey 1 than chose prey 2 after a 7-day fast. Weak preference for prey 1 (w): no significant choice after 7-day fast, but significantly more test spiders chose prey 1 than chose prey 2 after a 1-day pre-trial fast. Nil preference (n): no significant choice after 7-day and 1-day fasts. Strength of preference by adult test spiders indicated first, followed by strength of preference by juvenile test spiders. For details about prey, see table 3. Minus sign in front of w (2 instances, both with blood female *Culex* as prey 1): significantly more test spiders chose prey 2 after 1-day fast (contributes to preference index for prey 2 instead for prey 1: see table 10); otherwise s & w mean preference for prey 1. For Nelson and Jackson [12] data, juveniles 2.0 mm in body length; 3.0 mm in all other instances.

	blood *Culex* female	no-blood *Anopheles* female	no-blood *Culex* female	*Anopheles* male	*Culex* male	ghost midge	chironomid midge	vinegar fly	mayfly	fruit fly	cricket	caterpillar	aphid	oecobiid spider
blood female *Anopheles*	w[1], s[1]	s[1], s[1]	s[1], s[1]	s[1], s[1]	s, s	s, s	s[2], s[2]	s, s	s, s	s, s	s, s	s, s	s, s	s, s
blood *Culex* female	-	s[1], -w[1]	s[1], s[1]	s, -w	s[1],s[1]	s, s	s[2], s[2]	s, s	s, s	s, s	s, s	s, s	s, s	s, s
no-blood *Anopheles* female		-	w[1], s[1]	w[1], s[1]	w[1], s[1]	w, s	w[2], s[2]	s, s	s, s	s, s	s, s	s, s	s, s	s, s
no-blood *Culex* female			-	n[1], n[1]	n[1], n[1]	n, n	n[2], n[2]	w, w	w, w	s, s	s, s	s, s	s, s	s, s

Anopheles male	-	n[1], s[1]	n, w	n[2], w[2]	w, w	w, w	s, s	s, s	s, s	s, s	s, s
Culex male		-	n, n	n[2], n[2]	w, w	w, w	s, s	s, s	s, s	s, s	s, s
ghost midge			-	n, n	w, w	w, w	s, s	s, s	s, s	s, s	s, s
chironomid midge				-	w, w	w, w	s, s	s, s	s, s	s, s	s, s
vinegar fly			-	-		n, n	n, n	s, s	s, s	s, s	s, s
mayfly							n, n	w, w	s, s	s, s	s, s
fruit fly							-	s, w	s, s	s, s	s, s
cricket			-	-				-	n, n	n, n	n, n
caterpillar			-	-				-	-	n, n	n, n
aphid			-	-				-	-	-	n, n

[1]from Nelson and Jackson [12]. s in this table corresponds to medium in table 2.
[2]same for another chironomid midge, *Clinotanypus claripennis*, in Nelson and Jackson [12].

Table 8. Findings for adult females and juveniles of *Evarcha culicivora* ('test spiders') in mosquito series of simultaneous-presentation prey-choice experiments. For each experiment (row), 25 test spiders chose one of the two prey (i.e., $n = 25$ for each row). Blood: blood *Anopheles* female. No-blood: no-blood *Anopheles* female. See table 3 for details pertaining to prey. For all experiments, there was a pre-trial fast of 7 days. Data analysis: tests of goodness of fit (null hypothesis: as likely to choose prey 2 as to choose prey 1).

prey 1	prey 2	adult female test spiders		juvenile test spiders	
		chose prey 1	test of goodness of fit	chose prey 1	test of goodness of fit
blood	assassin bug	24	$\chi^2 = 21.16$, $p<0.001$	25	$\chi^2 = 25.00$, $p<0.001$
blood	barklouse	20	$\chi^2 = 9.00$, $p = 0.003$	20	$\chi^2 = 9.00$, $p = 0.003$
blood	brown rice hopper	25	$\chi^2 = 25.00$, $p<0.001$	22	$\chi^2 = 14.44$, $p<0.001$
blood	clubionid spider	25	$\chi^2 = 25.00$, $p<0.001$	24	$\chi^2 = 21.16$, $p<0.001$
blood	cockroach	23	$\chi^2 = 17.64$, $p<0.001$	20	$\chi^2 = 9.00$, $p = 0.003$
blood	green leaf hopper	22	$\chi^2 = 14.44$, $p<0.001$	24	$\chi^2 = 21.16$, $p<0.001$
blood	hersiliid spider	25	$\chi^2 = 25.00$, $p<0.001$	25	$\chi^2 = 25.00$, $p<0.001$
blood	house fly	22	$\chi^2 = 14.44$, $p<0.001$	23	$\chi^2 = 17.64$, $p<0.001$
blood	jumping spider	23	$\chi^2 = 17.64$, $p<0.001$	25	$\chi^2 = 25.00$, $p<0.001$
blood	long-legged fly	21	$\chi^2 = 11.56$, $p<0.001$	21	$\chi^2 = 11.56$, $p<0.001$
blood	mantis	25	$\chi^2 = 25.00$, $p<0.001$	24	$\chi^2 = 21.16$, $p<0.001$
blood	moth fly	23	$\chi^2 = 17.64$, $p<0.001$	23	$\chi^2 = 17.64$, $p<0.001$
blood	nephilid spider	23	$\chi^2 = 17.64$, $p<0.001$	25	$\chi^2 = 25.00$, $p<0.001$
blood	whitefly	19	$\chi^2 = 6.76$, $p = 0.009$	25	$\chi^2 = 25.00$, $p<0.001$
blood	wolf spider	22	$\chi^2 = 14.44$, $p<0.001$	24	$\chi^2 = 21.16$, $p<0.001$
no-blood	assassin bug	24	$\chi^2 = 21.16$, $p<0.001$	22	$\chi^2 = 14.44$, $p<0.001$
no-blood	barklouse	20	$\chi^2 = 9.00$, $p = 0.003$	23	$\chi^2 = 17.64$, $p<0.001$
no-blood	brown rice hopper	19	$\chi^2 = 6.76$, $p = 0.009$	21	$\chi^2 = 11.56$, $p<0.001$
no-blood	clubionid spider	24	$\chi^2 = 21.16$, $p<0.001$	25	$\chi^2 = 25.00$, $p<0.001$
no-blood	cockroach	25	$\chi^2 = 25.00$, $p<0.001$	25	$\chi^2 = 25.00$, $p<0.001$
no-blood	green leaf hopper	21	$\chi^2 = 11.56$, $p<0.001$	22	$\chi^2 = 14.44$, $p<0.001$
no-blood	hersiliid spider	21	$\chi^2 = 11.56$, $p<0.001$	24	$\chi^2 = 21.16$, $p<0.001$
no-blood	house fly	23	$\chi^2 = 17.64$, $p<0.001$	23	$\chi^2 = 17.64$, $p<0.001$
no-blood	jumping spider	23	$\chi^2 = 17.64$, $p<0.001$	25	$\chi^2 = 25.00$, $p<0.001$
no-blood	long-legged fly	20	$\chi^2 = 9.00$, $p = 0.003$	21	$\chi^2 = 11.56$, $p<0.001$
no-blood	mantis	23	$\chi^2 = 17.64$, $p<0.001$	24	$\chi^2 = 21.16$, $p<0.001$
no-blood	moth fly	24	$\chi^2 = 21.16$, $p<0.001$	21	$\chi^2 = 11.56$, $p<0.001$
no-blood	nephilid spider	22	$\chi^2 = 14.44$, $p<0.001$	24	$\chi^2 = 21.16$, $p<0.001$
no-blood	whitefly	20	$\chi^2 = 9.00$, $p = 0.003$	21	$\chi^2 = 11.56$, $p<0.001$
no-blood	wolf spider	21	$\chi^2 = 11.56$, $p<0.001$	25	$\chi^2 = 25.00$, $p<0.001$

Table 9. Findings for *Evarcha culicivora* ('test spiders') in the non-mosquito series of prey-choice experiments (see text). For each experiment (row), simultaneous presentation testing was used and 25 spiders chose one of the two prey (i.e., $n = 25$ for each row) for each row. For details concerning prey, see table 3. For all experiments, there was a pre-trial fast of 1 day. Data analysis: tests of goodness of fit (null hypothesis: as likely to choose prey 2 as to choose prey 1).

prey 1	prey 2	adult female test spiders		juvenile test spiders	
		chose prey 1	test of goodness of fit	chose prey 1	test of goodness of fit
Chaoborus sp.	*Ablabesmyia nilotica*	11	$\chi^2 = 0.36$, $p = 0.549$	10	$\chi^2 = 1.00$, $p = 0.317$
Chaoborus sp.	*Chironomus imicola*	14	$\chi^2 = 0.36$, $p = 0.549$	12	$\chi^2 = 0.04$, $p = 0.841$
Chaoborus sp.	*Clinotanypus claripennis*	11	$\chi^2 = 0.36$, $p = 0.549$	13	$\chi^2 = 0.04$, $p = 0.841$
Chaoborus sp.	*Conochironomus acutistus*	14	$\chi^2 = 0.36$, $p = 0.549$	14	$\chi^2 = 0.36$, $p = 0.549$
Nilodorum brevibucca	*Ablabesmyia nilotica*	15	$\chi^2 = 1.00$, $p = 0.317$	15	$\chi^2 = 1.00$, $p = 0.317$
Nilodorum brevibucca	*Chironomus imicola*	10	$\chi^2 = 1.00$, $p = 0.317$	9	$\chi^2 = 1.96$, $p = 0.162$
Nilodorum brevibucca	*Clinotanypus claripennis*	15	$\chi^2 = 1.00$, $p = 0.317$	14	$\chi^2 = 0.36$, $p = 0.549$
Nilodorum brevibucca	*Conochironomus acutistus*	16	$\chi^2 = 1.96$, $p = 0.162$	13	$\chi^2 = 0.04$, $p = 0.841$
Ablabesmyia nilotica	*Chironomus imicola*	12	$\chi^2 = 0.04$, $p = 0.841$	14	$\chi^2 = 0.36$, $p = 0.549$
Ablabesmyia nilotica	*Clinotanypus claripennis*	12	$\chi^2 = 0.04$, $p = 0.841$	8	$\chi^2 = 3.24$, $p = 0.072$
Ablabesmyia nilotica	*Conochironomus acutistus*	14	$\chi^2 = 0.36$, $p = 0.549$	11	$\chi^2 = 0.36$, $p = 0.549$
Chironomus imicola	*Clinotanypus claripennis*	12	$\chi^2 = 0.04$, $p = 0.841$	9	$\chi^2 = 1.96$, $p = 0.162$
Chironomus imicola	*Conochironomus acutistus*	9	$\chi^2 = 1.96$, $p = 0.162$	11	$\chi^2 = 0.36$, $p = 0.549$
Clinotanypus claripennis	*Conochironomus acutistus*	11	$\chi^2 = 0.36$, $p = 0.549$	13	$\chi^2 = 0.04$, $p = 0.841$
oecobiid spider	wolf spider	13	$\chi^2 = 0.04$, $p = 0.841$	12	$\chi^2 = 0.04$, $p = 0.841$
oecobiid spider	jumping spider	14	$\chi^2 = 0.36$, $p = 0.549$	9	$\chi^2 = 1.96$, $p = 0.162$
oecobiid spider	nephilid spider	16	$\chi^2 = 1.96$, $p = 0.162$	13	$\chi^2 = 0.04$, $p = 0.841$
wolf spider	nephilid spider	10	$\chi^2 = 1.00$, $p = 0.317$	9	$\chi^2 = 1.96$, $p = 0.162$
wolf spider	jumping spider	13	$\chi^2 = 0.04$, $p = 0.841$	13	$\chi^2 = 0.04$, $p = 0.841$
jumping spider	nephilid spider	15	$\chi^2 = 1.00$, $p = 0.317$	12	$\chi^2 = 0.04$, $p = 0.841$

Table 10. Preference indexes for 15 prey categories used in complete series of simultaneous-presentation prey-choice experiments (see text & table 7). For each pairing with another prey type, each prey category given a score of 0 when no preference was expressed (NS after 7-day & 1-day prey-trial fast), 1 when only a weak preference was expressed (significantly more test spiders chose this prey type after a 1-day, but not 7-day, fast) and 2 when a strong preference was expressed (significantly more chose this prey type after 7-day fast). Preference index for prey category: sum of scores for that prey category paired with each other category.

preference index	juvenile test spider	adult test spider
28	blood *Anopheles* female	-
27	-	blood *Anopheles* female
26	-	blood *Culex* female
25	no-blood *Anopheles* female	-
24	-	-
23	-	-
22	blood *Culex*	-
21	-	-
20	-	-
19	-	no-blood *Anopheles* female
18	-	-
17	*Anopheles* male	-
16	-	-
15	-	-
14	-	-
13	-	-
12	no-blood *Culex* female; *Culex* male, ghost midge & chironomid midge	no-blood *Culex* female, *Anopheles* male, *Culex* male, ghost midge & chironomid midge
11		
10	-	-
9	-	-
8	vinegar fly	vinegar & fruit fly
7	mayfly & fruit fly	mayfly
6	-	-
5	-	-
4	-	-
3	-	-
2	-	-
1	-	-

0	cricket, caterpillar, aphid, spider	cricket, caterpillar, aphid, spider

Table 11. Sample sizes in studies on the prey of salticids in the field.

salticid species	number of records of prey	source
Aelurillus m-nigram	58	[31]
Aelurillus muganicus	64	[32]
Cyrba algerina	59	[33]
Heliophanus dunni	50	[34]
Menemerus semilimbatus	96	[35]
Menemerus taeniatus	62	[36]
Mexcala elegans	64	[37]
Phidippus johnsoni	33	[38]
Phidippus audax	21	[39]
Portia fimbriata	24	[40]
Portia fimbriata	61	[41]
Paracyrba wanlessi	84	[42]
Salticus tricinctus	40	[43]
Salticus austinensis	46	[44]
Siler cupreus	24	[45]

Table 12. Prey categories for *Evarcha culicivora* juveniles and adult *E. culicivora* females, determined from complete series of simultaneous-presentation prey-choice experiments. See text for category-derivation procedure and table 3 for details pertaining to prey. When applicable, preference index listed for each category (see table 10). Each category given a letter code followed by listing of constituent prey types. Categories a, b, c, f and g applicable to juvenile and adult female test spiders. For juveniles, but not for adult females, d and e are distinct categories. Category de for adult females is inclusive of prey in categories d and e of juveniles. NA: not applicable.

prey category	juvenile preference index	adult female preference index
a. blood *Anopheles*	28	27
b. blood *Culex* female	22	26
c. no-blood *Anopheles* female	25	19
d. *Anopheles* male (17)	17	NA
e. no-blood *Culex* female, *Culex* male, ghost midge, chironomid midge (12)	12	NA
de. *Anopheles* male, no-blood *Culex* female, *Culex* male, ghost midge, chironomid midge (12)	NA	12
f. vinegar fly, fruit fly, mayfly (7-8)	7-8	7-8
g. cricket, caterpillar, aphid, spider (0)	0	0

APPENDIX TWO: ADJUSTING FOR REPEATED USE OF DATA SETS

Number of comparisons (K) made using data from different groups (data sets) in Chapter 3. For Bonferroni adjustments of alpha (i.e., the threshold p-value for judging a difference between two groups to be "significant"), $p<0.05/K$ corresponds to an alpha of $p<0.05$. For more information about Bonferroni adjustments, see: Sokal & Rohlf 1995, Hardin et al. 1996). Rows here correspond to rows in Table 4.

Row	Group	K for longevity	K for completion success	K for completion time
1	Water-only control for prey & plants (C1)	8	-	-
2	Water-only control for artificial nectar (C2)	5	-	-
3	No-blood female on Day 1	15	-	-
4	Blood female on Day 1	4	-	-
5	Male on Day 1	4	-	-
6	No-blood female on Day 5	1	-	-
7	No-blood female on Day 1 & Day 5	3	4	10
8	Blood female on Day 1 & Day 5	3	4	8
9	Male on Day 1 & Day 5	3	4	3
10	No-blood female on Day 1, 5 & 9	2	3	7
11	Blood female on Day 1, 5 & 9	2	3	7
12	Male on Day 1, 5 & 9	2	3	3
13	*Lantana camara*, no prey	11	4	4
14	*Lippia kituensis*, no prey	6	2	4
15	*Parthenium hysterophorus*, no prey	6	-	-
16	*Ricinus communis*, no prey	6	-	-
17	*Hibiscus rosa-sinensis*, no prey	6	-	-
18	*Lantana camara*, no-blood female on Day 1	6	1	4
19	*Lippia kituensis*, no-blood female on Day 1	5	1	4
20	*Parthenium hysterophorus*, no-blood female on Day 1	5	-	-
21	*Ricinus communis*, no-blood female on Day 1	5	-	-
22	*Hibiscus rosa-sinensis*, no-blood female on Day 1	5	-	-

23	*Lantana camara*, no-blood female on Day 1 & Day 5	-	4	9
24	*Lantana camara*, blood female on Day 1 & Day 5	-	4	8
25	*Lippia kituensis*, no-blood female on Day 1 & Day 5	-	3	8
26	*Lippia kituensis*, blood female on Day 1 & Day 5	-	3	7
27	*Parthenium hysterophorus*, no-blood female on Day 1 & 5	-	3	6
28	*Parthenium hysterophorus*, blood female on Day 1 & 5	-	3	6
29	*Ricinus communis*, no-blood female on Day 1 & 5	-	3	6
30	*Ricinus communis*, blood female on Day 1 & 5	-	3	6
31	1% sucrose, no prey	2	-	-
32	5% sucrose, no prey	5	-	-
33	10% sucrose, no prey	3	-	-
34	20% sucrose, no prey	10	-	-
35	30% sucrose, no prey	2	-	-
36	40% sucrose, no prey	1	-	-
37	20% glucose, no prey	5	-	-
38	20% fructose, no prey	6	-	-
39	20% maltose, no prey	4	-	-
40	5% glucose, no prey	4	-	-
41	5% fructose, no prey	4	-	-
42	5% glucose-fructose blend, but no prey	1	-	-
43	10% glucose-fructose blend, but no prey	1	-	-
44	20% glucose-fructose blend, but no prey	1	-	-
45	4% amino-acid blend (Glu,Gly,Pro,Ser) but no prey	1	-	-
46	20% glucose+amino-acid blend, but no prey	4	-	-
47	20% fructose+amino-acid blend, but no prey	4	-	-
48	20% maltose+amino-acid blend, but no prey	4	-	-
49	20% sucrose+amino-acid blend, but no prey	5	-	-
50	Sugar-only blend, but no prey	3	-	-
51	Full blend, but no prey	7	-	-
52	Full blend & ate one no-blood female	5	-	-
53	Full blend & ate one blood female	4	-	-

54	Full blend & ate two no-blood females	4	3	3
55	Full blend & ate two blood females	4	3	3

APPENDIX THREE: CONFERENCES SLIDES

These are slides that were prepared for presentations at scientific conferences. The idea is that these are here for people who might want to have a quick look to get a general understanding of my research Chapters 2 & 3.

Adaptive foraging periodicity by a mosquito-specialist predator

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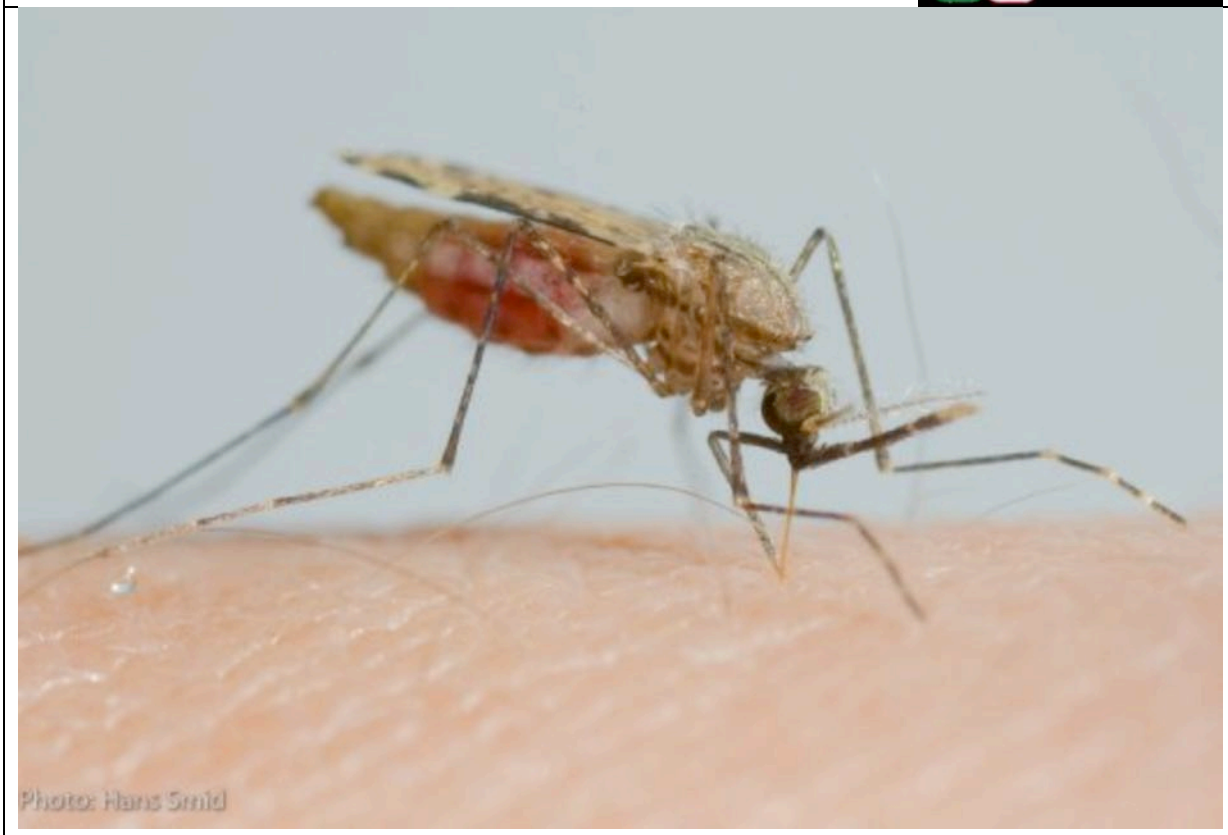
Objective & Specific Aims

To **understand a complex system of predatory specialization.**

Using spiders that feed indirectly on vertebrate blood by choosing blood-carrying *Anopheles* mosquitoes (malaria vectors) as preferred prey.

Credit: R. Jackson.
Evarcha culicivora female

Credit: R. Jackson.
Evarcha culicivora male



Credit: H. Smid.
Anopheles gambiae s.s.

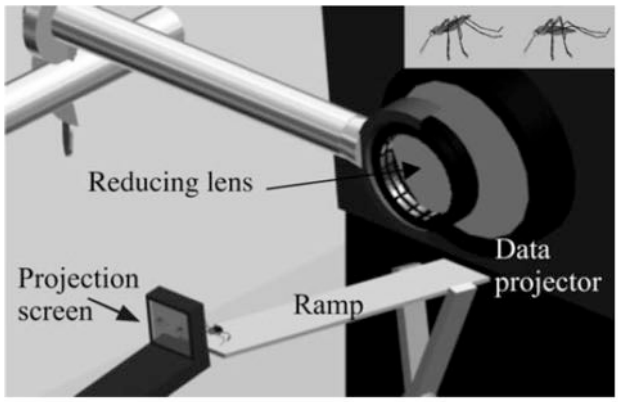
What is predatory specialization?

A predator being especially well adapted with respect to specific types of prey

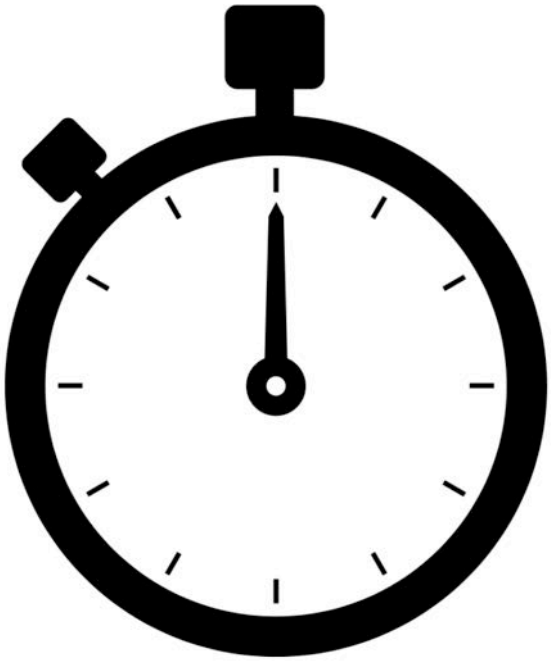
Broader interest
Understanding specific ways predators can specialize

The ways *E. culicivora* is known to be specialized include:

- A special way small juveniles capture *Anopheles*
- *Anopheles* specific feature detection system
- Finely tuned mosquito related prey-choice behaviour



Credit: X. Nelson.
Apparatus for virtual-prey testing



Adaptive timing

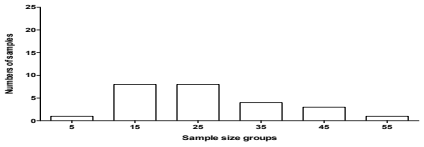
Hypothesis: *E. culicivora* has an innate activity pattern that facilitates being particularly effective **as a predator** of night-feeding anthropophilic mosquitoes

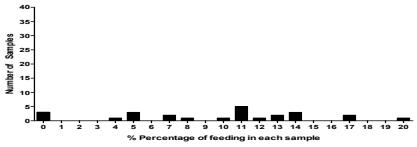
Hypothesis 1: Under natural conditions, *E. culicivora* tends to be **active as a predator** primarily in the early morning when this particular type of prey is available for *E. culicivora*

Hypothesis 2: *E. culicivora* is innately predisposed to be more responsive in the morning than in the afternoon to **vision-based and odour-based cues** from its preferred prey

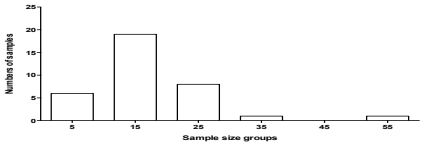
Hypothesis 3: *E. culicivora* is innately predisposed to express stronger preference in the morning than in the afternoon for **blood-carrying mosquitoes**

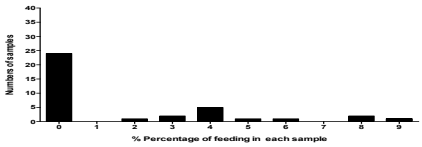
Morning



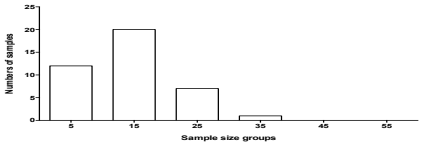


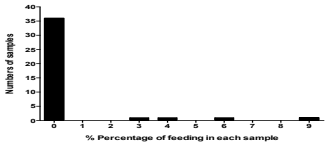
Mid-day





Afternoon





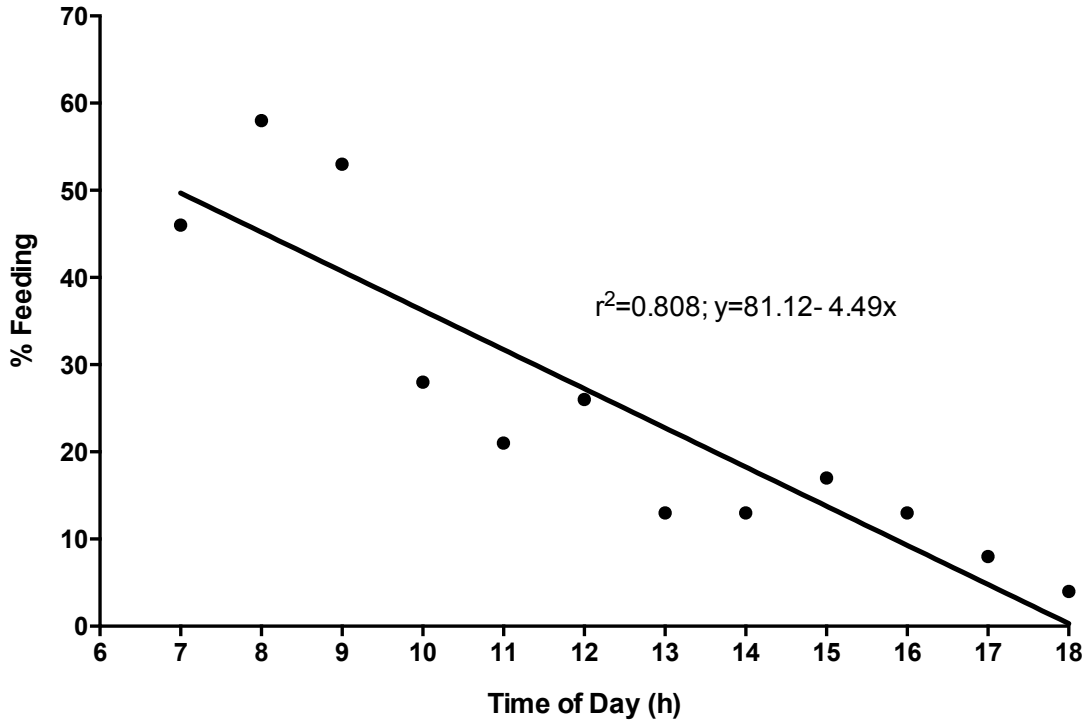
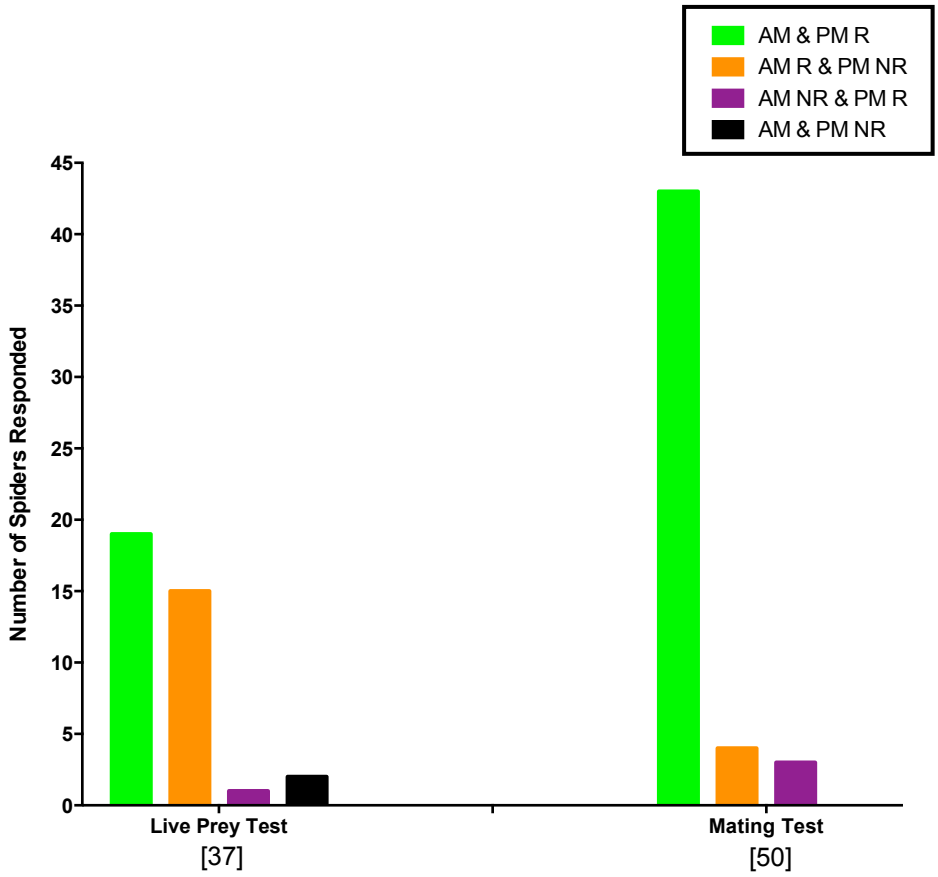


Figure 2. Simple linear regression analysis. Relationship between the different times of day (0700-1800 h) changes in the feeding rate of spiders.

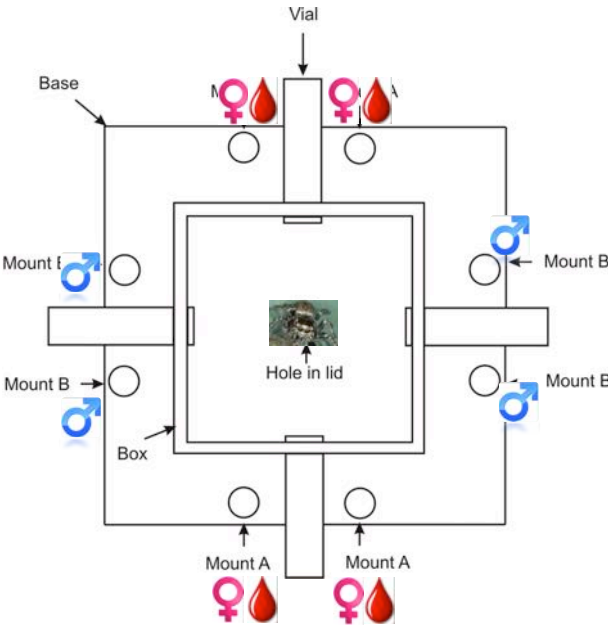
Question: Whether peak responsiveness in the morning was specific to predation instead of being a general feature of *E. culicivora*'s activity pattern?

living mates vs.. living prey

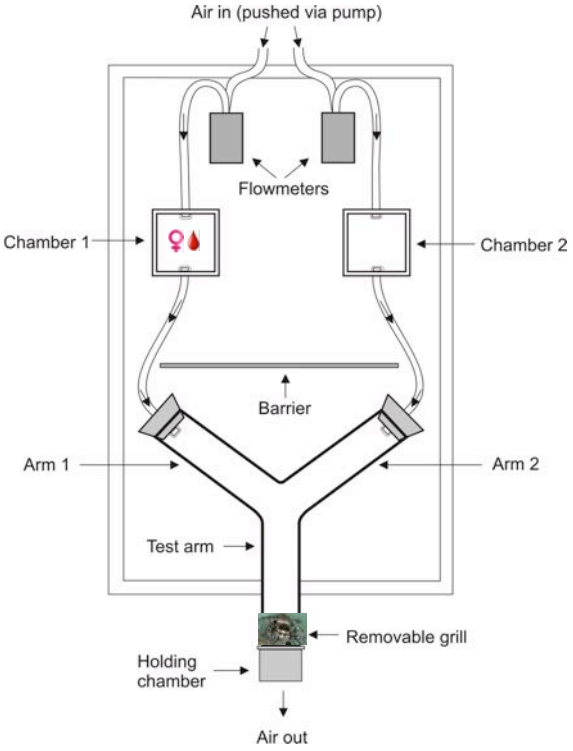


Hypothesis 2: *E. culicivora* is innately predisposed to be more responsive in the morning than in the afternoon to **vision-based and odour-based cues** from its preferred prey

Hypothesis 3: *E. culicivora* is innately predisposed to express stronger preference in the morning than in the afternoon for **blood-carrying mosquitoes**

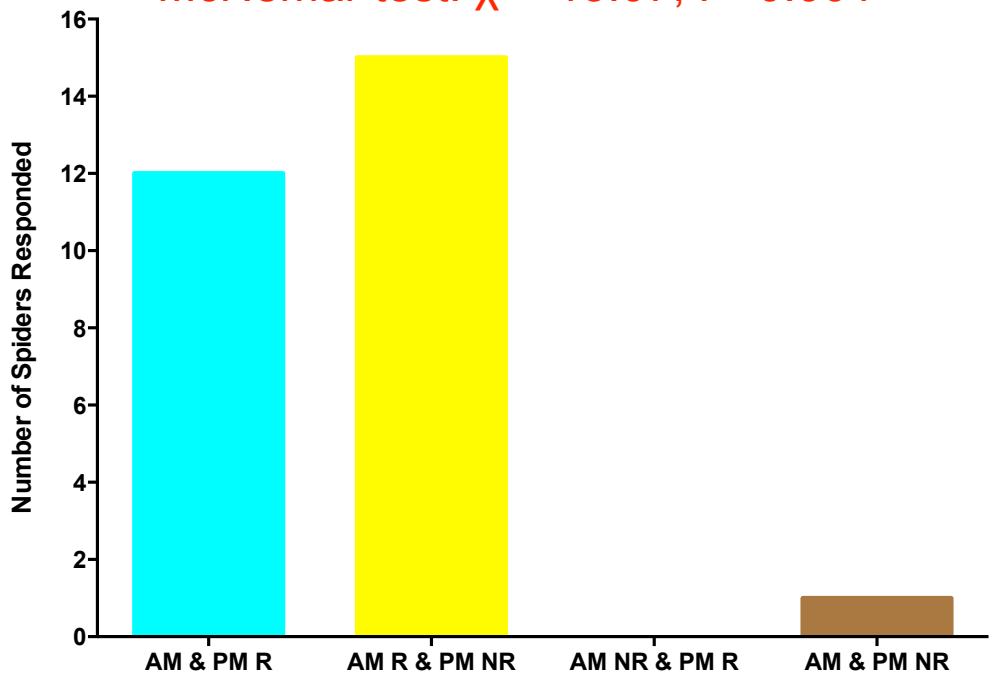


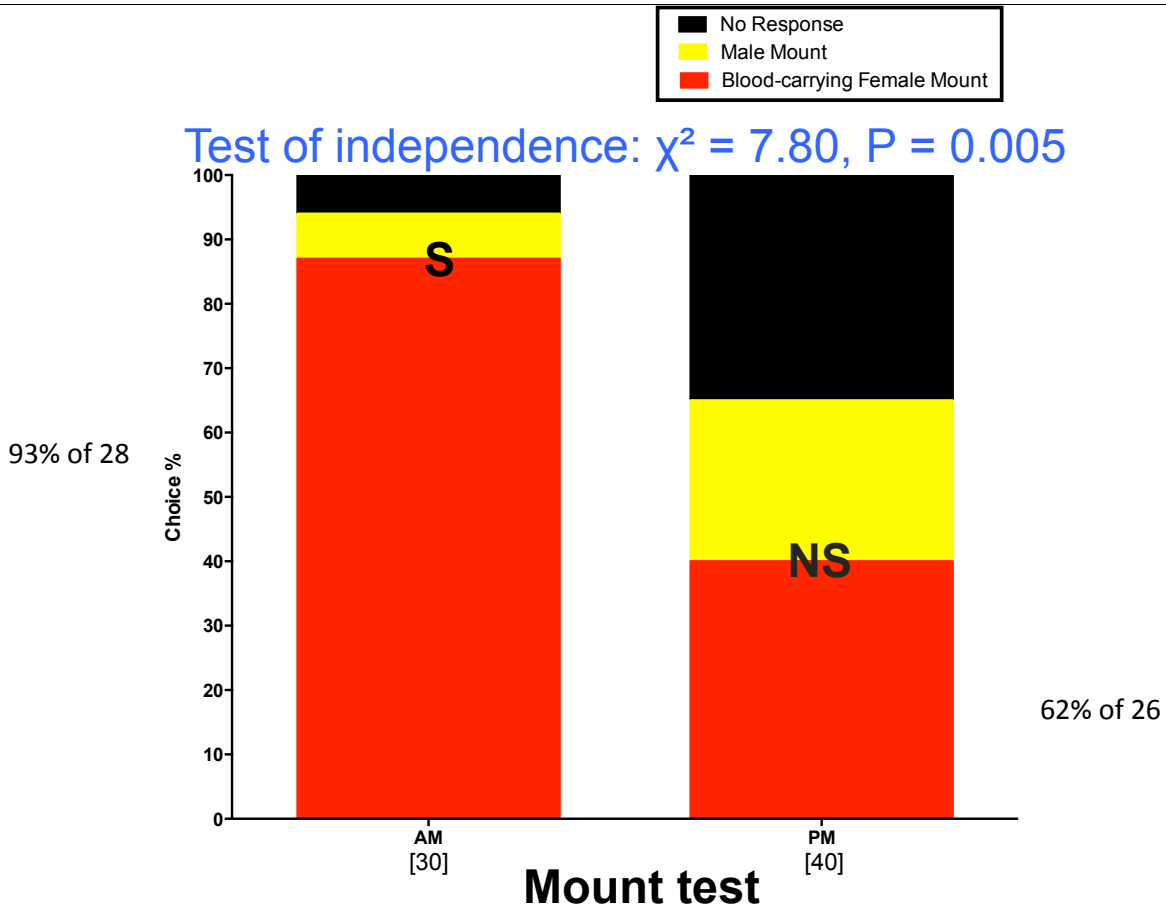
Mount Choice



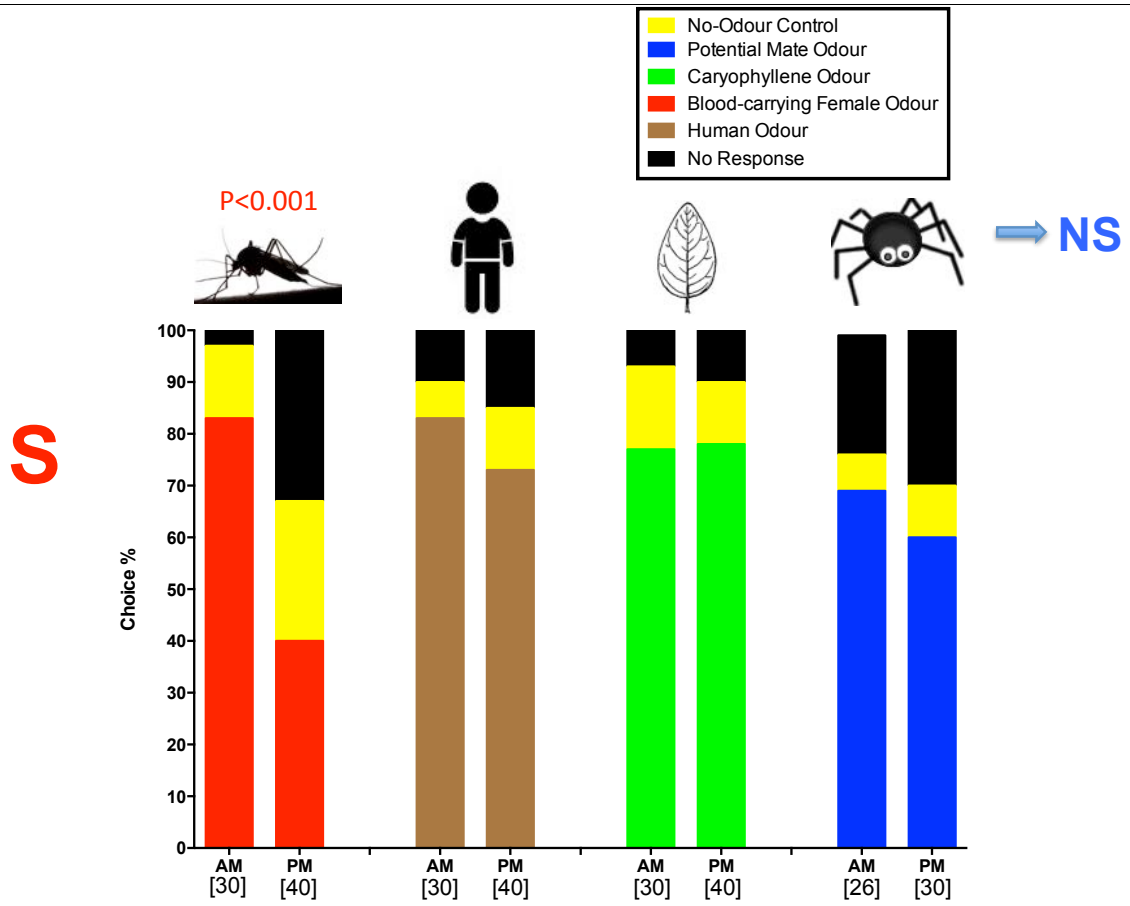
Odour Choice

McNemar test: $\chi^2 = 13.07$, $P<0.001$





Question: Is it that this predator more active and responsive in general during the morning or is it that peak seen in the early morning is specific to predation on blood-carrying mosquitoes?



Acknowledgement

Prof. Robert Jackson

Prof. Ximena Nelson

Dr. Fiona Cross

Spider team: Aynsley Macnab, Yinnon Dolev, Stephene Aluoch, Maurice Awayo , David Omondi

Insectary

Effects of prey and nectar meals on the capacity of a mosquito-specialist predator to complete the first active stage in its life cycle

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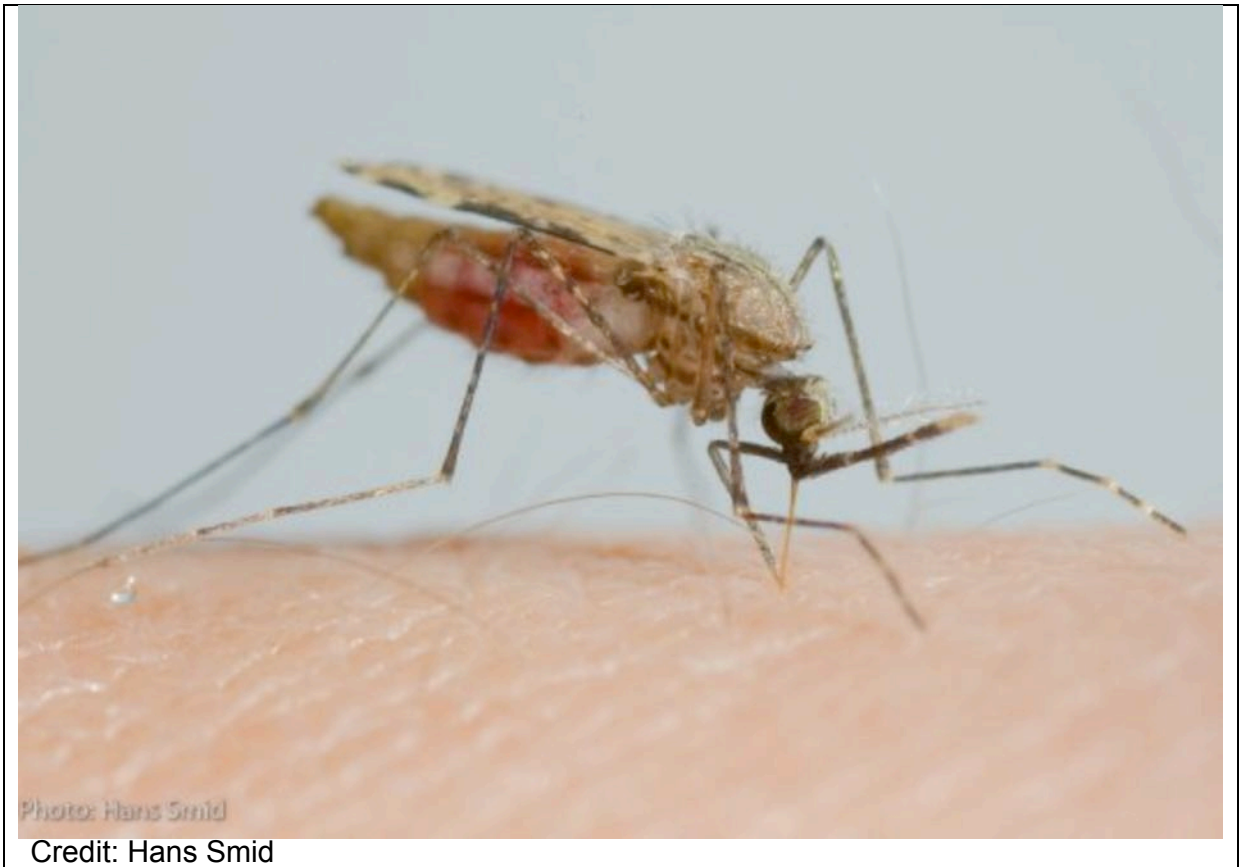
Objective & Specific Aims

- My research is a part of a larger research programme for which the central aim is to understand a complex system of adaptation. The system includes, besides people and the malaria parasite (*Plasmodium*), mosquitoes (especially *Anopheles gambiae*), certain plant species (e.g., *Lantana camara*) and *Evarcha culicivora*, a jumping spider (Salticidae) that feeds indirectly on blood by choosing blood-carrying *Anopheles* females as prey.

Credit: R. Jackson.
Evarcha culicivora female

Credit: R. Jackson.
Evarcha culicivora male

Credit: R. Jackson.
Evarcha culicivora juvenile



Credit: Hans Smid
Anopheles gambiae s.s.

Lantana camara

Hibiscus stramonium

Lippia kituensis

Ricinus communis

Parthenium hysterophorus

Objective & Specific Aims

- The spider and the mosquito both are also known to feed on nectar and both are attracted to the odour of particular plant species, but we do not understand in detail the ways in which, or the level to which, the spider and the mosquito are adapted to specific plant species.

- As a step toward filling this gap, I am focusing my PhD research on two specific hypotheses:

Hypothesis 1: *E*. *culicivora* has a plant-related activity budget that facilitates being particularly effective as a predator of *An*. *gambiae*.

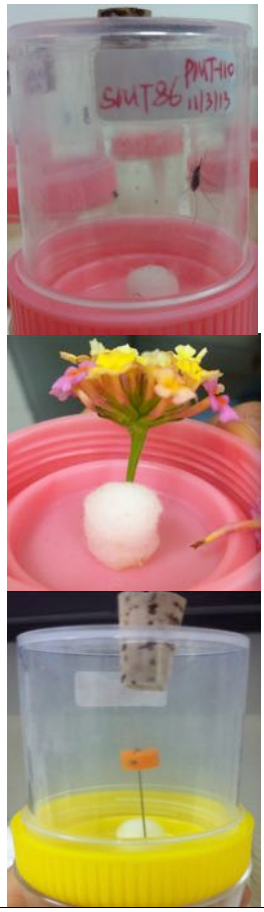
Hypothesis 2: *E*. *culicivora* is metabolically adapted to particular plant species.

Hypothesis: *E. culicivora* is metabolically adapted to particular plant species.

specific hypothesis

Plant-derived nutrients, acquired independently of predation; make an important contribution to the performance of 1st instar spiders.

Understanding the effect of nutrients on the performance of the 1st-instar spiders requires a thorough understanding of the effects of different feeding regimes.



Feeding regimes included:

First step:

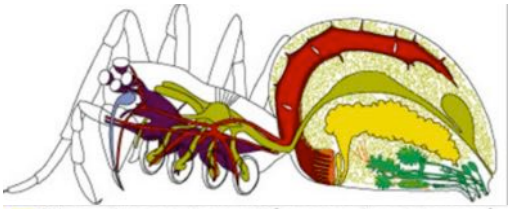
How different feeding regimes with prey-only influence the capacity of newly emerged juveniles of *E. culicivora* to complete the 1st instar?

Hypothesis: Feeding on a single blood female mosquito suffices for newly emerged juveniles to complete the 1st instar. ♀ 🩸

Does the type of mosquito matter? ♂ ♀

Answer: **NO**

Digestive tract may not developed well enough on day 1 for processing this prey.



Digestive system with pumping stomach

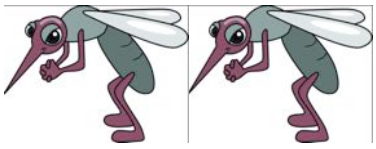
Creator: Design Unit
Rights: Australian Museum

NBF- ♀

BF- ♀🩸

M- ♂

→ 0



→

Group	1st moult rate
2 M	23%
2 NBF	57%
2 BF	57%

→

Group	1st moult rate
3M	78%
3NBF	92%
3BF	80%

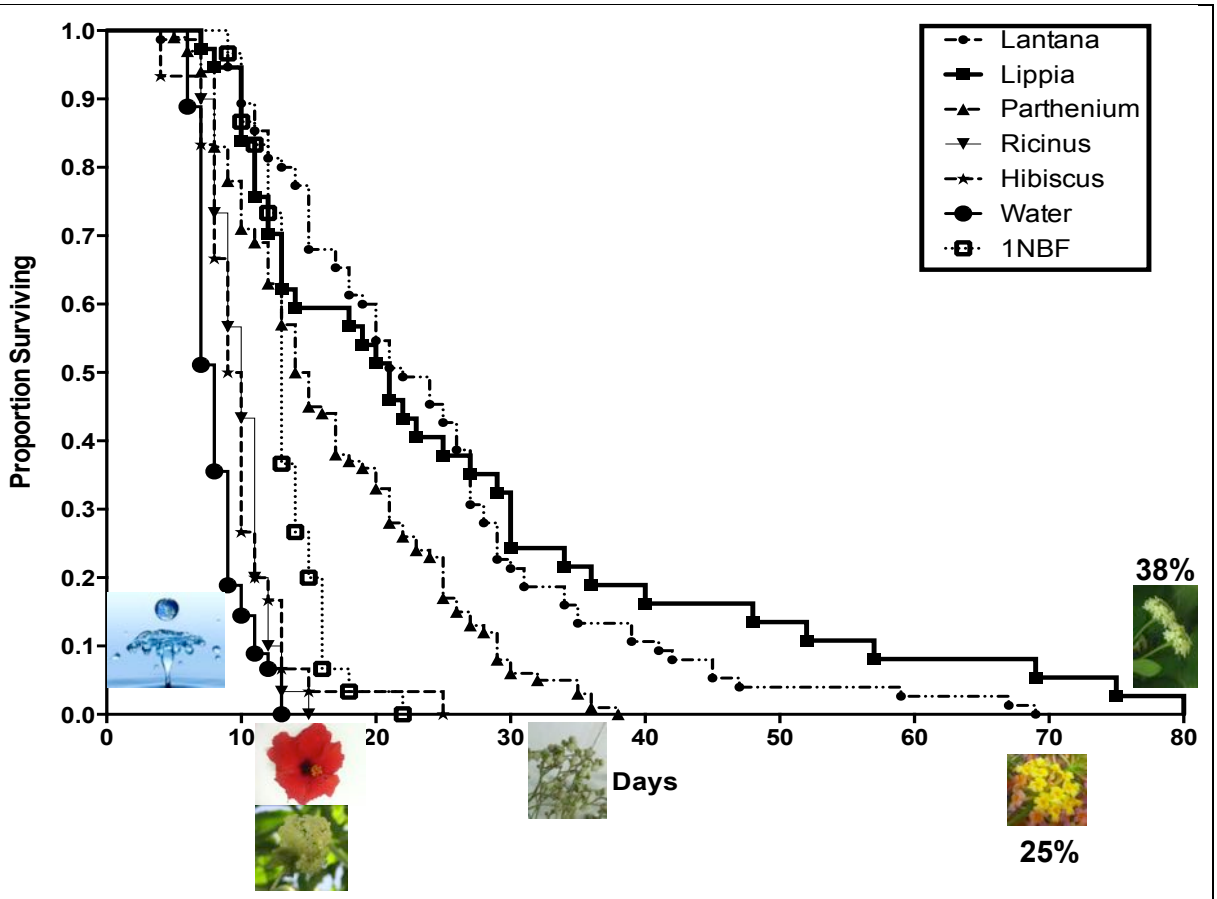
100%

First step:

The effects of plant nutrients. Does the plant species matter?

plant only

Hypothesis: for *E.culicivora*, access to nutrients from plants can, in the absence of prey, suffice for enabling the spider to complete the first instar.



Second step:

Hypothesis: Plant nutrients can be important because, by feeding on plant nutrients, the spider can stay alive, thereby increasing its chance of acquiring prey-meals in time to complete the 1st-instar.

	n	Moult rate before 15 days	Moult rate after 15 days
Mimic *Lantana* Nectar	30	0	0
Mimic *Lantana* Nectar 15 days + unlimited prey	84	0	46%

Third step:

Hypothesis: an optimal diet for 1st instar spiders is a combination of prey and plant nutrients.

NBF-♀

BF-♀

Group	1st moult rate
1NBF+*Lantana*	23%
1NBF+*Lippia*	23%
1NBF+*Parthenium*	3%
1NBF+*Ricinus*	3%
1NBF+*Hibiscus*	3%

Group	1st moult rate
2NBF+*Lantana*	97%
2BF+*Lantana*	90%
2NBF+*Lippia*	93%
2BF+*Lippia*	97%
2NBF+*Parthenium*	50%
2BF+*Parthenium*	53%
2NBF+*Ricinus*	43%
2BF+*Ricinus*	70%
2NBF	57%
3NBF	92%

100%

Next step:

To investigate what specific nutrients of plants matter? (e.g., sugar, amino acid, mimic *Lantana* nectar?)

Acknowledgement

Prof. Robert Jackson

Dr. Ximena Nelson

Dr. Fiona Cross

Spider team: Aynsley, Yinnon, Amber, Stephene, Ayaowo, Omondi, Jane, Kevin

Insectary

My friends