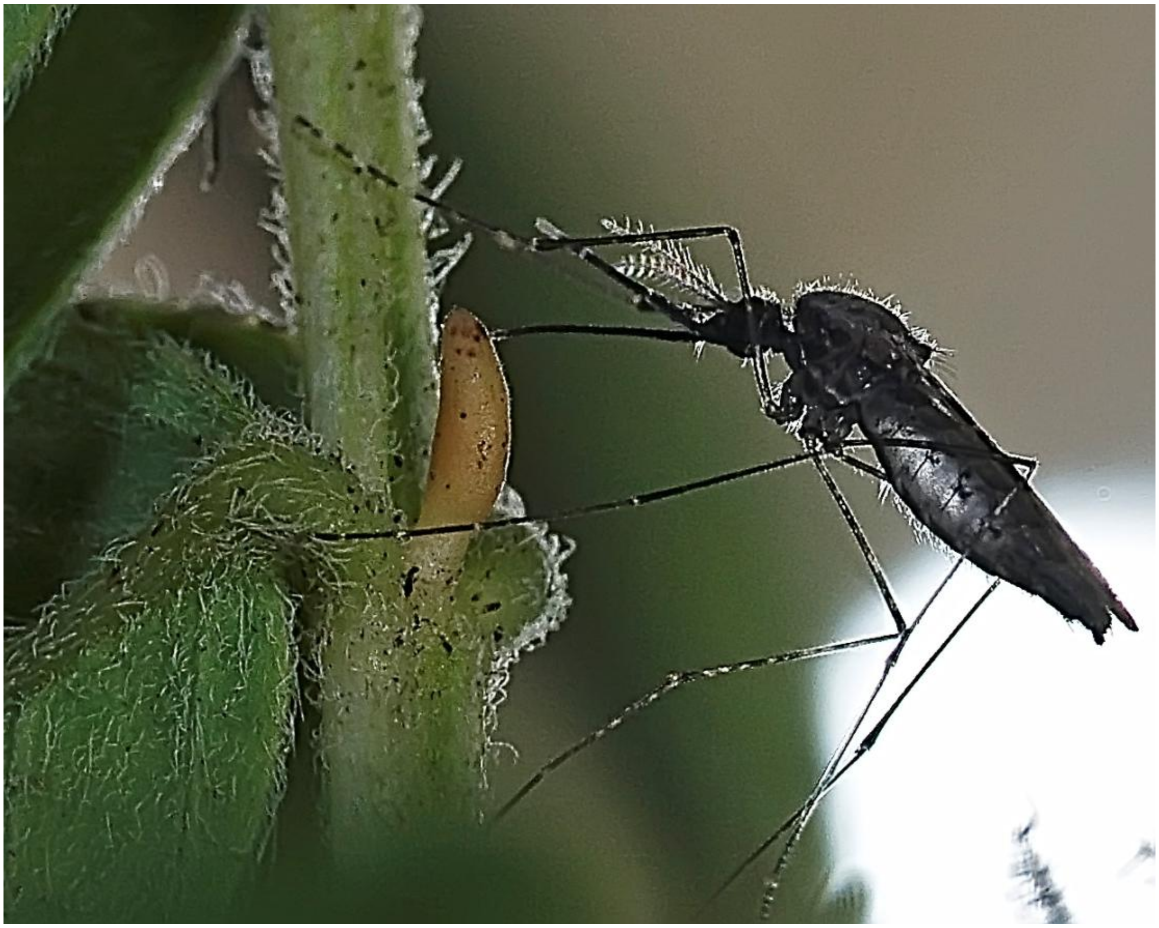

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THE ROLE OF MOSQUITO BEHAVIOUR ON PARASITE TRANSMISSION

by

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Abstract

I use a combination of theory and experiments to explore the role of various aspects of mosquito behaviour on the ability of mosquitoes to transmit parasites. Special focus is given to the mosquito *Anopheles gambiae s.s.*, the principal vector for *Plasmodium falciparum*, a parasite that causes human malaria. Female mosquitoes require host blood for egg production, but also use sugar from nectar sources; however, the extent of sugar use is poorly understood. Sugar can be used to fuel somatic maintenance and activity. Blood, on the other hand, is a poor resource for fueling somatic maintenance and activity because of its low conversion efficiency to energy reserves. Furthermore, although blood is necessary for egg production, obtaining blood is risky. Thus, there is a trade-off between reproduction and survival when using these two resources. I use an energy-explicit dynamic state variable model where the availability of both blood and sugar is varied and ask whether energetic condition is important for the feeding choices of mosquitoes. I then test this theory using two independent behavioural bioassay experiments. Both the experiments and theory agree that energetic state will have an impact on mosquito behavior – mosquitoes with lower reserves choose sugar over blood, and the availability of blood and sugar have non-linear effects on these choices. Furthermore, I examine the evolution of egg-laying behavior in the context of a co-evolving complex of adult and larval traits and find that adult mosquitoes evolve very specific preferences for habitats that correspond to larval characteristics, but that adults try to minimize larval interactions. Lastly, I use well established metrics of parasite transmission ability to demonstrate that the availability of both blood and sugar have a large impact on the ability of a mosquito population to transmit parasites. The availability of sugar in the environment may thus play a larger role in a mosquito's life, and subsequently its ability to transmit parasites, than traditionally thought. I discuss this finding in light of current vector control strategies.

Keywords: mosquito; parasite transmission; disease epidemiology; malaria; dynamic state variable model; genetic algorithm.

Dedication

To my loving wife, Iris Kim; my parents, Timothy and Dorothy Ma; my sister, Jennifer Ma; and all of the friends I have made along the way. Without your love and support, I could not have done this.

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I would like to thank my committee members, Dr. Carl Lowenberger and Dr. Eirikur Palsson, for their many contributions to my thesis. Dr. Lowenberger provided me with very fruitful discussions on all things pathology and mosquito biology. His very thorough reading of my work, and hard-hitting questions always kept me on my toes. His advice and support over the years has been a constant source of motivation for me. Dr. Palsson has made me double-check my equations and rationalize my modelling approaches. My thesis is stronger because of my committee’s contributions.

I would also like to thank my examiners, Dr. Mark Lewis and Dr. Wendy Palen for their thoughtful questions and comments on my work. These contributions have led to a much better thesis.

The many members of the Roitberg lab have been very helpful in getting to this end goal. I would especially like to thank Alex Chubaty for keeping me on my toes with my modelling and Kelly Walker for showing me that chemistry isn’t that bad. Thanks go to Kelly, Conan Phelan, Christie McDougall, Sigrid Keiser, Simon Zappia and the

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1: Introduction

1.1 Introduction

Blood-feeding mosquitoes are medically important pests because many of them are vectors for parasites that cause diseases in humans. Of particular importance is the mosquito, *Anopheles gambiae s.s.*, which acts as a primary vector of the protozoan parasite, *Plasmodium falciparum*, which causes human malaria. Despite our best efforts to control malaria, many humans across the world still suffer from the effects of malaria – a disease that causes over 300 million clinical cases and 1 million deaths a year (WHO 2008). One of the principal components of the Global Malaria Control Strategy initiative by the World Health Organization (WHO) is vector control.

There are four *Plasmodium* species that cause malaria in humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*. Of these four parasites, *P. falciparum* is the most deadly to humans, leading to most deaths associated with malaria. Symptoms of malaria include chills and sweats, high fevers, headaches, diarrhea, nausea, low blood pressure causing dizziness, muscle aches, a poor appetite, and an enlarged spleen.

The life cycle of *P. falciparum* spans across a human host and a mosquito vector (*Anopheles gambiae*). When a female mosquito infected with *P. falciparum* bites a human host, it injects sporozoites into the blood stream of the host. These sporozoites infect hepatocytes in the liver, where they eventually differentiate into merozoites. These merozoites enter the blood stream and bind to erythrocytes. Once the erythrocyte is invaded, the parasite forms a trophozoite which grows in the red blood cell cytoplasm before forming a schizont. In this stage, merozoites are formed and burst from the red blood cell to infect other erythrocytes. This process is generally well-sychronized and takes ~48h, leading to the characteristic cycles of chills and fevers associated with malaria. A small proportion (0 – 20%) of these merozoites develop into male and female gametocytes (Carter and Miller 1979). These gametocytes require ~8-10 days to become mature. When an uninfected female mosquito successfully acquires a blood meal from an infectious human host (i.e., with mature gametocytes), it ingests the gametocytes – the sexual stage of the *Plasmodium*. These gametocytes invade the midgut wall of the mosquito and then enter the basil lamina, forming an oocyst. After approximately 10 – 12 days, the oocyst maturation is complete and it divides into sporozoites. These sporozoites then migrate to the salivary glands where they can be passed on to a human host following a mosquito blood meal.

Anopheles gambiae, like most mosquito species, are anautogenous (i.e., require a blood meal before they can develop eggs). The life cycle of this mosquito is as follows: Female adult mosquitoes lay their eggs in batches in body of stagnant water. Larvae emerge from these eggs. These larvae feed on organic matter (generally algae) in the aquatic habitat, although sometimes they have been known to cannibalize smaller counterparts. After transitioning through four larval instars, a process that takes 7 to 10 days, the larvae pupate, at which point feeding stops. Individuals emerge as adults within 2 days (Clements 1992). The emerging (teneral) adults then mate in swarms, where males form a swarming cloud, and females enter the cloud (Takken 2005, Clements 1999). Males then locate females based on their lower wing beat frequency (Belton

1994). Following mating, a female must then acquire a blood meal to produce eggs. *Anopheles gambiae* in particular is highly anthropophilic and endophilic and is most active during the mid-scotophase. Mosquitoes locate blood hosts using a variety of cues. Long-range attraction is stimulated by CO_2 (Gillies 1980), and short-range attraction is stimulated by odour and temperature profiles of blood hosts. *Anopheles gambiae* are foot and ankle biters, and they use the micro-fauna and -flora of the feet to locate suitable biting regions (Knols and De Jong 1996). Once the female has successfully taken and digested the blood meal, the mosquito then selects a pool of stagnant water to lay her eggs. The cycle between taking at least one blood meal, egg laying (oviposition), and then a subsequent blood feed is known as the gonotrophic cycle.

My thesis focuses on behaviour of the mosquito vector, with the end goal of considering the epidemiological implications of mosquito behaviour. I focus on female *Anopheles gambiae s.s.* – the principal vector of *Plasmodium falciparum*. Although the modeling chapters presented in the thesis refer to a simulated mosquito, where possible, the environment and parameters used are for *A. gambiae*. The broad questions I am interested in are

1. How does a female mosquito's condition regulate her feeding and egg-laying decisions?
2. What is the likely impact of her decisions on parasite transmission?

The approach I take to address these questions is a combination of models and experiments.

One aspect of the feeding behaviour of a female mosquito that is often overlooked is her use of sugar as an energetic resource. Despite the increasing awareness of sugar as an important resource for female mosquitoes, the dietary importance of sugar is poorly understood. Although blood is a prerequisite for egg production, sugar can be used as a resource to sustain flight and somatic maintenance (Foster, 1995). Throughout my thesis, I incorporate the understanding that mosquitoes are omnivores that exploit two disparate resources – blood and sugar. There are several tradeoffs between acquiring a blood or sugar meal, and these are discussed at length in Chapter 2.

In Chapter 2, I consider the role of physiological condition on the behavioural strategy of female mosquitoes when faced with differing availability of resources. *Anopheles gambiae* tend to be very fragile: females will die within a few days without sugar or blood. Therefore, it is reasonable to explore the idea that the choices these mosquitoes make are energy state driven. I explicitly consider the trade-offs between taking sugar and blood meals, the principal among these is that taking a blood meal can lead to a direct contribution to fitness, whereas a sugar meal can prolong life and therefore lead to future contributions to fitness. As with any organism that forages on two distinct resources, there should be some sort of diet choice behaviour that modulates when they should take each of the different resources. I examine the influence of physical and energetic constraints on the feeding decisions of female mosquitoes, with special reference to *Anopheles gambiae s.s.*. Specifically, I use a dynamic state variable model to examine how a female mosquito's energetic condition, abdominal condition, and egg load may affect her decision between taking a blood host or a sugar host. The dynamic state variable model is a suitable approach for this problem because it can explicitly consider the effect of states. I vary the resource availability (i.e., the probability of finding a resource when sought) and use Monte Carlo simulations of the solution to the dynamic state variable model to examine the effect this has on the life of a mosquito.

In Chapter 3, I test the energy-based theory from Chapter 2 with two laboratory experiments using *A. gambiae* females. Specifically, I address the prediction that energetic condition of a mosquito influences their choice between sugar and blood hosts. In the first experiment, which I call the ‘no choice’ experiment, I present a single female mosquito with a blood host (a mouse), and measure the energetic reserves of individual mosquitoes to determine the relationship between energetic condition and host acceptance. In the second experiment, which I call the ‘choice’ experiment, I present mosquitoes of different energetic condition with a choice between olfactory cues for a sugar host and a blood host.

In Chapter 4, I concentrate on the evolution of egg-laying (i.e., oviposition) choices. The oviposition site selection of adults and the ability of their offspring to survive in the environment of their mother’s choosing are both important factors in the ultimate success of an individual; however, these two life stages are often considered separately. I argue that to better understand the oviposition strategies of mosquitoes both larval and adult life characteristics must be considered concurrently. I use an evolutionary game simulation model called a genetic algorithm and allow adult and larval traits to vary (and possibly form links) under different environmental conditions with frequency- and density-dependent processes occurring. This allows me to ask the question of what traits are best suited to handle different environments in light of the intimate link between adult and larval fitness. This will allow me to better understand oviposition behaviour.

In Chapter 5, I apply the understanding of mosquito behaviour developed throughout this thesis to well-accepted measures of a mosquito's ability to transmit the parasite. Specifically, I scale up the role of behaviour from an individual level to the level of disease epidemiology, where the ability of *A. gambiae* to transmit *P. falciparum* in a mosquito-human system. I extend the questions of the effect of resource availability (both sugar and blood) on the ability of a population of mosquitoes to transmit parasites.

Individual behaviour can have a profound impact on population dynamics; however, most models of parasite transmission usually treat mosquitoes as 'flying syringes' (Ribeiro 1995), with no consideration of mosquito behaviour. Throughout the thesis, I have developed an understanding of mosquito behaviour and how this may mitigate the effects of changes in the environment. Importantly, the role of the environment may have a significant impact on feeding decisions, oviposition behaviour, and ultimately survivorship and reproduction of female mosquitoes. All of these facets of a mosquito's biology are likely to play a large role in the ability of the mosquito to transmit parasites, and therefore this thesis allows us to make recommendations on how to better control mosquitoes and vector-borne diseases.

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1.3 Connecting statement

I begin by exploring the state-dependent choices of a female mosquito using a dynamic state variable model. These models are useful in exploring how an individual's state may affect its decisions. This model is used as a building block for subsequent chapters that further explore the role of energy reserves on behaviour.

2: The role of resource availability and state-dependence in the foraging strategy of blood-feeding mosquitoes

A previous version of this chapter was published in Evolutionary Ecology Research. 2008. 10: 1111-1130.

Brian O. Ma and B.D. Roitberg

Brian O. Ma implemented the model, ran the simulations and wrote this manuscript. B.D. Roitberg provided key input on model design and analysis.

2.1 Abstract

Background: Blood-feeding female mosquitoes are omnivores that face tradeoffs and constraints between taking blood meals, sugar meals, and ovipositing their eggs.

Questions: (1) How does a mosquito allocate her time and energy to blood and sugar feeding? (2) How does the availability of sugar and/or blood influence her decisions?

Method and key assumptions: We use a dynamic state variable model to address this question. The model is parameterized from primary literature using *Anopheles gambiae* for reference where possible. The model assumes that female mosquitoes have evolved to make decisions that maximize her lifetime reproductive success.

Conclusions: Blood and sugar have important roles in the life history of blood-feeding female mosquitoes. In the presence of blood hosts, the decision to search for a blood meal is almost always chosen over the search for sugar. However, away from the blood host microhabitat, sugar is readily used. Survivorship and fecundity are increased with an increase in sugar availability. Blood feeding frequency, mosquito fecundity and survivorship are only marginally decreased with decreases in blood availability.

Keywords: Mosquitoes, omnivores, foraging behavior, resource availability, state-dependence, *Anopheles gambiae*, *Aedes aegypti*, vector-borne disease control, dynamic state variable model.

2.2 Introduction

Organisms that consume more than one resource are faced with the dilemma of what to eat and when to eat it. In nature, we see many different ways of solving the problem of diet selection and have come to understand that there are many factors that may cause differences in the decisions an organism makes about how to forage for resources. For instance, patchy resources (MacArthur and Pianka 1966), predator foraging inaccuracies (Fryxell and Lundberg 1998), time-lagged predator responses (Ma et al. 2003) and diet balancing (Pulliam 1975, Westoby 1978) may all play a role in changing the way an individual chooses to forage. One interesting life history that does not fall under any of these typical diet choice scenarios is that of anautogenous blood-feeding female mosquitoes, which require a blood meal before they are capable of laying eggs.

Blood-feeding female mosquitoes are omnivores that exploit two disparate food sources, namely sugar and blood, and therefore must employ a strategy that allows them to allocate time and energy between acquiring the two resources. Sugar, predominately obtained from floral nectaries (Foster 1995) and extra-floral nectaries (Gary and Foster 2004), provides a source of readily available energy for flight and for the accumulation of glycogen (Clements 1955, Nayar and Sauerman 1975). Blood, on the other hand, is primarily used as a source of protein required for the production of eggs. However, blood can also be used as a source of energy reserves but at a lower efficiency than from a sugar meal (Van Handel 1965). Clearly, sugar is the more profitable resource for the accumulation of energy reserves, and blood meals can be used for the accumulation of energy reserves but only at the expense of egg production.

To produce offspring, a female must take a blood meal, develop eggs, and lay her eggs at a suitable oviposition site. For many species of mosquitoes, blood hosts and oviposition sites are spatially segregated; thus, a mosquito must travel from one habitat to another in order to successfully lay eggs (Kauffmann and Briegel 2004, Le Menach et al. 2005). Furthermore, there is a physical tradeoff for abdominal space between blood in the midgut and sugar in the crop; i.e., a replete blood meal excludes the possibility of a replete sugar meal and vice versa (Foster and Eischen 1987, Takken et al. 2001, Fernandes and Briegel 2005), which is further complicated by spatial constraints imposed by fully developed eggs (Klowden 1981). Blood feeding is associated with a higher risk of death than sugar feeding because of host defensive behavior (Walker and Edman 1985). Engorged mosquitoes are heavier, thereby limiting their ability to fly (Nayar and Sauerman 1971, Foster 1995) and escape predators (Roitberg et al. 2003).

Feeding decisions of a female mosquito are affected by her physiological state (Briegel 2003), and studies on the nutritional status of a mosquito are of importance when considering the tradeoff between blood and sugar feeding. Small-bodied mosquitoes face relatively higher energetic deficits, and therefore may behave very differently than large-bodied, energy-rich conspecifics (Takken et al. 1998b), and starved mosquitoes are more likely to approach a blood host than a mosquito engorged on blood (Klowden 1986).

Resource availability also may affect the decisions of a mosquito. Sugar feeding depends on the seasonal availability of floral nectaries (Andersson 1990), but there has been little work done on how varying sugar availability might affect the tradeoff between blood and sugar feeding of mosquitoes.

In light of our understanding of the tradeoffs and constraints on blood and sugar feeding as well as the state-dependence of these feeding decisions, the biological complexity associated with diet choice in the blood-feeding female mosquitoes is not easily elucidated. For instance, reducing the number of blood hosts should lead to, on average, a decrease in the blood meals taken by a population of female mosquitoes; however, mosquitoes with fewer opportunities to blood feed may instead feed at increased frequency relative to scenarios where blood meals are readily available (i.e., increased biting rate). Furthermore, decreasing the supply of sugar should lead to, on average, an increase in blood meals taken by a population of female mosquitoes because blood may be used to fuel somatic maintenance in the absence of readily available sugar sources (Takken et al. 1998b).

To better understand the interaction between foraging for these disparate resources, we present a dynamic state variable model of mosquito foraging behavior where the tradeoffs, constraints, and state-dependence of feeding decisions are explicit (Mangel and Clark 1988, Mangel and Ludwig 1992, Houston and McNamara 1999, Clark and Mangel 2000). We apply the model to answer two questions of interest. (1) Under what physiological conditions should a female mosquito undertake a particular feeding decision? (2) How does the availability of sugar and/or blood influence these feeding decisions?

2.3 The Model

We construct a dynamic state variable (DSV) model of female mosquito feeding decisions over her lifetime. This type of model allows us to explicitly explore the importance of individual physiological states on flexible decision making policies (Clark and Mangel 2000). We assume that a female mosquito has evolved to make decisions that maximize her lifetime reproductive success given a particular combination of physiological states. Specifically, the physiological states we consider are blood in the midgut (B), sugar in the crop (S), nutritional reserves (R), and egg reserves (E). Additionally, we include a location state (L) because we consider a situation where resource availability is dependent on location – indoors or outdoors. Additionally, we assume that the mosquito is omniscient; i.e., has full knowledge of the availability of resources in the environment. These resources are assumed to be constant and do not vary with season. Furthermore, we assume that all blood hosts are indoors because they are most active when blood hosts are sleeping, and we do not explore 'alternate' blood hosts.

The activities that the female mosquito can do are (1) search for a blood host (denoted as the subscript *blo*), (2) search for a sugar host (*sug*), (3) search for an oviposition site (*ovip*), (4) rest (*rest*), or (5) leave her current location (*leave*). The outcome of a particular decision is denoted as either failure (=0) or success (=1) based on a probability, $\lambda_{k,m}$, of finding resource k in the location m. There is no $\lambda_{k,m}$ term associated with the decision to rest, as we assume that if an individual chooses to rest, it can do so at any time. For each decision (i) and outcome (j), there is an associated metabolic cost, $\alpha_{i,j}$, and time cost, $\tau_{i,j}$.

An adult mosquito acquires energy from the environment only when blood or sugar feeding. Sugar can only be used to produce energy reserves, but blood can be used to produce eggs and/or nutritional reserves. Ingested sugar (S) enters the crop, where it is transferred to nutritional reserves (R) at a constant rate (σ). Similarly, blood is transferred to nutritional reserves at a constant rate β_r and to eggs at β_e. Blood and sugar are converted into reserves with conversion efficiencies of c_b and c_s respectively. Blood is converted into eggs as a decelerating function of blood state (Anderson and Roitberg 1999, Roitberg and Gordon 2005) and at a conversion rate of c_e. For each foraging decision made, the mosquito also allocates her existing blood energy between reserves at a proportion ψ or egg production at a proportion of (1-ψ) to maximize her lifetime reproductive success. Thus, the general form of the basic flow of energy into and between states, which is the same regardless of activity, is described by the equations 1a-d.

$$B(t+\tau_{i,j}) = B(t) - \psi\beta_r B(t) - \left(1-\psi\right)\beta_e B(t) \qquad \text{(eq. 1a)}$$

$$S(t+\tau_{i,j}) = S(t) - \sigma S(t) \qquad \text{(eq. 1b)}$$

$$R(t+\tau_{i,j}) = R(t) + c_b\psi\beta_r B(t) + c_s\sigma S(t) \qquad \text{(eq. 1c)}$$

$$E(t+\tau_{i,j}) = E(t) + c_e\left(1-\psi\right)\beta_e B(t) \qquad \text{(eq. 1d)}$$

Furthermore, if a female chooses to leave her current location (L), then her state is updated to the other patch (e.g., if L = indoors, and the individual leaves then L' = outdoors).

There are several restrictions placed on the state values. Each state is bound by a ceiling (X_{max}) and floor value (X_{min}), where X represents a generic state variable. The floor value for all states is set to $X_{min} = 0$. In addition, to account for the tradeoff between midgut and crop space in the abdomen, we make the maximum volume of both blood (B_{lim}) and sugar (S_{lim}) that can be ingested functions of the current blood and sugar volumes. We additionally assume that B_{lim} is a linearly decreasing function of egg load because the presence of mature eggs imposes a small physical limitation on available midgut space (Klowden 1981). The limit for blood and sugar for a given feeding event are expressed using equations 2a-b respectively,

$$B_{lim} = B_{0\max} - (1 - v)\, S(t) - B(t) - E(t) \qquad \text{(eq. 2a)}$$

$$S_{lim} = S_{0\max} - (1 - v/\gamma)\, B(t) - S(t) \qquad \text{(eq. 2b)}$$

where $B_{0\max}$ and $S_{0\max}$ represent the capacity for blood in an empty midgut and sugar in an empty crop respectively, v represents the maximum proportion of midgut size available with a full crop, and γ represents the size ratio of the midgut and the crop. We use these phenomenological linear functions because they are simple ways of reflecting the abdominal space constraint, and, furthermore, we can find no evidence that suggests any non-linearity. Finally, we assume that a critical level of egg production (E_{crit}) must be reached for egg maturation, below which the mosquito is assumed to have immature ovarian follicles, and therefore cannot lay eggs (Briegel 1990).

Basic mortality is modelled as a linearly increasing function of weight (Roitberg et al. 2003), which in turn is a linearly increasing function of current resource states. The basic mortality associated with flight in location m to undertake decision i, including leaving, is represented by $\mu_i(b,s)$ (eq. 3),

$$\mu_i(b,s) = \left(\frac{\mu_{\max,i,m} - \mu_{\min,i,m}}{\gamma B_{0\max} + S_{0\max}} (\gamma b + s) + \mu_{\min,i,m} \right), \qquad \text{(eq. 3)}$$

where $\mu_{max,i,m}$ and $\mu_{min,i,m}$ represents the probability of dying at maximum and minimum weight respectively. This function is a simple representation of mortality that assumes a linear relationship between weight and mortality because we can find no studies suggesting that mortality is a non-linear function of body weight. Mortality is applied each time step in a geometric fashion. An additional mortality cost, $\mu'(b,s)$, is associated with blood feeding which is due to the defensive behavior of the blood host. This function follows the same form as eq. 3 but uses μ'_{max} and μ'_{min}.

An individual female mosquito's expected fitness, $F(b,s,r,e,l,t)$, can be decomposed into two components: the direct contribution to fitness between times t and t+1, and the expected contribution to fitness from time t+1 onwards (i.e., future fitness) given that the mosquito survives to until that time (Mangel and Clark 1988, Mangel and Ludwig 1992, Houston and McNamara 1999, Clark and Mangel 2000). Because of the spatial nature of the model, fitness indoors and outdoors mirror one another and are calculated independently of one another. The only decision that returns a direct contribution to fitness is the decision to oviposit, with a conversion of egg state into eggs of c_e. The fitness from blood feeding, sugar feeding, and resting are based on the expected contribution to fitness after time t+1 onward given that the mosquito survives. The fitness from leaving is based on the best choice from the other habitat from time t+1 onwards weighted by the probability of surviving the flight from one location to the other.

The basic change in state variables described in equations 1a-d are used below as $B(t-\tau_{i,j})=b'$, $S(t-\tau_{i,j})=s'$, $R(t-\tau_{i,j})=r'$, and $E(t-\tau_{i,j})=e'$. The expected future fitness values are weighted by the probability of that activity occurring ($\lambda_{i,j}$) as well as the probability of surviving that activity ($\mu_i(b,s)$). Furthermore, during each activity, the individual maximizes fitness by allocating blood energy between energy reserves or egg production based on the proportion ψ. The fitness gain for each activity is given, for a location *m*, below.

(1) Search for a blood host – A successful blood meal will increase the blood state (*B*) by $\varepsilon(b)$, the expected blood meal size, which is drawn from a binomial distribution and bounded between 0 and B_{lim}. The fitness value from seeking a blood host is

$V_{blo}(b,s,r,e,l,t) =$

$$\max_{\psi}[\lambda_{blo,m}(1-\mu_{blo}(b,s))^{\tau_{blo,1}}(1-\mu'(b,s))F(b'+\varepsilon(b),s',r'-\alpha_{blo,1},r',l,t+\tau_{blo,1}) \\ +(1-\lambda_{blo,m})(1-\mu_{blo}(b,s))^{\tau_{blo,0}}F(b',s',r'-\alpha_{blo,0},e',l,t+\tau_{blo,0})]$$

(eq. 4a)

where for the allocation level that yields the highest fitness, the first half of the equation represents the fitness accrued from successfully acquiring a blood meal discounted by the probability of surviving the acquisition of that blood meal (both predators and host defensive behaviour). The latter half of the equation represents the fitness accrued from the unsuccessful search of a blood meal discounted by the probability of surviving the unsuccessful search.

(2) Search for a sugar host – If the individual is successful at finding a sugar host, the sugar state (*S*) is increase by the expected sugar meal size, $\varepsilon(s)$, which is bounded between 0 and S_{lim}. The fitness value from seeking a sugar host is

$V_{sug}(b,s,r,e,l,t) =$

$$\max_{\psi}[\lambda_{sug,m}(1-\mu_{sug}(b,s))^{\tau_{sug,1}}F(b',s'+E(s),r'-\alpha_{sug,1},e',p,t+\tau_{sug,1}) \\ +(1-\lambda_{sug,m})(1-\mu_{sug}(b,s))^{\tau_{sug,0}}F(b',s',r'-\alpha_{sug,0},e',p,t+\tau_{sug,0})]$$

(eq. 4b)

where for the allocation level that yields the highest fitness, the first half of the equation represents the fitness accrued from successfully acquiring a sugar meal discounted by the probability of surviving the acquisition of that sugar meal. The latter half of the equation represents the fitness accrued from the unsuccessful search of a sugar meal discounted by the probability of surviving the unsuccessful search. (3) Search for an oviposition site – Successful oviposition differs from the other actions because successful oviposition means that there is a direct contribution to fitness through egg production, where $c_e E$ is the number of eggs produced. When a successful oviposition has occurred, the egg state, $E = 0$ because all reproductive energy has gone to egg production. The fitness value for seeking an oviposition site is

$V_{ovip}(b,s,r,e,l,t) =$

$$\begin{aligned}&\max_{\psi}[\lambda_{ovip,m}(c_e e' + (1-\mu_{ovip}(b,s))^{\tau_{ovip,1}} F(b', s', r' - \alpha_{sug,1}, 0, l, t + \tau_{ovip,1})\\&+(1-\lambda_{ovip,m})(1-\mu_{ovip}(b,s))^{\tau_{ovip,0}} F(b', s', r' - \alpha_{ovip,0}, e', l, t + \tau_{ovip,0})]\end{aligned}$$

(eq. 4c)

where for the allocation level that yields the highest fitness, the first half of the equation represents the fitness accrued from successfully utilizing an oviposition site discounted by the probability of surviving the act of ovipositing. The latter half of the equation represents the fitness accrued from the unsuccessful search for an oviposition site discounted by the probability of surviving the unsuccessful search.

(4) Rest – Individuals that rest are assumed to always be able to locate a resting location and the fitness value of resting (discounted by the probability of surviving) is

$V_{rest}(b,s,r,e,l,t) =$

$$\max_{\psi}[(1-\mu_{rest}(b,s))^{\tau_{rest}} F(b', s', r' - \alpha_{rest}, e', l, t + \tau_{rest})]$$

(eq. 4d)

(5) Leave – If an individual successfully leaves its current location, the location state, *l*, is updated to be l'. The fitness value of leaving the current habitat is

$V_{leave}(b,s,r,e,l,t) =$

$$\max_{\psi}[\lambda_{leave,m}(1-\mu_{leave}(b,s))^{\tau_{leave,1}}F(b',s',r'-\alpha_{leave,1},e',l',t+\tau_{leave,1}) \\ +(1-\lambda_{leave,m})(1-\mu_{leave}(b,s))^{\tau_{leave,0}}F(b',s',r'-\alpha_{leave,0},e',l,t+\tau_{leave,0})]$$

(eq. 4e)

where for the allocation level that yields the highest fitness, the first half of the equation represents the fitness accrued from successfully leaving discounted by the probability of surviving the act of leaving. The latter half of the equation represents the fitness accrued from the unsuccessful act of leaving discounted by the probability of surviving the unsuccessful act.

Lifetime reproductive success is maximized via choosing the best activity and allocation decisions at each time step for each state combination, yielding

$$F(b,s,r,e,l,t) = \max[V_{blo},V_{sug},V_{ovip},V_{rest},V_{leave}].$$

(eq. 5)

The optimal decision is calculated by backwards induction from a terminal fitness function $F(b,s,r,e,l,t,T) = 0$ for all combinations of states (Clark and Mangel 2000). We use a large value for our time horizon that is well beyond the expected lifetime of an individual mosquito (T=2500), and allow the model to converge to stationary states. Our convergence criterion is that the decisions for each combination of states do not change for the last 25 time steps. For non-integer state values, we use a four-state linear interpolation to calculate fitness (Clark and Mangel 2000).

The solution to the DSV assumes that the mosquito has evolved to maximize lifetime reproductive success in a given environment with known resource availability. We use Monte Carlo simulations to determine how mosquitoes that have evolved in that environment might behave in novel environmental conditions.

2.3.1 Parameterization of the model

Where possible, we parameterized the model using primary literature sources for the hematophagous endophilic anthropophilic mosquito species *Anopheles gambiae*. *Anopheles gambiae* is arguably the most important parasite-vectoring mosquito because of its role in the transmission of *Plasmodium falciparum*, a parasite that causes malaria in humans. Furthermore, this species is of particular interest to our research group as we maintain and perform empirical studies using this species.

A typical environment for *A. gambiae* has blood hosts and oviposition sites spatially segregated. Blood hosts are predominately found in and around domiciles (which we refer to as 'inside') (Gillies 1954), whereas oviposition sites are predominately found away from houses (which we refer to as 'outside') and near more dense vegetation and thereby a source of sugar hosts. Thus, for our baseline parameter set, we use values of $\lambda_{ovip,out} = 1$, $\lambda_{ovip,in} = 0$, $\lambda_{blo,in} = 1$, and $\lambda_{blo,out} = 0$. All other $\lambda_{k,m}$ values are set to 1 for simplicity but $\lambda_{blo,in}$, $\lambda_{sug,out}$ and $\lambda_{sug,in}$ are varied during our sensitivity analysis.

We set all metabolic costs ($\alpha_{i,j}$) as linear functions of time, where resting and unsuccessful decisions have $\alpha_{i,j} = 1$, and the metabolic energy required for successful actions have a 1:1 relationship with the time spent for each decision ($\tau_{i,j}$). We break the day into 15 minute steps, where unsuccessful decisions are associated with a $\tau_{i,0} = 1$ and successful decisions have $\tau_{i,1} = 2$, except for the decision to leave, which we assume takes one hour (i.e., $\tau_{leave,1} = 6$).

The maximum volume of blood in the midgut (B_{0max}) and sugar in the crop (S_{0max}) for *A. gambiae* are estimated at 4 μL and 1 μL respectively (Briegel 1990), which also yields a relative size ratio of midgut to crop of $\gamma = 4$ and a maximum proportion of midgut size with a full crop of $v = 0.8$. Without access to energy resources, a female mosquito will die within a few days due to starvation (Briegel 1990); thus, we use R_{max}=64 which means that mosquitoes that do not feed (or conversely, rest the entire time) will die within a few days. This also allows for sufficient resolution for energy lost to metabolism from activity as well as for differences between the conversion of blood and sugar to reserves.

Blood must be broken down by proteolytic enzymes and therefore is processed more slowly than sugar; Van Handel (1965) estimated that reserve accumulation by sugar is ten times faster than it is for blood. Nayar and Van Handel (1971) determined that *Aedes taeniorhynchus* and *Aedes sollicitans* mosquitoes flown to exhaustion could fly almost immediately after a sugar meal but required 4-6h to fly after imbibing a blood meal and could only do so for short periods. To capture this difference, we use a conversion rate of blood to reserves (β_r) of 1 and a conversion rate of sugar to reserves (σ) of 10. We also include a conversion rate of blood to egg production (β_e) set to unity. The conversion efficiency of blood to reserves (c_b) is considered to be lower than the conversion efficiency of sugar to reserves (c_s) because of the additional processes necessary for digesting a blood meal. An unmetabolized sugar meal supplies over 10 times the flight range of blood meals (Nayar and Van Handel 1971), and thus, we use c_b = 1 and c_s = 10 for our analysis.

The conversion of the egg reserve state to eggs for *A. gambiae* taking a complete blood meal yields an eggload of 90 – 120 eggs (Briegel 1990, Fernandes and Briegel 2005, Roitberg and Gordon 2005). We discretize the egg state into ten units (E_{max} = 10) and assume that the conversion of egg state into eggs is c_e = 10; thus, a female with a full egg state can produce 100 eggs. Briegel (1990) determined that no eggs matured when the energy invested in egg development was less than 1.3 cal for *A. gambiae*, which as a ratio of the total calories from a blood meal, corresponds to a threshold egg reserve state of E_{crit} = 4.

For *A. gambiae*, the daily mortality rate estimated from a mark-recapture study is 0.22 (Takken et al. 1998a) which, given our 15 minute time steps and assuming that mortality is independent of time, translates to a 0.005 hourly mortality rate. We use this as our baseline mortality rate ($\mu_{min,i}$) for all decisions except resting, which we assume is very safe and does not change with weight (μ_{rest}=0.001). Accurate measures of mortality rates for each decision are difficult, and thus, we can only make qualitative assumptions about the relative risk of death for each decision a mosquito makes. As weight increases, we assume that a mosquito is five times as likely to die, yielding a mortality rate at full weight ($\mu_{max,i}$ = 0.025). We assume that there is no difference in the mortality rate functions for the search for a sugar host, blood host, or oviposition site because we have no data that states otherwise. Additional mortality associated with blood-host defensive behavior is assumed to be μ'_{min} = 0.05 and μ'_{max} = 0.25. For simplicity, we also assume that the mortality rate function for leaving is the same as for the other decisions.

2.4 Methods

We develop the decision-making policy of a female mosquito for the DSV model coded using the programming language C (source code available upon request). We focus our analysis on the availability of resources in the environment, with most of our attention given to the availability of sugar indoors ($\lambda_{sug,in}$), outdoors ($\lambda_{sug,out}$), and the availability of blood hosts indoors ($\lambda_{blo,in}$). We also explore the model's robustness by performing a sensitivity analysis on survivorship, where we vary the steepness of the mortality curve with respect to weight, and we vary the risk of host defensive behavior while blood-feeding. We also examine the robustness of our results to changes in the time ($\tau_{leave,1}$) and costliness ($\alpha_{leave,1}$) of leaving. Our interpretation of the results is divided into what a mosquito chooses indoors and outdoors, and we focus on the decisions to blood- and sugar-feed, as for the most part, the trends for where the other two decisions, oviposition and resting, occur remain qualitatively similar. The methodology and corresponding results are divided into two major sections: the DSV deterministic model and the Monte Carlo simulations.

2.4.1 DSV Model

Because of the complex nature of state space (i.e., excluding time as a state, we have five states over which decisions can vary), we first divide the analysis into indoor and outdoor components. We summarize the proportion of the total state space where making each of the five possible decisions maximizes lifetime reproductive success for a given parameter set. However, this does not tell us everything about the physiological conditions under which a mosquito should blood or sugar feed. Thus, we also take two-dimensional slices of state space, where two states vary and two states are fixed. We concentrate on three scenarios that we think are the most biologically interesting: (1) blood (*B*) and reserve (*R*) states vary (and sugar (*S*) and egg (*E*) states are fixed at 0), (2) *S* and *R* vary (and $B = E = 0$), and (3) *E* and *R* vary (and $B = S = 0$).

2.4.2 Monte Carlo simulations

We also simulate a female's lifetime using a Monte Carlo simulation that uses the solution to the DSV model. We assume that mosquitoes have evolved in a world where blood hosts are spatially separated from oviposition sites; this mimics a typical environment in which *A. gambiae* is found. Sugar is readily available outside near oviposition sites but is in limited supply near the blood host habitat.

We simulate mosquitoes in several novel environments, using 5000 individuals per run. Individuals begin relatively malnourished (Beier 1996), where their initial energy reserve state is drawn from a normal distribution with a mean of ~10% of the maximum energy; i.e. $R = 5 \pm 0.5$ (mean ± S.E.)(Walker 2008). The quantity of blood or sugar taken on a given feeding event is randomly drawn from a binomial distribution where the proportion of large meal sizes is high; i.e., we assume that most mosquitoes feed to near repletion (n=10, p=0.7).

We incrementally increase and decrease the availability of blood (inside) and sugar (inside and outside) and track several key outcomes of the simulations. We track the frequency of each decision made over the lifetime of the mosquito; this is analogous to the proportion of the total state space results from the DSV component but gives us a better idea of the state space that is most commonly experienced by an individual. Because the decisions an individual makes will likely affect its survivorship and fecundity, we also track mean values for longevity (i.e., life expectancy from birth), number of gonotrophic cycles per individual, number of blood and sugar meals per gonotrophic cycle, and egg production.

2.5 Results

2.5.1 DSV model

Several general patterns emerge from the results of the DSV model. We first examine the entire decision space across the blood, sugar, reserve and egg states, keeping in mind that much of the decision space included in these summary statistics may not be readily attained by the mosquito. Then, from the examination of the two-dimensional plots of state space, we gain a better understanding of what the best decisions are under different state combinations.

The decisions made around the houses (inside) and around the vegetation (outside) are very different. In the habitat ‘inside’ most of the decision space is dominated by resting (97%). Of the remaining decision space, 48% is to leave the inside, 22% is to search for a blood meal, and 30% is to search of a sugar meal. In the habitat ‘outside’ the decision to rest is the best for 89% of the decision space. Of the remaining decision space, the most frequent decision is to oviposit (93%) whereas searching for a sugar meal is 5% and the decision to leave is 2%.

Generally, a female mosquito will begin its life outside at low energy reserves and an empty abdomen. Under these conditions, the best decision is to search for a sugar meal (Fig. 2.1 a,b). If an individual successfully imbibes a sugar meal, and that sugar meal is large, it will rest and allow for the sugar meal to be processed (Fig. 2.1 b). However, if the individual is unsuccessful at finding a sugar meal or the sugar meal is small, it will attempt to take a sugar meal again. Once an individual has successfully imbibed and processed a sugar meal, its reserve state will increase and therefore the best decision is to move inside.

In the inside habitat (Fig. 2.1 d,e), an individual at very low energy reserves with no blood or sugar in the abdomen will search for a sugar meal. Conversely, with higher energy reserves, an individual will take a blood meal. With a small amount of sugar in the crop, at low energy reserves a mosquito will blood feed, and with high energy reserves, an individual will rest (Fig. 2.1 e). Conversely, with blood in the midgut, at low energy reserves an individual will search for a sugar meal, and with higher energy reserves, an individual will rest (Fig. 2.1 d). When egg reserves have accumulated to a sufficient level, which is greater than the critical egg state for egg production to occur, mosquitoes leave the inside provided that they have sufficient energy reserves to make the trip to the outside.   If an individual has insufficient energy to make the trip, it will take a sugar meal to supplement its energy reserves (Fig. 2.1 f).

Once outside with high egg reserves, an individual will oviposit (Fig. 2.1c), then if her energy reserves are low she will take a sugar meal and then rest until her sugar meal is processed; if her energy reserves are high she will immediately return indoors.

Although we have only presented a single parameter set with fixed probabilities of finding resources, the results from the DSV with different probabilities of finding resources follow similar qualitative trends: the changes in the (historical) probability of finding sugar inside does not dramatically change the frequency of each decision. The decisions to blood feed and sugar feed inside are particularly invariable to changes in resources.

2.5.2 Monte Carlo simulations

The frequency of blood feeding declines only when blood becomes very difficult to find (Fig. 2.2). The increase in frequency of blood feeding from low blood availability ($\lambda_{blo,in}$ = 0.1) to high blood availability ($\lambda_{blo,in}$ = 1.0) is ~10%. Conversely, the frequency of blood feeding is highly invariable to changes in sugar availability ($\lambda_{sug,in}$). Note that we do not consider the case where $\lambda_{blo,in}$ = 0 because if no blood is available in the environment, individuals cannot develop eggs and therefore do not contribute to the next generation.

The frequency of sugar feeding inside increases in a decelerating manner with increasing sugar availability inside ($\lambda_{sug,in}$), but is relatively invariable to changes in sugar available outside ($\lambda_{sug,out}$) (Fig. 2.3). However, this relationship only exists when blood availability is low ($\lambda_{blo,in}$ = 0.1). As blood availability increases (i.e., when $\lambda_{blo,in}$ = 0.4 to 0.5), sugar feeding inside becomes negligible, and stops entirely at values of $\lambda_{blo,in}$ > 0.5. Furthermore, the frequency of sugar feeding inside, even at its greatest value (when $\lambda_{sug,in}$ = 0.7; $\lambda_{sug,out}$ = 0.1; $\lambda_{blo,in}$ = 0.1) is ~20 times less frequent than the decision to blood feed.

The frequency of sugar feeding outside increases when blood is scarce (i.e., when $\lambda_{blo,in}$ is low), but does not change with changes in sugar availability inside ($\lambda_{sug,in}$) or outside ($\lambda_{sug,out}$) except when sugar outside is absent (Fig. 2.4). Because the availability of sugar inside ($\lambda_{sug,in}$) also has very little effect on the frequency of sugar feeding outside, we only show a single example of $\lambda_{sug,in}$ = 1.0 (Fig. 2.4). The percent increase in the frequency of sugar feeding outside is at most ~50% from high blood availability to low blood availability.

The mean longevity of an individual is largely unaffected by the availability of sugar inside ($\lambda_{sug,in}$). However, increasing availability of sugar outside ($\lambda_{sug,out}$) increased longevity in an accelerating manner, and increasing blood availability inside ($\lambda_{blo,in}$) increased longevity asymptotically (Fig. 2.5). Overall, the effect of increasing $\lambda_{sug,out}$ had a larger effect on longevity than did increasing $\lambda_{blo,in}$. The most frequent cause of death was starvation, except when resources are abundant (i.e., $\lambda_{sug,out}$ and $\lambda_{blo,in}$ are high) where death resulted from either background mortality while searching for blood hosts, or host defensive behavior.

The mean fecundity was only affected by the availability of blood hosts inside ($\lambda_{blo,in}$) and sugar hosts outside ($\lambda_{sug,out}$) (Fig. 2.6 a). The effect of the availability of sugar hosts inside ($\lambda_{sug,in}$) was negligible. Overall, the effect of increasing $\lambda_{sug,out}$ had a larger effect on fecundity than did increasing $\lambda_{blo,in}$. The increases in fecundity are likely attributable to a combination of an increase in the mean number of gonotrophic cycles (Fig. 2.6 b) and to a lesser degree, an increase in the mean number of eggs per gonotrophic cycle with increasing in $\lambda_{sug,out}$ (Fig. 2.6 c). Additionally, the mean length of the gonotrophic cycle increases with an increase in $\lambda_{sug,out}$, decreases with an increase in $\lambda_{blo,in}$, and is relatively insensitive to changes in $\lambda_{sug,in}$. Even though the gonotrophic cycle is longest when $\lambda_{blo,in}$ is low and $\lambda_{sug,out}$ is high, the overall effect is that there are a greater number of gonotrophic cycles at high $\lambda_{blo,in}$ levels.

2.6 Discussion

We present a model of blood-feeding female mosquito foraging decisions which explicitly considers the tradeoffs between feeding on sugar and blood. The model predicts that both blood and sugar are utilized throughout the mosquito's lifetime, but that the conditions under which each is taken are very different. Although at any given time the acquisition of blood is in direct conflict with the acquisition of sugar, our results suggest that a female blood-feeding mosquito solves the tradeoff between blood and sugar feeding sequentially across the blood host and oviposition microhabitats. The search for blood hosts occurs in the blood host microhabitat. However, in this microhabitat, sugar is largely ignored except when individuals are starved. The search for sugar, on the other hand, generally occurs when mosquitoes are low on energy and away from the blood host microhabitat (and near oviposition sites).

The availability of different resources has implications for different feeding frequencies, survivorship, and fecundity. Our study suggests that a decrease in the availability of blood hosts does not have a large effect on fecundity and little effect on longevity. An increase in blood availability increases fecundity but only marginally increases longevity, even though we explicitly allow mosquitoes to allocate energy from a blood meal towards energy reserves. The frequency of blood feeding is not affected by sugar availability in the blood host habitat, which suggests that within the microhabitat near blood hosts, female mosquitoes prioritize egg production (i.e., direct fitness payoff) in lieu of future fitness. To mitigate the effects of lower host availability, female mosquitoes likely become more persistent in their search for a blood host and sugar feed to avoid starvation. Sugar near oviposition sites is heavily relied upon because after emergence or oviposition, nutritional reserves tend to be low. This is reflected in the response to increased sugar availability – there is a sharp increase in longevity coupled with an increase in fecundity. However, sugar available near blood hosts will only be used when blood is difficult to acquire, and even then, only when reserve levels are very low regardless of the availability.

Our results suggest that sugar is an important component of a female mosquito's life history, even in species that are closely associated with humans and capable of allocating energy derived from blood to fuel activity. Mosquitoes are more likely to search for a sugar source when they are low on energy reserves; this energy-deprived condition is especially prevalent at emergence (Walker 2008) where individuals emerge outside (i.e., at oviposition sites and away from blood hosts). During this initial period, the risk of starvation is very high but can be ameliorated through feeding on sugar (Foster and Takken 2004). When energy reserves are sufficient, our theory says that sugar should be ignored and the mosquito should leave the emergence habitat in search of a blood meal.

The importance of sugar to blood-feeding female mosquitoes has often been overlooked (Foster and Takken 2004), especially for anthropophilic and endophilic mosquitoes such as *Anopheles gambiae* and *Aedes aegypti* (Briegel 1990). Many studies have demonstrated that sugar intake increases the lifespan of mosquitoes (Nayar and Sauerman 1975, Andersson 1992, Straif and Beier 1996, Okech et al. 2003, Gary and Foster 2004), yet sugar feeding in anthropophilic endophilic mosquitoes is often thought to be infrequent and incidental (Edman et al. 1992, Foster 1995, Beier 1996). For instance, in a census study, a very low proportion of sugar-positive *A. gambiae* female mosquitoes was found around blood host microhabitats (Beier 1996). The reason for the under-representation of sugar feeding may be due to the location of sugar feeding. The reliance on sugar away from blood host microhabitats predicted by the model suggests that census data for sugar-positive females captured near blood host microhabitats (e.g.

Beier 1996) will grossly under-represent the frequency of sugar-feeding by mosquitoes throughout their lifetime.

The resource acquisition dilemma faced by mosquitoes is similar to the dilemma faced by some host-feeding parasitoids. Like mosquitoes, these parasitoids use sugar sources to fuel maintenance (Jervis and Kidd 1986, Jervis and Kidd 1999), and also use their hosts as a source of nutrients required for egg maturation (Heimpel and Collier 1996). Empirical evidence suggests that in host-feeding parasitoids, host-feeding occurs when energy reserves are low, and oviposition occurs when energy reserves are higher (Heimpel and Collier 1996). Furthermore, studies have shown that sugar is required by some host-feeding parasitoids in order to experience the benefit of host-feeding (Heimpel et al. 1997). Unlike the mosquito system, egg-laying in the host and the potential source of egg-laying nutrients (i.e., the host) are within the same microhabitat, and potential sources of sugar such as floral nectaries are spatially separate (Bernstein and Jervis 2008). For female mosquitoes, the microhabitat in which the direct fitness payoff of oviposition is linked with sugar sources, and the microhabitat linked with the resource required to oviposit (i.e., blood) are spatially separate. There have been numerous models of the tradeoff between host-feeding and oviposition (reviewed in Jervis and Kidd 1986, Heimpel and Collier 1996, Jervis and Kidd 1999), and at least one model of the tradeoff between sugar feeding and oviposition in non-host-feeding parasitoids (Clark and Mangel 2000, Bernstein and Jervis 2008), but we know of no models that explicitly consider the interaction between sugar feeding, host-feeding, and oviposition behavior.

The model presented in this paper complements and expands on existing models of mosquito decision making (e.g. Roitberg and Friend 1992). Previous models have considered limited decisions over a single gonotrophic cycle, whereas the model presented in this paper presents a mosquito with an entire suite of decisions over her entire lifetime. We explicitly track the egg state of a female mosquito, which is important because of the direct fitness benefit that oviposition entails. We also allow for the possibility of multiple blood or sugar meals within a single gonotrophic cycle, as well as carryover of nutritional reserves from one gonotrophic cycle to the next. This latter characteristic can lead to different decisions being made with each gonotrophic cycle, which becomes especially important in light of the fact that the decisions made during the first gonotrophic cycle are sometimes different than in subsequent gonotrophic cycles (Takken et al. 1998b). Furthermore, considering more than one gonotrophic cycle is important because parasites such as *Plasmodium spp.* have an incubation period that is greater in duration than a gonotrophic cycle.

Our model could be expanded in several ways. Physiological states we did not consider that may affect feeding decisions are age (Xue et al. 1995, Anderson and Roitberg 1999), body size (Takken et al. 1998b), and the presence of parasites (Koella 1998, Anderson and Roitberg 1999). Our analysis could be expanded to include the availability of oviposition habitat; we recognize that reduction in oviposition sites reduce the ability of the mosquitoes to transmit malaria (Gu et al. 2006). Finally, we caution against extrapolating our model predictions to situations where blood hosts and oviposition sites are not spatially segregated. We only consider a resource distribution scenario that is representative of anthropophilic endophilic mosquitoes such as *A. gambiae*.

2.6.1 Implications for mosquito and vector-borne disease control

The results from our model have implications for mosquito and vector-borne disease control. Our results suggest that controlling the availability of sugar near oviposition sites would be the best way of controlling mosquito populations, as even a small decline in the availability of this resource has a noticeable effect on female mosquito fecundity and survivorship. If sugar was removed completely, even in the presence of blood, some theory developed by Stone et al. (2009) suggests that a population of mosquitoes would decline because of reduced insemination of females by sugar-deprived males. Conversely, there has to be a significant reduction in blood host availability to reduce the survivorship and fecundity of the mosquitoes. In practice, bed nets have been used to limit a mosquito's access to blood hosts; i.e., reduce blood host availability. However, our results suggest that a very large proportion (~50%) of individuals would have to be covered by bed nets for there to be a significant reduction in mosquito survivorship and fecundity.

For vector-borne disease control, mosquito control is further complicated because the biting rate of the mosquito must be considered. In the case of *A. gambiae* transmitting *Plasmodium falciparum*, the parasite that causes malaria in humans, the parasite can be transmitted to and from the mosquito even from an unsuccessful blood feed; i.e. when the mosquito has not taken a complete blood meal or has only been allowed to probe. Furthermore, once inside the mosquito, *P. falciparum* requires approximately 10 days to reach the infectious stage. Therefore, the increased persistence of individuals when blood meals are difficult to acquire may in fact mitigate the effect of lower availability. However, both unsuccessful blood meals and multiple blood meals within a gonotrophic cycle can increase the likelihood of transmitting the disease (Briegel and Horler 1993). We do not explicitly consider disease transmission in our model, but the predictions from other studies are conflicting. Some have suggested that reduced sugar feeding can drastically reduce the chances of transmitting the disease (Impoinvil et al. 2004), while others have suggested that blood feeding in lieu of a sugar meal can lead to a higher transmission rate (Gary and Foster 2001).

Given these advances in our understanding of the link between physiological state of the mosquito and feeding decisions, we need to reconsider the assumption of constant biting rates prevalent in most disease-vector models. Incorporating more complex vector behavior into analytical models of mosquito-host dynamics may give us a better understanding of how to control vector-borne diseases because it will more accurately account for how environmental and physiological factors influence the resulting dynamics. The tradeoffs in the acquisition and allocation of resources result in nonlinear functions relating the resource availability and feeding decisions which may have important implications for disease epidemiology.

2.6.2 Summary

We designed a state-dependent model of mosquito behavior based on first principles using the problems that a female mosquito faces. Specifically, given the tradeoffs and constraints associated with blood, sugar, and egg acquisition, when should a blood-feeding female mosquito sugar feed, blood feed or oviposit? Our results emphasize the intimate link between survivorship and fecundity in female mosquitoes and demonstrate the importance of sugar feeding in a female mosquito's life history. With more sugar available, mosquitoes live longer and therefore take more blood meals and complete more gonotrophic cycles. Even with this simple model, the behavior of the mosquitoes is consistent with our understanding of mosquito behavior in nature.

2.7 Acknowledgements

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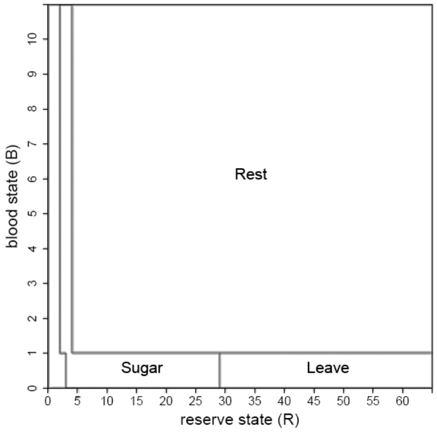
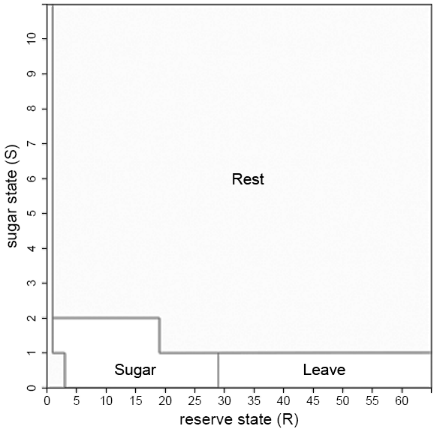
2.8 Figures

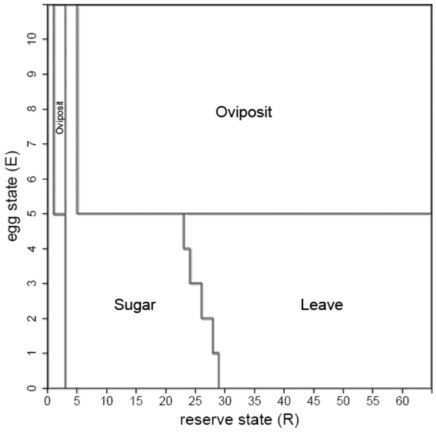
Figure 2.1: The best decision for a mosquito under different physiological state combinations based on the DSV results where two states vary and the other two states are fixed. The spaces where each decision is best are labeled on each figure: ‘Blood’ is the decision to search for a blood host; ‘Sugar’ is the decision to search for a sugar host; ‘Rest’ is the decision to rest; ‘Oviposit’ is the decision to search for an oviposition site; ‘Leave’ is the decision to leave the current habitat. Panels a-c represent decision space ‘outside’; panels d-f represent decision space ‘inside’. In panels a and d, sugar state (S) = egg state (E) = 0; in panels b and e, blood state (B) = egg state (E) = 0; in panels c and f, blood state (B) = sugar state (S) = 0.


blood state (B)
reserve state (R)
Rest
Sugar
Leave
0 1 2 3 4 5 6 7 8 9 10
0 5 10 15 20 25 30 35 40 45 50 55 60

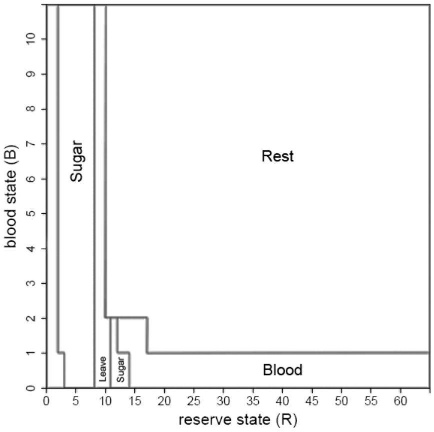
a)


sugar state (S)
reserve state (R)
Rest
Sugar
Leave
0 1 2 3 4 5 6 7 8 9 10
0 5 10 15 20 25 30 35 40 45 50 55 60

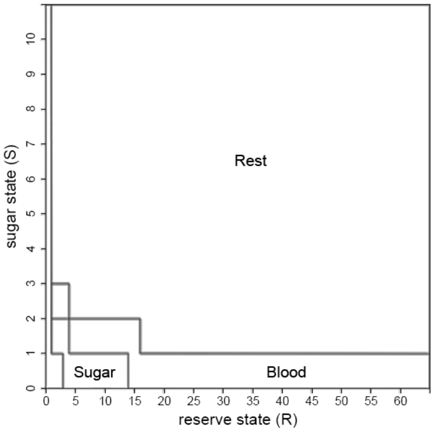
b)


egg state (E)
reserve state (R)
Oviposit
Oviposit
Sugar
Leave
0 1 2 3 4 5 6 7 8 9 10
0 5 10 15 20 25 30 35 40 45 50 55 60

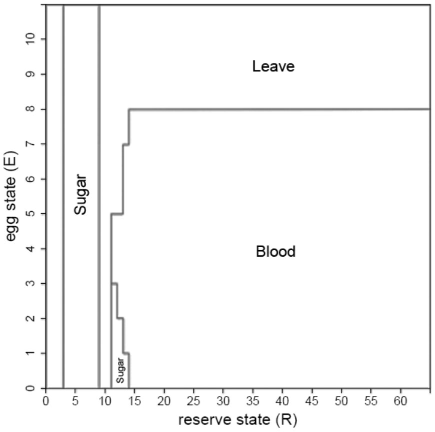
c)


blood state (B)
reserve state (R)
Sugar
Rest
Leave
Sugar
Blood
0 1 2 3 4 5 6 7 8 9 10
0 5 10 15 20 25 30 35 40 45 50 55 60

d)


sugar state (S)
reserve state (R)
Rest
Sugar
Blood
0 1 2 3 4 5 6 7 8 9 10
0 5 10 15 20 25 30 35 40 45 50 55 60

e)


egg state (E)
reserve state (R)
Leave
Sugar
Blood
Sugar
0 1 2 3 4 5 6 7 8 9 10
0 5 10 15 20 25 30 35 40 45 50 55 60

f)

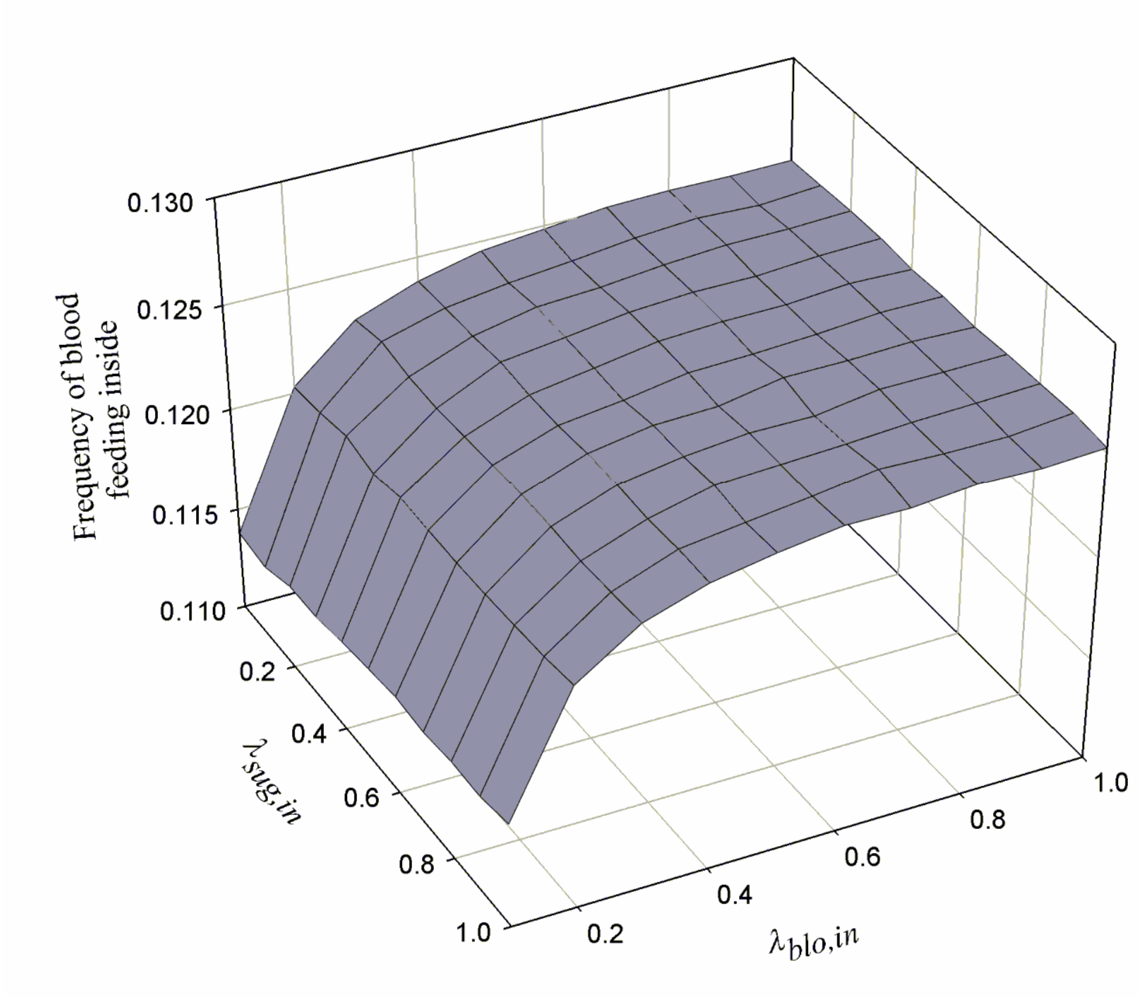


Figure 2.2: The frequency of blood feeding by a mosquito in the inside habitat as a function of the availability of sugar ($\lambda_{sug,in}$) and blood in the inside habitat ($\lambda_{blo,in}$). The results presented are for the probability of finding sugar outside ($\lambda_{sug,out}$) of 1.0. This figure is representative of the same relationship for values of $\lambda_{sug,out}$ from 0.1 to 1.0.

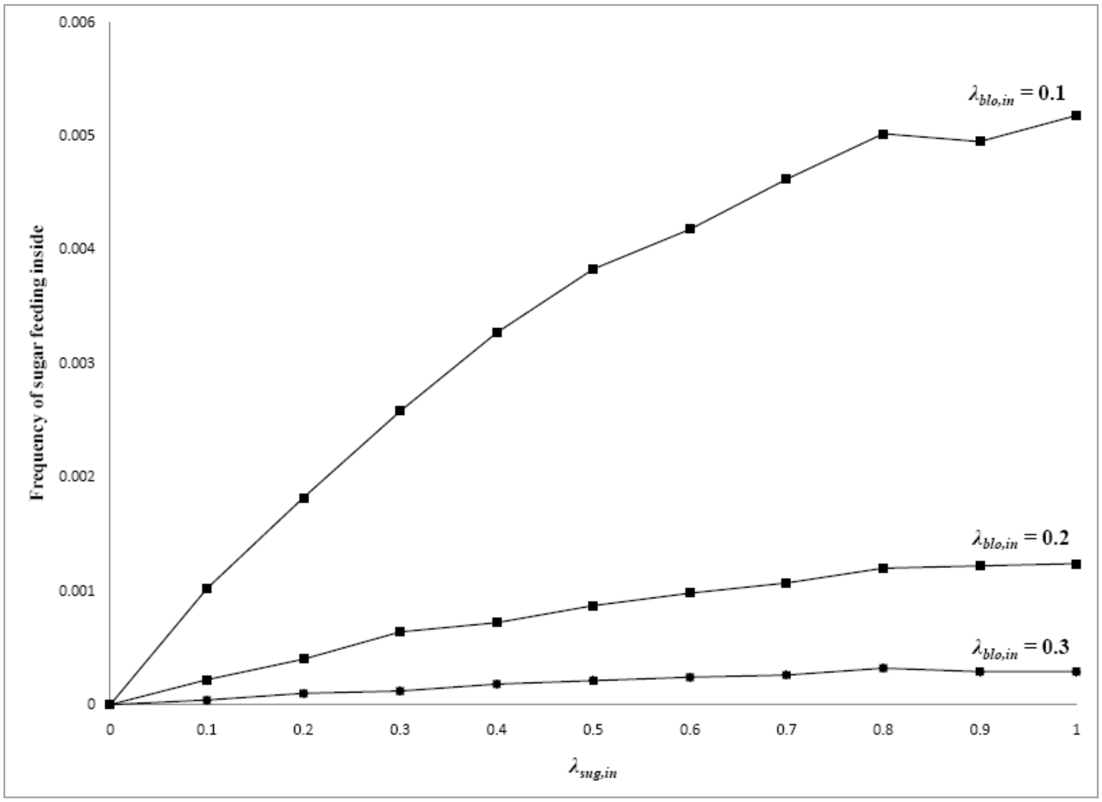


Figure 2.3: The frequency of sugar feeding by a mosquito in the inside habitat as a function of sugar availability inside ($\lambda_{sug,in}$). The sugar availability outside ($\lambda_{sug,out}$) is 1.0. This figure is representative of the same relationship for values of $\lambda_{sug,in}$ from 0.0 to 1.0.

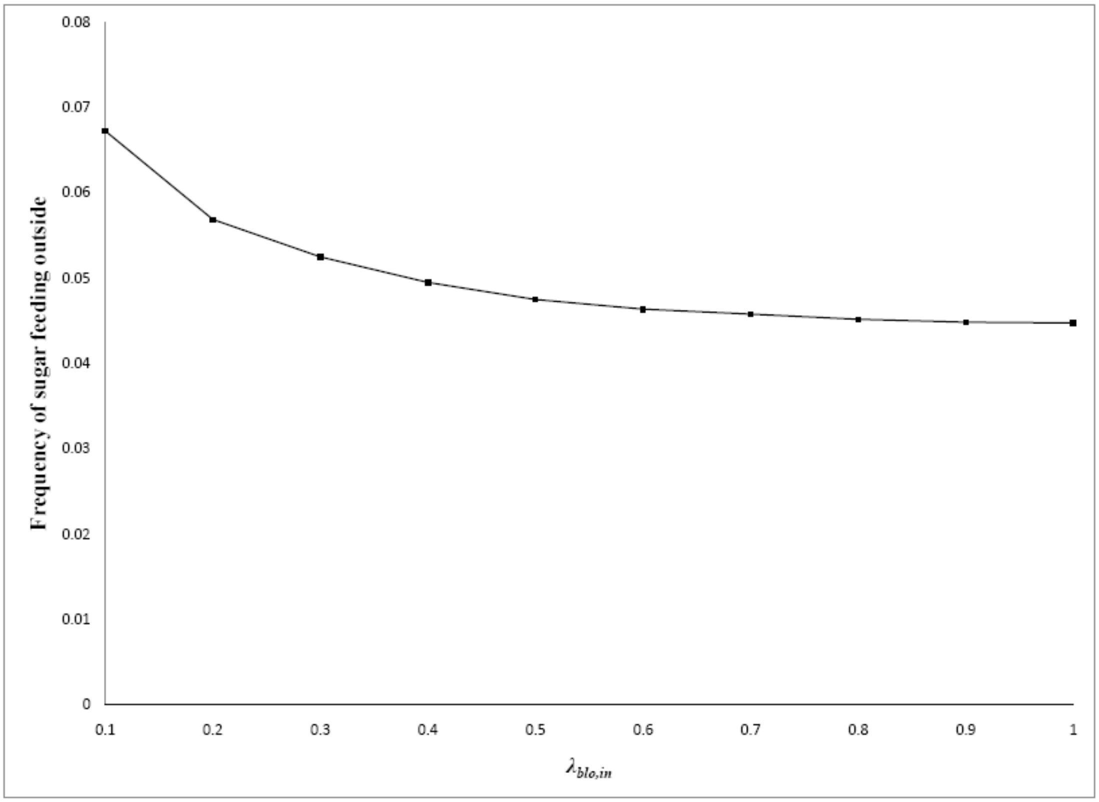


Figure 2.4: The frequency of sugar feeding by a mosquito in the outside habitat as a function of blood availability ($\lambda_{blo,in}$) at a sugar availability inside ($\lambda_{sug,in}$) of 1.0. This figure is representative of the same relationship for values of $\lambda_{sug,in}$ from 0.0 to 1.0.

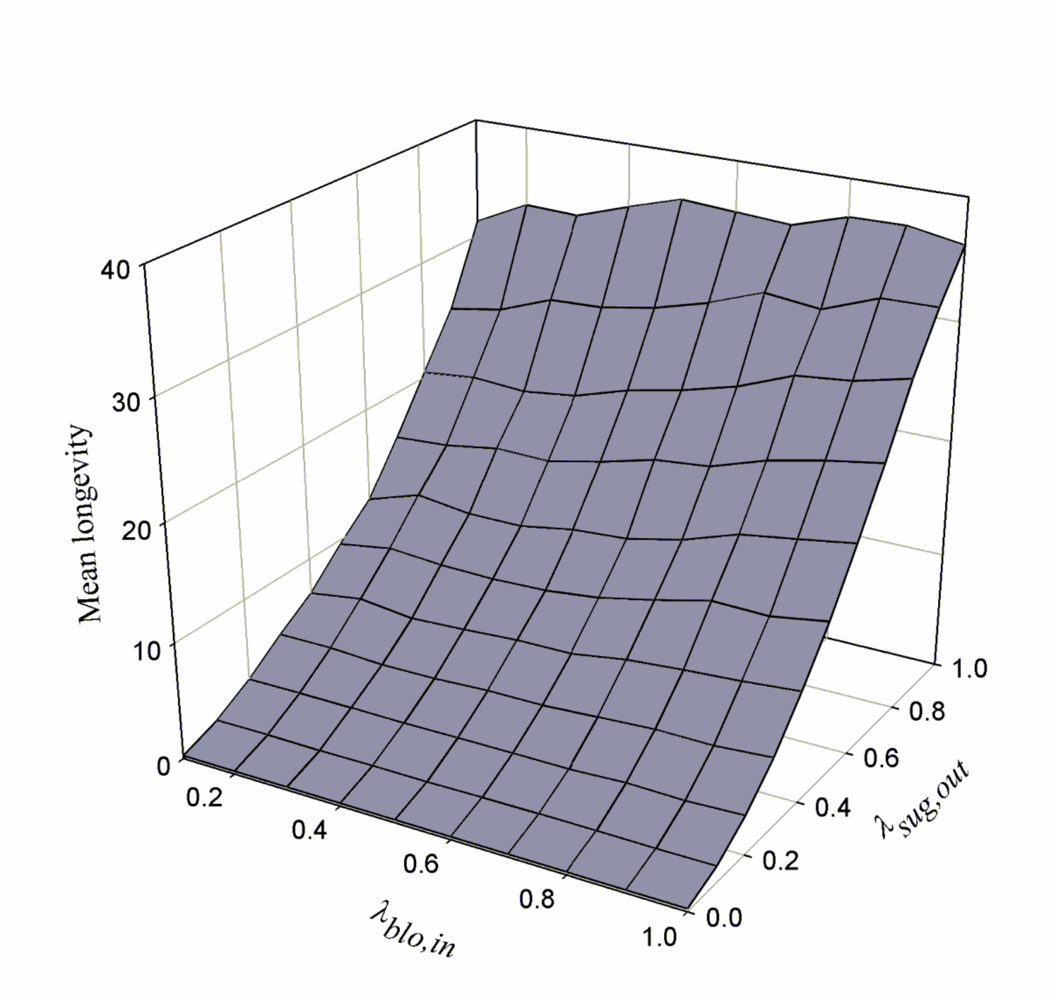
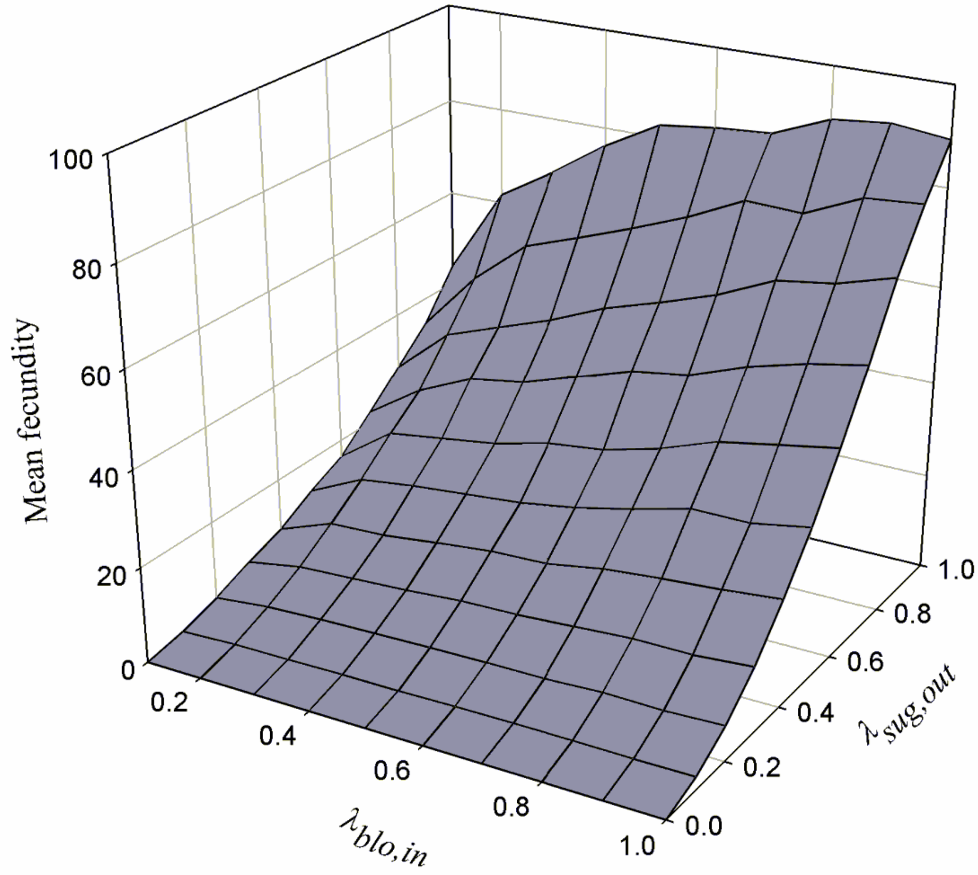


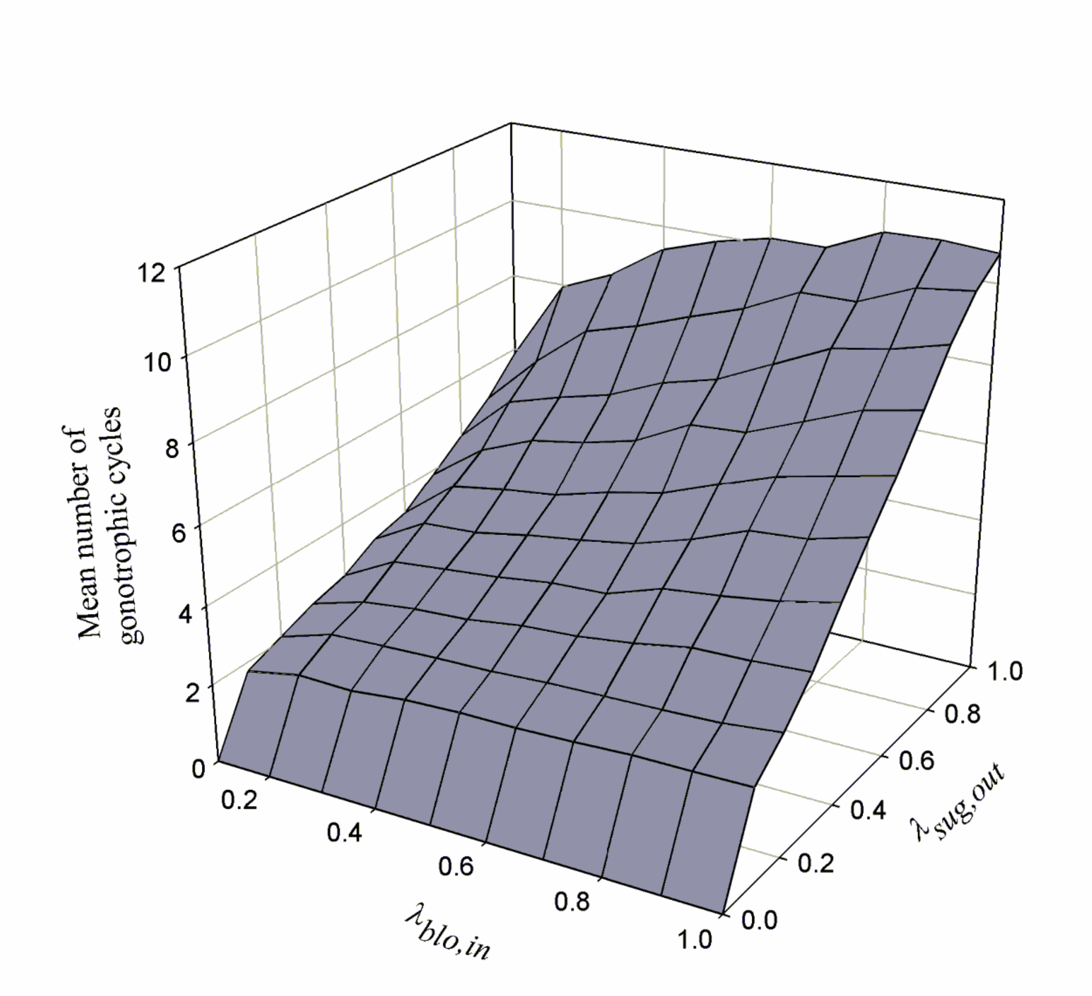
Figure 2.5: Mean longevity of a mosquito as a function of blood availability inside ($\lambda_{blo,in}$) and sugar availability outside ($\lambda_{xug,out}$) based on the Monte Carlo simulation results at $\lambda_{sug,in} = 1.0$. This figure is representative of the same relationship for values of $\lambda_{sug,in}$ from 0.0 to 1.0.

Figure 2.6: Reproductive capacity of individuals as a function of blood availability inside ($\lambda_{blo,in}$) and sugar availability outside ($\lambda_{sug,out}$) based on Monte Carlo simulation results at $\lambda_{sug,in} = 1.0$. These results are representative of the same relationship for values of $\lambda_{sug,in}$ from 0.0 to 1.0. Panel a shows the mean fecundity, panel b shows the mean number of gonotrophic cycles, and panel c shows the mean egg production per gonotrophic cycle of an individual over her lifetime.

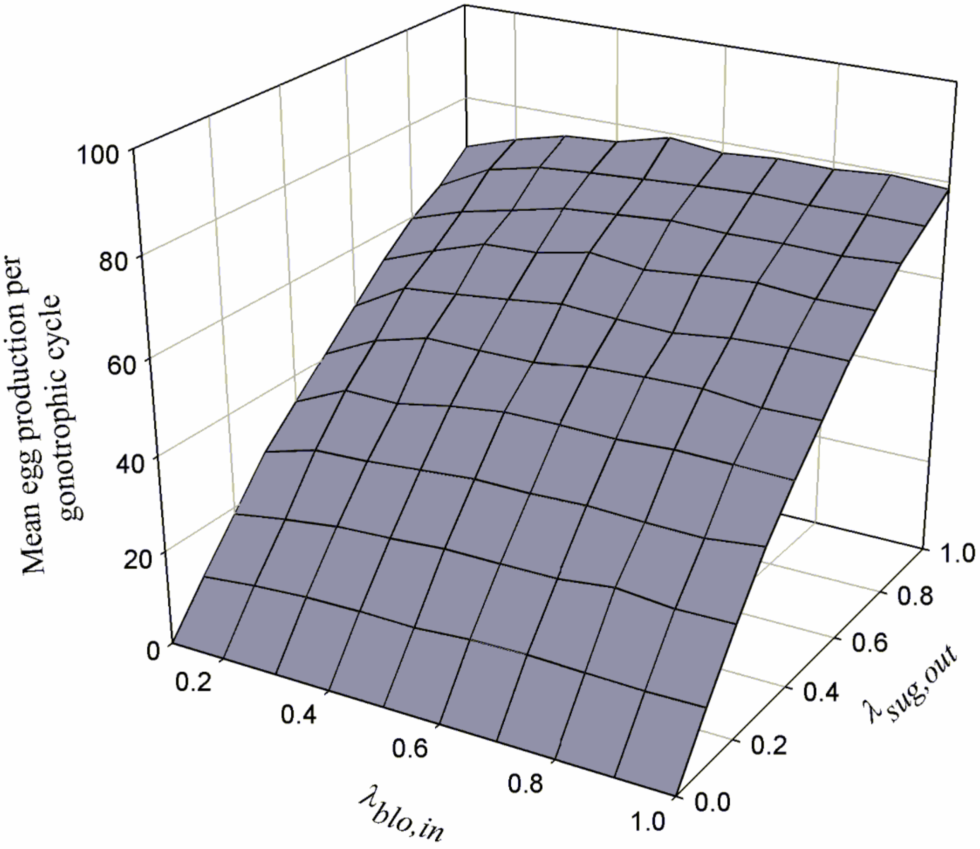
a)


Mean fecundity
100
80
60
40
20
0
0.2
0.4
0.6
0.8
1.0
$\lambda_{blo,in}$
0.0
0.2
0.4
0.6
0.8
1.0
$\lambda_{sug,out}$

b)


Mean number of gonotrophic cycles
12
10
8
6
4
2
0
0.2
0.4
0.6
0.8
1.0
$\lambda_{blo,in}$
0.0
0.2
0.4
0.6
0.8
1.0
$\lambda_{sug,out}$

c)


100
80
60
40
20
0
Mean egg production per gonotrophic cycle
0.2
0.4
0.6
0.8
1.0
$\lambda_{blo,in}$
0.0
0.2
0.4
0.6
0.8
1.0
$\lambda_{sug,out}$

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2.10 Connecting statement

In the following chapter, I present two independent laboratory experiments using *Anopheles gambiae s.s.* females that test the energy-based theory from Chapter 2. This theory states that females that are low on energy reserves, they will choose sugar over a blood meal.

3: Acceptance of sugar and blood hosts by the mosquito, *Anopheles gambiae s.s.* (Diptera: Culicidae), depends on energetic condition

3.1 Abstract

The role of energetic condition on the acceptance of blood and sugar hosts by *Anopheles gambiae s.s.* mosquitoes was explored. In the absence of a choice (i.e., only exposed to a blood host), there was a positive correlation between glycogen levels of large-bodied nulliparous female mosquitoes in their first gonotrophic cycle and the acceptance of the blood host (mouse). There was no relationship between the remaining sugar component (trehalose and free glucose) and host acceptance decisions. We then further explored the role of energetics on host acceptance decisions when female mosquitoes were given a choice between a blood host proxy (nylon sock with incubated human sweat) and a sugar host proxy (honey). We used female mosquitoes that were either newly-emerged, given access to sugar, or given access to sugar and blood, and found that newly emerged, energy poor female mosquitoes were more likely to choose a sugar host than a blood host, but sugar-fed and blood + sugar-fed mosquitoes were more likely to choose blood hosts. This work highlights the importance of energetic condition on host acceptance and choice in female mosquitoes.

Keywords: Mosquito, *Anopheles gambiae*, host choice, host acceptance, energetic condition

3.2 Introduction

There has been increasing awareness that sugar is an important component of a female mosquito's diet (Foster 1995). In nature, both female and male mosquitoes are known to feed on sugar, which is imbibed from floral and extrafloral nectaries and is processed into energy reserves in the form of glycogen and trehalose (Clements 1992). Glycogen is the main source of energy for mosquito flight and somatic maintenance (Clements 1955, Naksathit et al. 1999). Sugar availability, and subsequently energetic condition, affects survival and reproduction (Nayar and Sauerman 1971, 1975b, a, Foster 1995, Gary and Foster 2001, Okech et al. 2003, Ma and Roitberg 2008). In female mosquitoes, sugar is a seemingly important resource for survival and activity, but the search for and consumption of sugar has many trade-offs with the search for and consumption of blood (Ma and Roitberg 2008). Host blood is an important resource because it is necessary for the production of eggs; however, the acquisition of a blood meal is relatively costly. Blood is riskier to acquire than sugar because of a higher risk-of-death via host defensive behaviour (Walker and Edman 1985). Furthermore, blood is a relatively poor resource for the maintenance of soma and flight activity because of its lower efficiency of conversion into resources for these processes than sugar (Van Handel 1965). Ma and Roitberg (2008) explored the implications of the trade-offs between sugar and blood feeding on life-history characteristics and behaviour. Here, we further explore these trade-offs by evaluating the interaction between energetic condition and host-seeking and acceptance behaviour using the anautogenous (blood feeding) female mosquito, *Anopheles gambiae s.s.*. In this study, we pose two questions. First, in the absence of choice, how does an adult female mosquito's energetic condition affect its willingness to accept a blood host? This is measured by the concentration of glycogen – the key component of energy that is involved in flight and somatic maintenance (Nayar and Van Handel 1971). Second, following the logic that blood poses a riskier and less efficient somatic resource than sugar, in the presence of both a sugar and blood host, how does a mosquito of varying energetic condition choose between a sugar- and blood-host?

We predict that as the glycogen level of a female mosquito increases, it is more likely to accept a blood host. This is because blood is thought to be a costlier resource to acquire than sugar because it is associated with higher risk of death as well as lower conversion efficiency than sugar, and a mosquito should therefore be more hesitant to take a blood meal when low on reserves. Thus, we further predict that, when given a choice, a mosquito of lower energetic condition will choose sugar over a blood meal. Theory developed by Ma and Roitberg (2008) supports this prediction, suggesting that at low energetic conditions, seeking a sugar meal maximizes lifetime reproductive success.

Several studies have demonstrated that energetic condition can affect blood feeding behaviour in mosquitoes. Some work has centered around the immediate effect of engorging on sugar, for example with *Aedes aegypti*, where mosquitoes that engorged to repletion on sugar required several hours before they resumed spontaneous flight (Colvard Jones and Madhukar 1976). Before being converted into energy reserves, a sugar meal is stored in the crop, which can constrain the amount of blood that can be taken into the midgut (Foster and Eischen 1987, Takken et al. 2001, Fernandes and Briegel 2005). As such, there may be a confounding effect from sugar feeding if it has occurred so recently that the sugar has not been processed. In our study, we allowed sufficient time for a sugar meal to be processed and therefore removed this confound.

Other work on sugar deprivation has involved the comparison of *ad libitum* access and complete denial of sugar solution to the mosquitoes as a crude measure of different energetic conditions (Khan and Maibach 1970, Klowden 1986, Straif and Beier 1996).

In this study, we performed a detailed examination of the role of energetic condition on host seeking and acceptance behaviour. We performed two independent experiments in direct tests of the two aforementioned questions. First, we conducted a 'no choice' experiment, where a female of varying energetic condition was offered access to a blood host (mouse), and we measured the glycogen and free sugar (i.e., unprocessed sugar and trehalose) levels of each mosquito. Second, we performed a 'choice' experiment, where females of varying energetic condition were offered both a blood and sugar host.

3.3 Methods

3.3.1 Mosquito rearing conditions

Anopheles gambiae s.s. (Ifakara strain) were reared in a Conviron growth chamber at Simon Fraser University where the colony has been maintained for approximately 10 years. This colony originated from Njag, Tanzania. The growth chamber was kept at a temperature of 28°C ± 2°C, relative humidity of 75% (±10%), and a reverse light cycle with a photoperiod of 12h dark and 12h light beginning at 10 am and 10 pm PST respectively. Larvae were reared at 200 adults/3L distilled water in a 32 × 46 × 6 cm plastic tray to produce large-bodied mosquitoes (Lyimo et al. 1992) and fed Nutrafin® Basix Staple Tropical Fish Food *ad libitum* daily. Distilled water was periodically added to the larval trays to maintain a volume of 3L, and excess food was siphoned off the bottom of the tray to maintain growth conditions. Pupae were collected using a plastic pipette and transferred to glass bowls that were placed in a 30 × 30 × 30 cm Plexiglas™ cage where adults emerged (approximately 200 adults per cage). Adults were allowed to emerge into the cages for three days, after which the glass bowl was removed.

3.3.2 'No Choice' experiment

In the 'no choice' experiment, we generated variability in adult female mosquito energetic conditions by offering mosquitoes in each cage access to 2.5%, 10%, or 25% w/v sucrose solution. Fernandes and Briegel (2005) demonstrated that mosquitoes reared under these different sucrose solutions have different survivorship, and this difference suggests that these treatment levels may generate variability in glycogen levels between the tested mosquitoes. One day before experiments, the sucrose solution was removed from each cage and replaced with distilled water. On the day of the experiment, five to seven day old adult female mosquitoes from each of the different treatment cages (2.5%, 10%, and 25% w/v sucrose solution) were captured and placed in glass vials. The sample size for each of the three sucrose solutions was n=14, n=11, and n=12 respectively. The mosquitoes were taken to the experiment room, which was brought to 28°C and 75% r.h. using a ceramic heater (Titan®) and a hot air humidifier (Holmes®) one hour before experiments were performed. All replicates were performed between 2 to 6 pm PST, which corresponded to the period of night where *A. gambiae* most actively seek blood hosts (i.e., the mid-scotophase period).

We performed a behavioural bioassay using a small glass wind tunnel (5.5 cm diameter × 32 cm length), which consisted of a mosquito release chamber (7 cm length) separated from the main chamber by a metal mesh, a main chamber (25 cm length), and a mouse-holding chamber (4.5 cm diameter × 15 cm length), which detached from the main chamber (Figure 3.1). This wind tunnel was similar in design to one constructed by Geier et al. (1999). A female mosquito was randomly chosen and placed at the proximal end of a glass cylinder, separated by a removable metal mesh. A laboratory mouse, *Mus musculus* (CD-1 strain), was placed in a removable glass holding cylinder situated at the distal end of the wind tunnel. The mouse was separated from the mosquito by a metal mesh. Both ends of the wind tunnel were sealed using a rubber stopper with a port for air in the center, and air was passed through the wind tunnel from the mouse towards the mosquito at 1 L/min. Light was provided by a monochromatic red light.

After an acclimatization period of two minutes, the mosquito was allowed to exit the release chamber by removing the metal mesh and quickly sealing the chamber using Teflon tape. The actions of the mosquito were recorded for two minutes; we recorded the behavioural response of the mosquito: no response; host seeking, which corresponded to flight activity by the mosquito; host acceptance, which corresponded to landing on the mesh and/or probing through the mesh. If there was a positive response (i.e., landing on mesh or probing through mesh), we also measured the latency of the response as the time to landing on the mesh and the time at first probe.

After the experiment, tested mosquitoes were anaesthetized using a stream of CO_2 gas and placed in a -20 °C freezer. At a later date, the wing length of the mosquito was measured. The glycogen and free sugar levels of each mosquito was measured using the hot anthrone procedure (Van Handel 1985). The free sugar component consists of trehalose and unprocessed sugar meals. Each mosquito was placed in a microcentrifuge tube to which 0.2 mL of sodium sulfate solution was added. The mosquito was then ground up using a glass rod, which was then rinsed with 1 mL MeOH. The samples were then centrifuged at 13000 rpm for 10 min. The supernatant and precipitate separated into two 16 × 125mm glass test tubes. The test tube containing the supernatant was placed in a hot water bath at 92 °C to evaporate to ~0.1 mL. A series of standard glucose solutions were also created by placing 25, 50, 100, 150, and 200 μg glucose solution (1 mg/mL) in test tubes and filling up the test tubes to 5 mL with anthrone reagent. Test tubes containing the precipitate (glycogen component) and the supernatant (free sugar component) were then filled to a final volume of 5 mL with anthrone reagent. All test tubes were then placed in the hot water bath at 92 °C for 17 min. The test tubes were then cooled. The absorbance of the solutions and standard solutions was measured using a Beckman Du 640 spectrophotometer at a wavelength of 625 nm. From the standard curve, we were able to calculate the μg glycogen and free sugar from each sample by plotting μg glucose against absorbance. These values were converted into calories using a conversion factor of 0.004 calories/μg (Takken et al. 1998).

3.3.3 'Choice' experiment

In the 'choice' experiment, we collected female *A. gambiae* at various stages of their adult life to generate mosquitoes of different energetic condition. Newly emerged mosquitoes were collected as our poor-energetic-condition treatment level. These mosquitoes are known to have lower energetic reserves than sugar- or blood-fed females (Briegel 1990, Walker 2008). We used sugar fed and sugar + blood fed mosquitoes as our high–energetic- condition treatment levels. We used both sugar fed and sugar + blood fed mosquitoes because we wanted to explore the different influences of blood and sugar on host selection as several studies have shown that at least with small females, the first blood meal may be used to make up for somatic protein and energy deficits (Takken et al. 1998, Fernandes and Briegel 2005).

In these experiments, we used a large two-port olfactometer that allowed us to test many mosquitoes at once. We used approximately fifty female mosquitoes per trial, which were transferred from a cage to a holding container using a small glass vial. The holding container consisted of a PVC tubing 10 cm in diameter and 15 cm long. One end of the holding container was covered by a fine nylon mesh, while the other was covered by a hinged disc made of monochromatic red Plexiglas™ that allowed us to release the mosquitoes. The two-port olfactometer was constructed of monochromatic red Plexiglas™, was 40 cm × 40 cm × 40 cm, and was divided horizontally into two chambers using another piece of Plexiglas™ (Figure 3.2). Capture tubes (10 cm diameter × 35 cm length) were placed on the distal end of the olfactometer. Capture tubes were sealed at the end and had a small opening at the top to introduce airflow. The entrance to the capture tube had a nylon mesh cone that allowed mosquitoes to easily enter the capture tube but made it difficult for mosquitoes to exit. One hour prior to the start of the experiment, the tent was brought to 28°C and 75% r.h. using a ceramic heater (Titan®) and a hot air humidifier (Holmes®). The olfactometer was kept under a tent constructed of sheets of plastic in order to maintain temperature and humidity conditions. To increase humidity inside the olfactometer, crumpled paper towels dampened with distilled water were placed inside the main chamber of the olfactometer. The holding container was placed at the proximal end of the olfactometer.

As our proxy for a blood host, we used incubated human sweat which was acquired via ankle length nylon socks (SECRET®) worn by BOM, MS, or RPT for 24h.

Incubated human sweat is known to be a good proxy for a blood host (Braks and Takken 1999), and preliminary tests using only worn socks (versus a blank) indicated that mosquitoes would fly towards the socks to a greater extent than a blank. As our proxy for a sugar host, we used alfalfa clover 10mL of honey diluted ten-fold with distilled water (Kidd Bros. Products Ltd.) placed on a crumpled paper towel. This has been shown to be a good proxy for a sugar host (Foster and Takken 2004). The two host cues were randomly placed in the left and right capture tubes. Conditioned air was passed through the capture tubes, over the attractants, and into the main chamber of the olfactometer at 1 L/min.

Mosquitoes were released from the holding container, and the mosquitoes were given the opportunity to fly into the main chamber. Experiments were allowed to run for 24 hours in night time conditions, beginning at 3 pm, which on our reverse photoperiod cycle corresponded to the peak activity period for *A. gambiae*. We ran the experiments for the extended period because preliminary trials of shorter duration had a low capture rate of mosquitoes. After a trial was finished, the chamber was flushed with CO_2 gas to knock out the mosquitoes. We counted the number of mosquitoes in the main chamber and each of the capture tubes. The numbers of replicates for each treatment group were as follows: newly-emerged mosquitoes – n = 8; sugar-fed mosquitoes – n = 10; sugar+blood-fed mosquitoes – n = 12.

3.3.4 Statistical analyses

The data from the experiments are analyzed using generalized linear models and an analysis of variance (ANOVA). Generalized linear models are a category of models that test the fit of a dependent variable against a known distribution (i.e., to test whether there is a significant relationship between the dependent variable and the continuous independent variable). In particular, I use linear regressions, which are useful when trying to determine if there is a significant relationship between two variables of interest and the data are normally distributed. I also use logistic regressions, which are useful when the dependent variable is binomial (e.g. a choice). ANOVAs compare the means of two or more treatment groups to test if they are statistically different. When one or more dependent variables are of interest, I also use multivariate analysis of variance (MANOVA), which is an extension of an ANOVA where there is more than one dependent variable.

For the ‘no choice’ experiment, all analyses considered body size, and sugar and glycogen levels. Wing length (x) was converted into an estimate of body weight (y) following the equation $y = 0.0037x^{4.08}$ (Koella and Lyimo 1996). We used a linear model to measure the effect of the different sucrose solution treatments on glycogen and free sugar levels. We used a logistic regression to evaluate how the behavioural response of the mosquito changed with changes in free sugar, glycogen, body size, and any interactions between these variables. We used a linear model to measure the effect of glycogen and body size on the time to land on the mesh or the time to probe through mesh. For both the logistic regression and the linear regression, we present the statistical model reduced by stepwise reduction in the results section. Statistics were performed using JMP 7.0 statistical software (SAS Institute, Cary, NC, USA).

For the choice experiment, to test the effect of treatment level (newly emerged, sugar-fed, or blood-fed) on the choice between sugar and blood host, we used a MANOVA on the arcsine-transformed proportions of responders to the blood host cue, sugar host cue, and non-responders. Separate ANOVAs with a post-hoc Tukey's HSD test were run for each response to determine the differences between each mosquito condition classes.

3.4 Results

3.4.1 No choice experiment

Sucrose solution and glycogen levels

The distribution of glycogen levels was not normal, so we used the Kruskal-Wallis test to measure the effect of sucrose solution treatment on glycogen levels and found that the sucrose solution had no effect on glycogen levels (Kruskal-Wallis test: χ^2 = 3.99, d.f. = 2, p = 0.1358) or free sugar levels (Kruskal-Wallis test: χ^2 = 2.53, d.f. = 2, p = 0.2822).

Behavioural response

As glycogen level increased, there was an increase in the willingness to fly towards and probe against the mesh (logistic regression: χ^2 = 10.3, d.f. = 3, p = 0.0165), and the response was independent of body size. There was no effect of free sugar on mosquito response (logistic regression: χ^2 = 5.1, d.f. = 3, p = 0.162). There was a bimodal distribution of glycogen levels in the data, so we split the data into two clear parts – high and low glycogen levels (low <0.00020 cal; high $\geq$ 0.0003 cal). We performed a linear regression on both high and low glycogen levels and body size, while incorporating the interaction between the glycogen levels and body size into the statistical model., We found no relationship between glycogen levels and body size ($F_{3,33}$ = 0.57, p = 0.64) (Figure 3.3a). There was also no relationship between free sugar levels and body size ($F_{1,35}$ = 0.000, p = 0.999) (Figure 3.3b).

Latency

There was no effect of body size (linear regression: $F_{1,16} = 0.1653$, $p = 0.6897$), free sugar level ($F_{1,16} = 0.16$, $p = 0.6928$) or glycogen level ($F_{1,16} = 1.01$, $p = 0.3304$) on the time it took for a mosquito to fly to the mesh. There was no effect of body size (linear regression: $F_{1,6} = 0.9756$, $p = 0.3614$) (Figure 3.4c) or free sugar level ($F_{1,6} = 1.40$, $p = 0.2812$) (Figure 3.4b) on the time it took for the mosquito to first probe through the mesh; however, as glycogen concentration increased, the time to first probe decreased ($F_{1,6} = 10.16$, adjusted $r^2 = 0.566$, $p = 0.0011$) (Figure 3.4a).

3.4.2 Choice experiment

There was a significant whole model effect of mosquito condition on the number of responders to each lure and the number of non-responders (MANOVA; $F_{6,50} = 13.47$; Wilks' Lambda $p<0.0001$) (Figure 3.5). A greater proportion of individuals that were sugar fed or sugar+blood fed (mean ± S.E.: 0.415 ± 0.085 and 0.366 ± 0.095 respectively) chose the sock lure than newly emerged individuals (0.085 ± 0.022) (ANOVA Tukey's HSD test, $F_{2,27} = 4.63$, $p = 0.0187$). This was coupled with a greater proportion of newly emerged individuals choosing the honey lure (0.659 ± 0.062) than the sugar-fed (0.091 ± 0.028) and sugar+blood-fed (0.077 ± 0.032) mosquitoes (ANOVA, Tukey's HSD test, $F_{2,27} = 41.03$, $p < 0.0001$). There was no difference in the proportion of non-responders between the three mosquito condition classes, although there was a marginally smaller proportion of non-responders in the newly-emerged class (newly-emerged: 0.256 ± 0.066; sugar-fed: 0.494 ± 0.084; sugar+blood-fed: 0.558 ± 0.098) (ANOVA, Tukey's HSD test, $F_{2,27} = 2.91$, $p = 0.0715$).

3.5 Discussion

The energetic condition of a mosquito had a significant impact on host acceptance and host seeking behaviour of female *Anopheles gambiae s.s.* mosquitoes. The results found in this study are qualitatively consistent with the predictions made by Ma and Roitberg (2008). In the absence of choice, as the glycogen level of a nulliparous female *Anopheles gambiae s.s.* mosquito in its first gonotrophic cycle decreased, they were less willing to seek or accept a blood host. Furthermore, the time it took for those mosquitoes to accept the blood host, which was measured as the latency to probing behaviour, was positively correlated with glycogen levels. However, when given a choice, a greater proportion of mosquitoes of a lower energetic condition tended to choose the sugar host cue over the blood host cue. Conversely, mosquitoes of higher energetic condition tended to choose the blood host cue over the sugar host cue.

The findings of our 'no choice' experiment are consistent with our predictions, which suggest that at low energetic conditions a mosquito should be less willing to seek or accept a blood host. This is likely because seeking and acceptance of a blood host by a mosquito is an innately costly activity that involves the high metabolic costs of flight and the risk of death because of host defensive behaviour (Day et al. 1983), respectively. In addition, there are physiological costs associated with taking blood for activity and somatic maintenance (Van Handel 1965). Trehalose levels (the sugar component) did not play a factor in host acceptance decisions, which is consistent with the fact that trehalose has been shown to not play a factor in flight or survival (Fernandes and Briegel 2005).

We found no correlation between sugar treatment level and glycogen concentration, and we therefore caution researchers from using sugar concentration as a proxy for energetic condition for *A. gambiae*. This is somewhat surprising because it has been demonstrated, that mosquitoes reared on different concentration sucrose solutions have different survivorship (Fernandes and Briegel 2005). However, the Kruskal-Wallis test is a low power test, and we therefore may not be detecting a difference when there is one. Our result is consistent with work done on *Aedes aegypti*, where it was found that sugar meal concentration had no effect on energy utilization (Naksathit et al. 1999). The lack of a difference between sugar treatment levels can be explained by a difference in behaviour between the individuals of each treatment group. For instance, Colvard Jones and Madhukar (1975) found that as female *Aedes aegypti* fed on sucrose solutions of higher concentrations were less likely to undergo flight activity. This suggests that higher concentration sugar might take longer to convert into energetic reserves but could be mediated by more frequent feeding by individuals offered lower concentration sugar. Despite the lack of difference in nutritional reserve levels found between individuals fed sucrose solution of different concentration, we were still able to generate mosquitoes of varying glycogen levels.

We found no correlation between body size and glycogen or carbohydrate levels. This may be because we generated large-bodied mosquitoes, and therefore most individuals are of ‘good condition’. Size can play an important role in determining the host seeking and blood meal utilization in *A. gambiae*, where smaller-bodied mosquitoes often require more than one blood meal to initiate egg production (Takken et al. 1998, Fernandes and Briegel 2005), and energy-deprived small female mosquitoes tend to more readily accept blood hosts than large females of the same energy state (Roitberg et al. *in* *review.*). However, at higher energetic conditions, larger females more readily accepted a blood host than their small-bodied counterparts.

Some studies have examined the period immediately following a sugar meal, and found that blood-host seeking is depressed in this initial post-sugar meal period (Colvard Jones and Madhukar 1976). This result is most likely driven by the physical distention of the crop from a sugar meal suppressing blood host seeking behaviour (Ma and Roitberg 2008). In our 'no choice' experiment, we allowed the crop to empty by providing a 1-day sugar deprivation period. Other studies on the effect of sugar on blood feeding generally found the opposite pattern to our results; i.e., sugar access results in reduced blood host seeking (Klowden 1986, Foster and Eischen 1987, Bowen and Romo 1995, Straif and Beier 1996), although in some cases sugar access led to smaller but more frequent blood meals (Gary and Foster 2006). The differences in the results between our study and other studies can be attributed to several key differences in the approaches. First, we directly measure the energetic condition (glycogen and free sugar levels) of individuals, whereas other studies involve completely denying sugar access to mosquitoes for one or more days but no direct measure of energetic condition. Second, these studies were long-term studies over a period of several days, where mosquitoes were also provided access to blood hosts as a resource, which leads to differences in their lipid and protein stores. In the 'choice' experiment, where mosquitoes were given a choice between a sugar- and blood-host cue, a greater proportion of newly-emerged, energy poor mosquitoes tended to choose the sugar-host cue than the blood-host cue; sugar fed and blood + sugar fed female mosquitoes tended to choose the blood-host cue. This result is consistent with our predictions that when given a choice, mosquitoes of lower energetic condition should favour a sugar meal over a blood meal. These findings are also consistent with the findings of Foster and Takken (2004), who demonstrated that sugar feeding is important for female *A. gambiae* early in life. Our methodology differs from the experiment performed by Foster and Takken (2004) in that we allowed the mosquitoes a full day to respond to odours, and furthermore, unlike the statistical techniques employed by Foster and Takken (2004), we treated each experimental run as a separate trial and explored the effect of mosquito condition on the average proportion of responders. We found no difference in the choices made by sugar fed (and nulliparous) and sugar + blood fed (parous) mosquitoes. Fernandes and Briegel (2005) point out the first blood meal may be used to make up for somatic protein and energy deficits in smaller mosquitoes, but our results suggest that this difference in physiological condition was not a factor in the choice between sugar and blood feeding in our experimental design. However, it is worth noting that despite the strong preference for blood hosts in these two groups of mosquitoes, there was still a small percentage (~10%) of mosquitoes that chose sugar from each group.

A potential confound in the ‘choice’ experiment was the age of individuals in each treatment group. For instance, newly-emerged mosquitoes were always younger than their sugar- or sugar+blood-fed counterparts. Because we dealt with only large-bodied mosquitoes, there is no reason to suspect that individuals require more than one blood meal to lay eggs (Takken et al. 1998).   However, there is some evidence that suggests that mosquitoes senesce (Styer et al. 2008, Dawes et al. 2009), which could lead to differences in responses being due to difference in age (and not necessarily energetic condition).

In the future, our ‘no choice’ experiment should be repeated with humans as a blood host. We use laboratory mice as a proxy for a blood host, but the natural blood host of *A. gambiae* is humans. Khan and Maibach (1969) found that *Aedes aegypti* mosquitoes that were sugar-fed would probe as avidly on an artificial feeder as they would a human arm, but sugar-starved mosquitoes probed avidly on a human arm but not on an artificial feeder. This may be evidence that the result we find in this study may only exist for mice and not for a better quality host like a human arm.

One drawback to the two-port olfactometer experiment was that the energetic condition of the mosquitoes was not measured. Because of the duration of the experiments (24h), the energy level of the mosquitoes was allowed to change during the course of a trial, making it difficult to estimate the energetic condition of individuals at the time that they made their choice.

Finally, despite the use of two different protocols and experimental apparatus, we obtained responses that were consistent with our energy state predictions; i.e., our results were not technique dependent. Of course, we worked with just one species of mosquito, *Anopheles gambiae*, so one must be careful before reaching broad conclusions, but given its energy budget challenges *A. gambiae* is a highly appropriate model for such studies (Fernandes and Briegel 2005).

3.5.1 Summary

How a mosquito responds to environmental stimuli is a factor of both the type and strength of the cue as well as endogenous stimuli such as age, presence of eggs, gonotrophic cycle, and nutritional state (Klowden 1996). There have been several studies examining the impact of sugar availability on blood host feeding behaviour in mosquitoes; however, to our knowledge, our study is the first to directly examine the energetic condition (i.e., glycogen and sugar levels) of the mosquito. This work highlights the importance of energetic reserves in female adult mosquito decision making, and demonstrates how low energetic condition can lead to reduced blood host seeking and acceptance behaviour in mosquitoes. Although *A. gambiae* are closely linked with humans, adult female mosquitoes are ultimately omnivores that utilize both blood and sugar, and sugar plays an important role in fueling somatic maintenance and activity while blood represents a costly resource. This study suggests that the energetic state of a female mosquito will mediate the conditions under which it feeds on blood or sugar. This study also highlights the need to explore energetic condition further with studies on mosquitoes because although sugar deprivation experiments will reduce energetic conditions a closer look at the energetic conditions leading to changes in behaviour is necessary.

3.6 Acknowledgements

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3.7 Figures

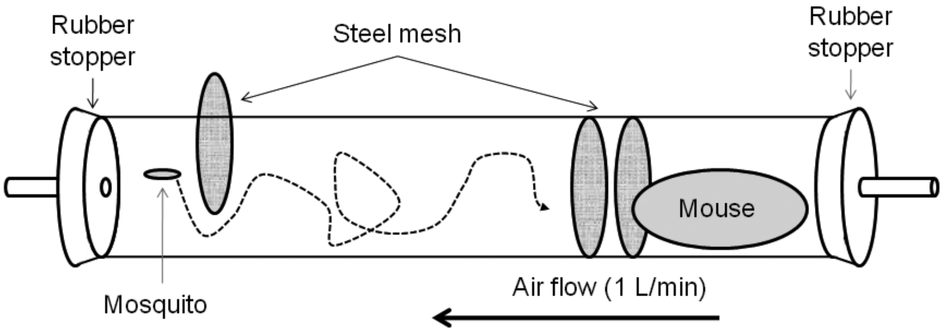


Figure 3.1: Simplified schematic of the small wind tunnel apparatus.

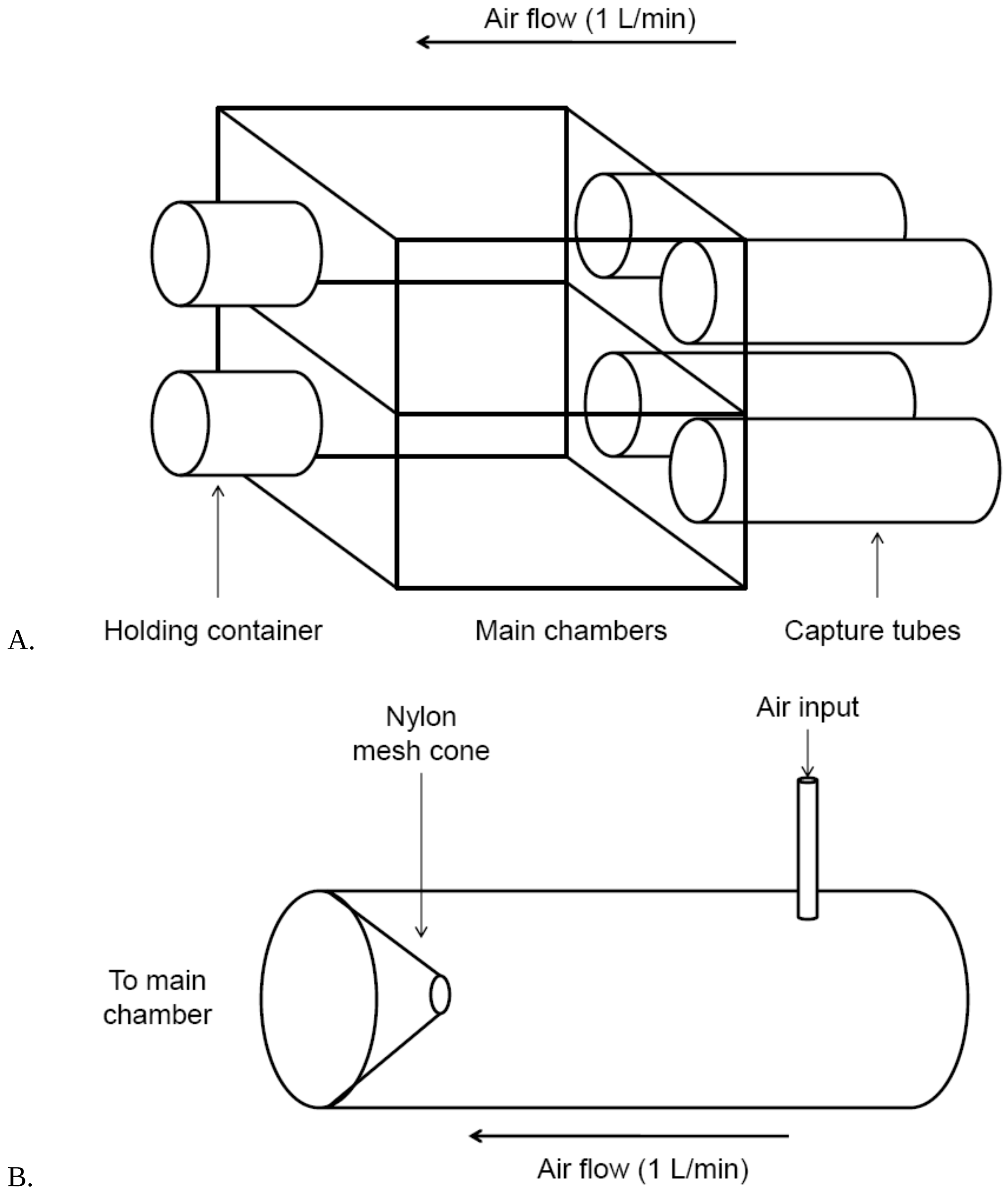
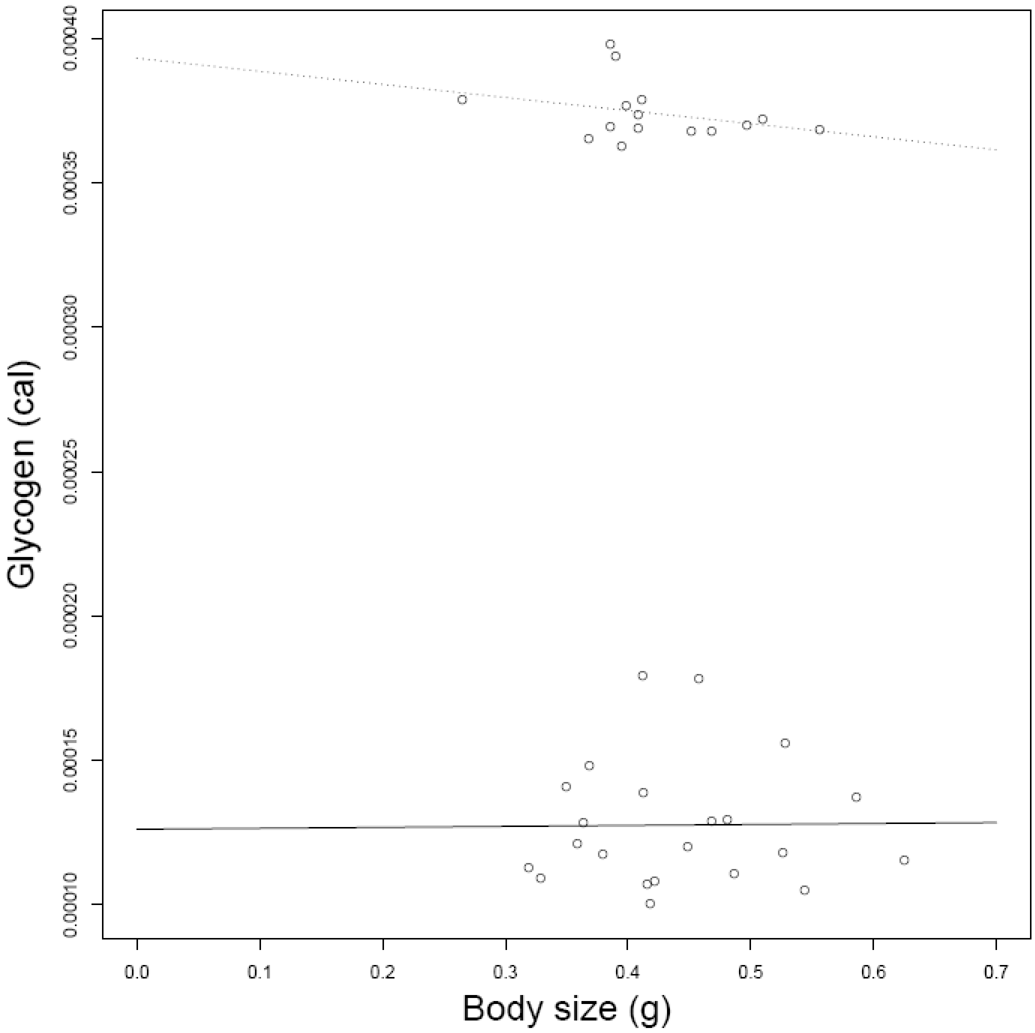
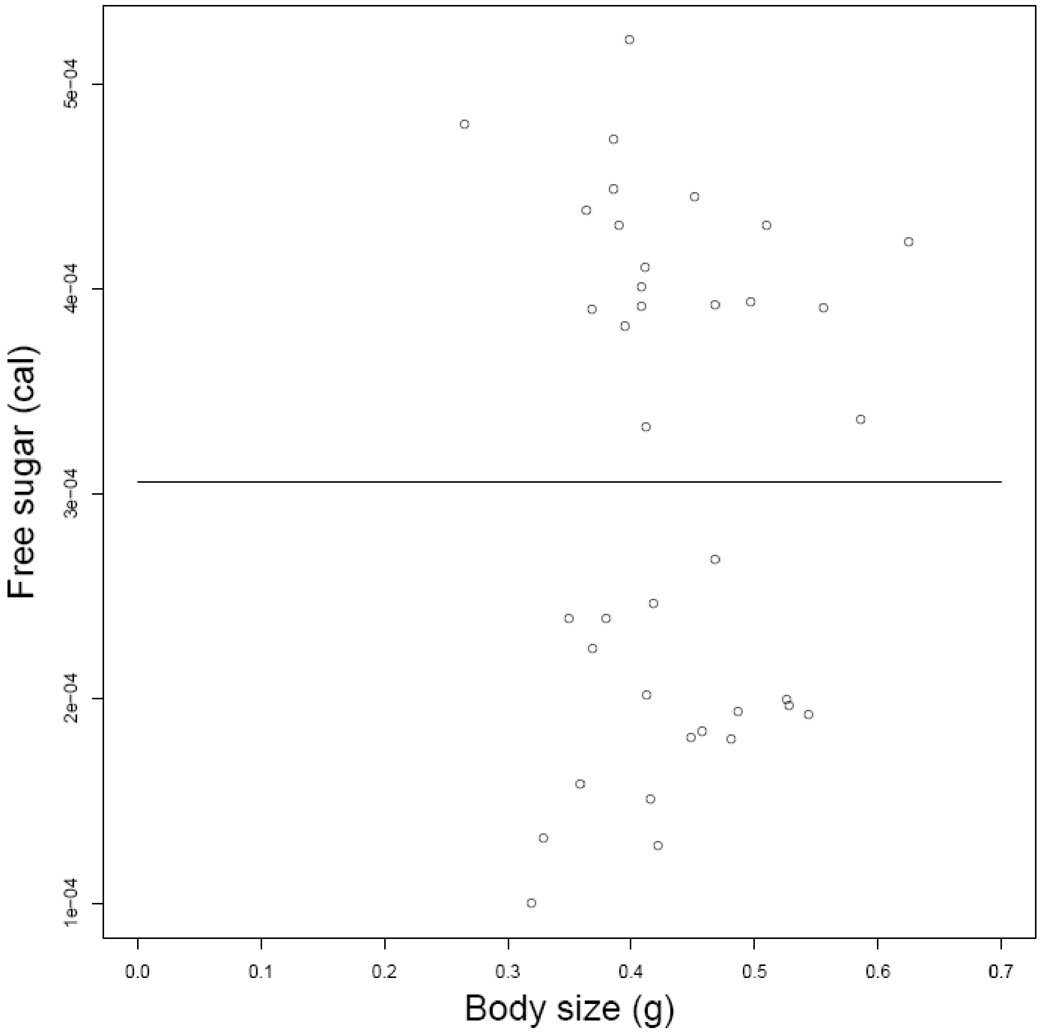


Figure 3.2: Simplified schematic of the two-port olfactometer (panel A) with a close up of a capture tube (panel B).

Figure 3.3: Glycogen (panel A) and free sugar (panel B) levels of female *Anopheles gambiae s.s.* mosquitoes as a function of body size as determined from the wind tunnel behavioural bioassay. In panel A, the solid line represents the best fit regression line for the ‘low’ glycogen level mosquitoes, and the dashed line represents the best fit regression line for the ‘high’ glycogen level mosquitoes.


Glycogen (cal)
0.00010
0.00015
0.00020
0.00025
0.00030
0.00035
0.00040
Body size (g)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

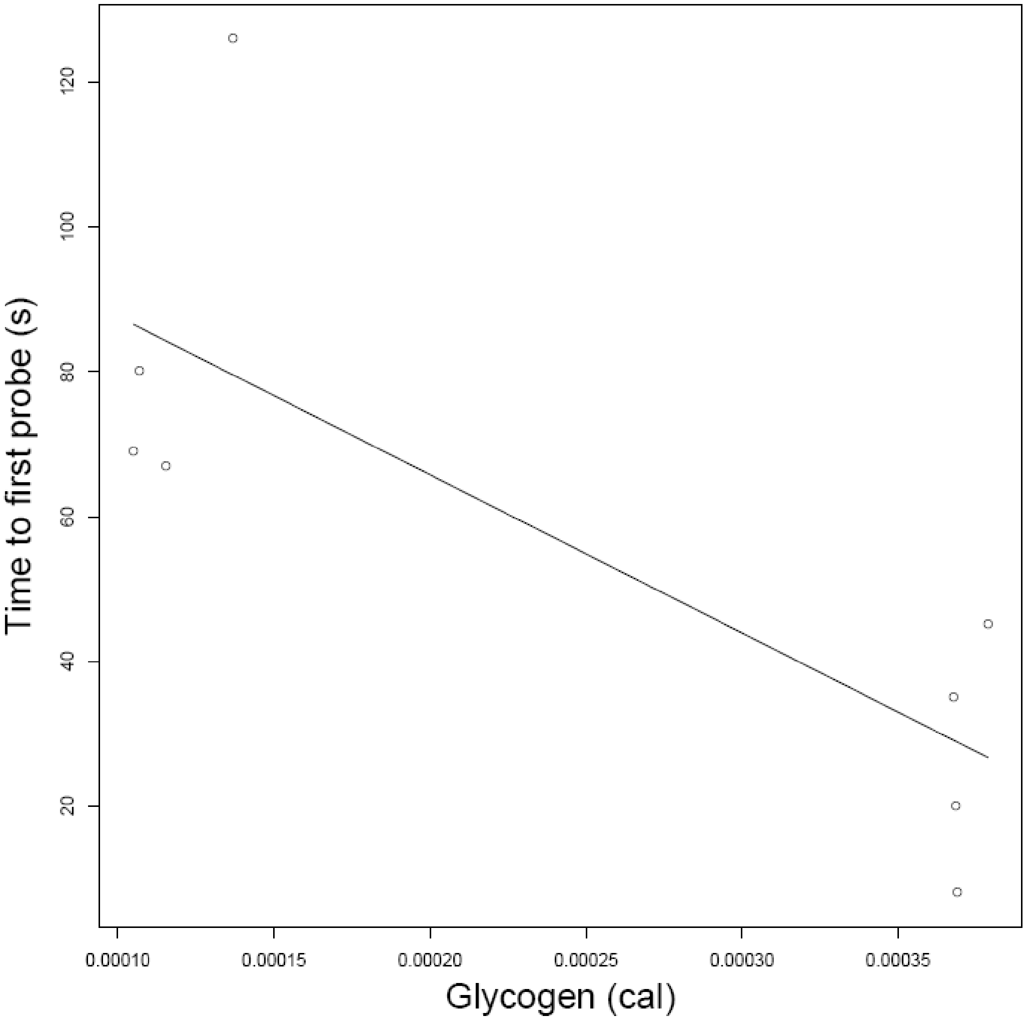
A.


Free sugar (cal)
5e−04
4e−04
3e−04
2e−04
1e−04
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Body size (g)

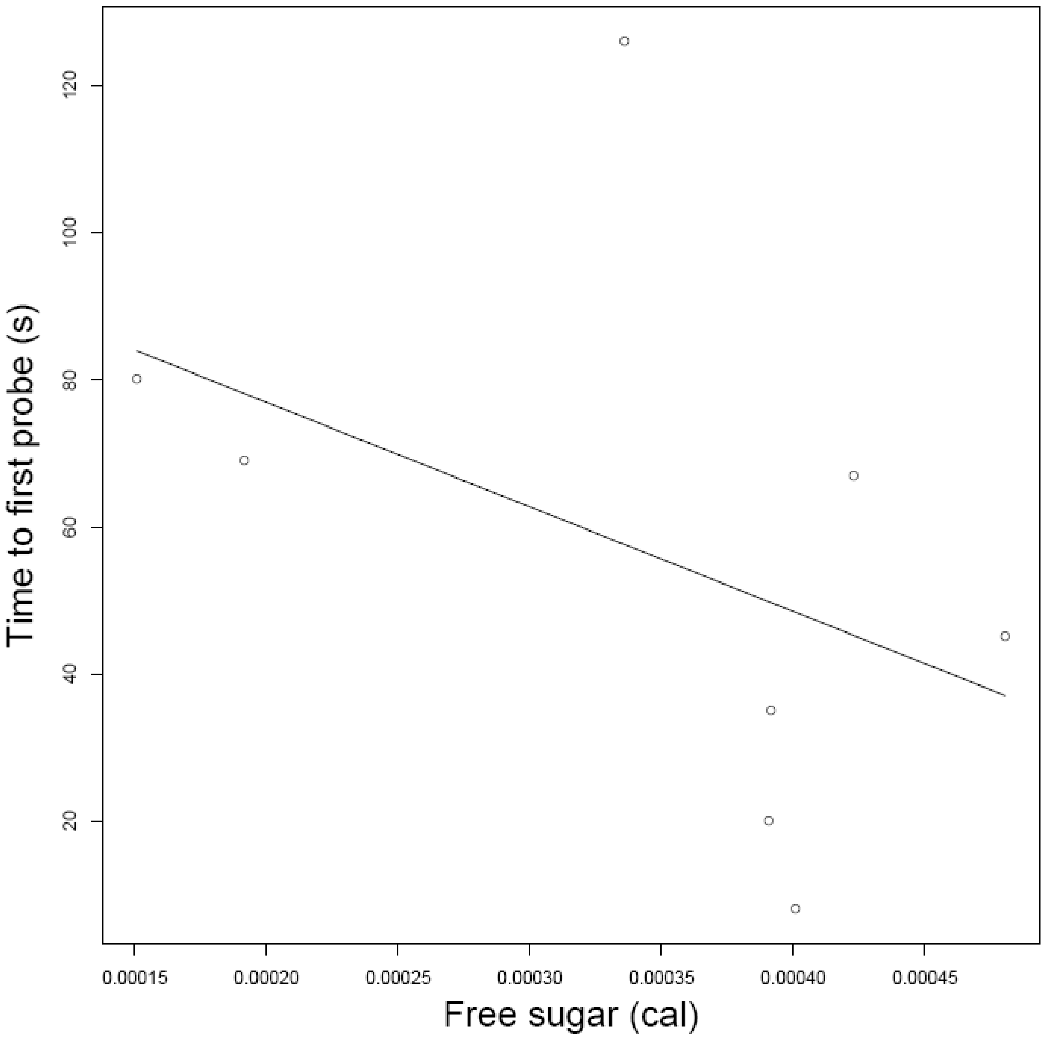
B.

Figure 3.4: Response time to first probe versus glycogen level (panel A), free sugar level (panel B), and body size (panel C) in female *Anopheles gambiae s.s.* mosquitoes as determined from the wind-tunnel behavioural bioassay.

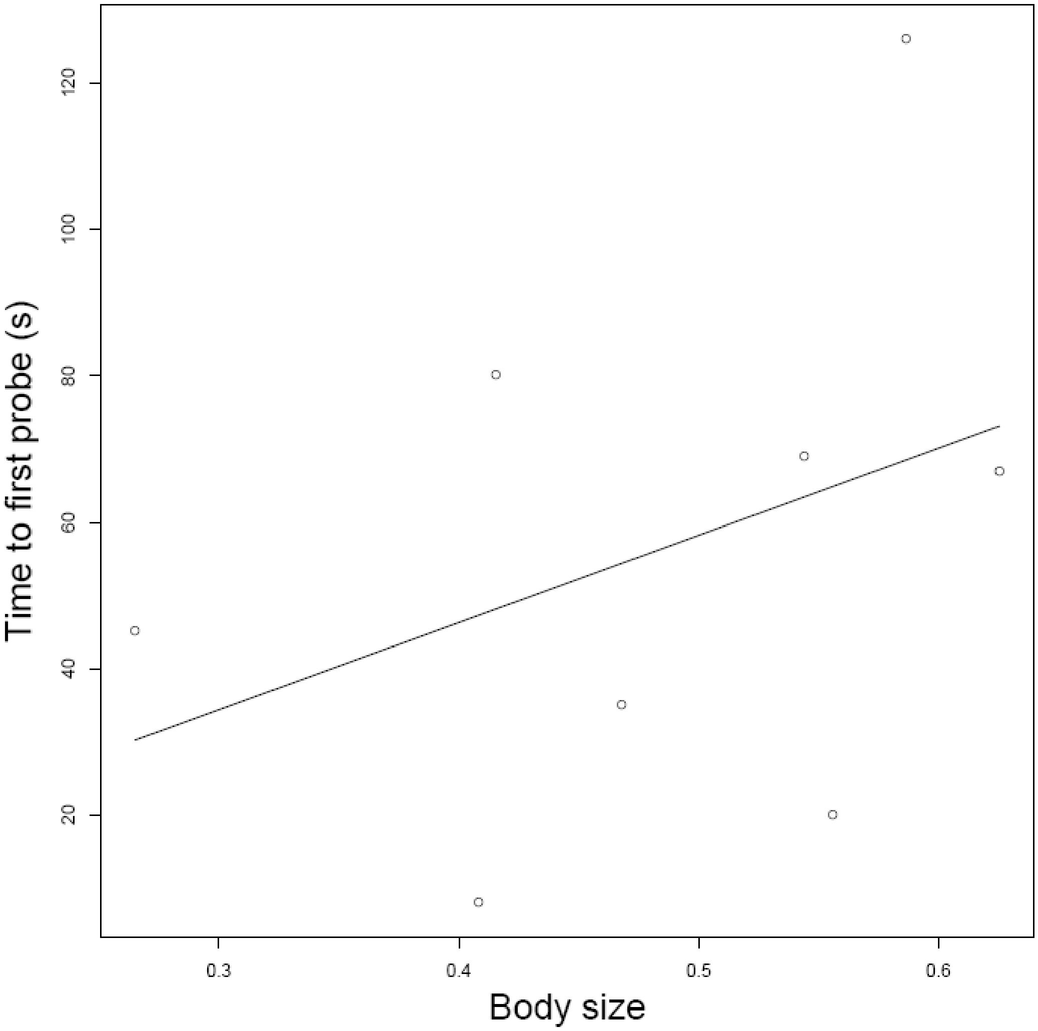
A)


Time to first probe (s)
120
100
80
60
40
20
0.00010
0.00015
0.00020
0.00025
0.00030
0.00035
Glycogen (cal)

B)


Time to first probe (s)
20
40
60
80
100
120
0.00015
0.00020
0.00025
0.00030
0.00035
0.00040
0.00045
Free sugar (cal)

C)



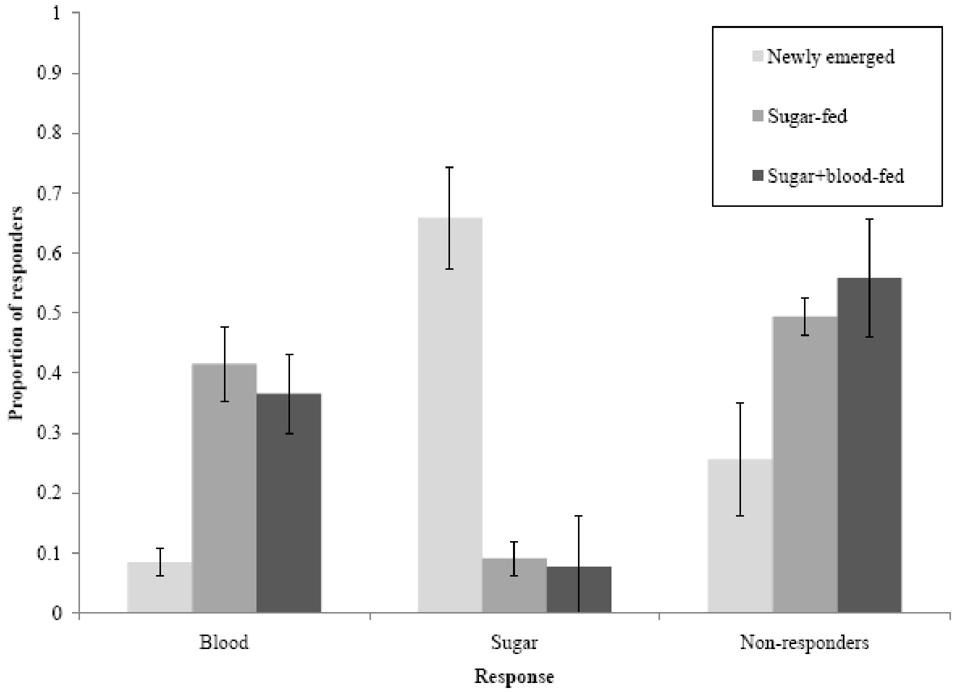


Figure 3.5: The proportion of *Anopheles gambiae s.s.* mosquitoes that responded to blood or sugar cues, or that did not respond in the two-port olfactometer bioassay where 50 mosquitoes were provided access to a blood host cue (nylon sock with incubated human sweat) and a sugar host cue (honey) for a period of 24h.

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3.9 Connecting statement

In the following chapter, I examine another aspect of mosquito behaviour: oviposition site selection. I employ an evolutionary game model called a genetic algorithm to explore the oviposition behaviour of mosquitoes when we consider adult and larval traits together. I use a simulation model called a genetic algorithm (GA) model, which is a simulation game theoretic model that measures the co-evolution of traits in a frequency- and density-dependent system.

4: An evolutionary game of larval habitat exploitation by mosquitoes

Submitted to The Journal of Evolutionary Biology

Brian O. Ma, B.D. Roitberg, and W. Takken

Brian O. Ma implemented the model, ran the simulations and wrote this manuscript. B.D. Roitberg provided key input on model design and analysis. B.D. Roitberg and W. Takken were involved in developing the original concept and comments on the submitted manuscript.

4.1 Abstract

We consider adult and offspring traits concurrently using an evolutionary game. We use mosquitoes as our model organism, focusing on the behavioural traits of adult female mosquitoes regulating oviposition site preference, and the larval traits that regulate how they interact with conspecifics and the environment. The model predicts that adult larval habitat site preference, the strength of that preference, and the ability of larvae to handle environmental stochasticity form a coalescing trait complex, but that there is no directional selection for larval aggression suggesting that female adults mediate larval interactions via larval habitat selection. Understanding the evolution of oviposition site selection in mosquitoes will have implications for mosquito control policy.

Keywords: Mosquitoes, egg-laying, habitat selection, genetic algorithm, evolutionary game

4.2 Introduction

The fitness of a mother is intimately linked to offspring fitness and consequently relies on the mother's ability to select an environment that offspring will thrive in (Perry & Roitberg, 2005, Roitberg & Mangel, 1993). Organisms with two distinct life stages are often associated with very different habitats (e.g., insect parasitoids: host-living larvae, terrestrial adults; mosquitoes: aquatic larvae, terrestrial adults). In these organisms, offspring are essentially locked in to the habitat of female adult's choice and must be well equipped (whether it be via physiology or behaviour) to cope with the environment to survive to adulthood. Although these two processes are usually considered separately, larval habitat exploitation is a function of a mother's ability to choose that habitat, and the ability of her larvae to cope with that habitat. Thus, adult performance and larval performance are both measures of the ability of the same individual to exploit a larval habitat, only over two distinct life history stages. Thus, we expect traits affecting adult egg-laying decisions and larval fitness to operate together wherein the performance from one life stage depends upon the actions of the other stage; there may also be within-stage games as well (i.e., frequency-dependence) wherein the performance (e.g. of a larval strategy) depends upon the strategies of others.

Using mosquitoes as our model organism, we focus on the combination of adult and larval traits. Adult females are terrestrial and lay eggs (i.e. oviposit) in aquatic habitats (Clements, 1999), and therefore, the emerging larvae are bound to the aquatic larval habitat that the female adult chooses. Studies on mosquitoes have generally focused on abiotic and biotic factors that influence either adult fitness gained through successful egg-laying (oviposition) or larval fitness gained through survival to adulthood;

however, there are surprisingly few studies that link these two together (*but see* Reiskind & Wilson, 2004, Rudolf & Wilson, 2005).

Several factors have been shown to be important for both oviposition site selection and for larval offspring fitness. Oviposition site selection is thought to be influenced by a number of abiotic and biotic cues (Bentley & Day, 1989). Abiotic cues include substrate moisture (Minakawa et al., 2001) and contrast (Huang et al., 2005, McCrae, 1984), habitat size (Service, 1977) and permanence (Mokany & Mokany, 2006), distance to hosts (Minakawa et al., 1999, Barker et al., 2003), and canopy coverage (Minakawa et al., 2005a). Biotic cues include the presence of natural enemies (Kiflawi et al., 2003, Munga et al., 2006), the presence of conspecifics (Kiflawi et al., 2003, Munga et al., 2006, McCrae, 1984, Sherratt & Church, 1994), the presence of other mosquito species (Kiflawi et al., 2003), the presence of parasitized larvae (Lowenberger, 1994), and food availability (Blaustein & Kotler, 1993, Bond et al., 2005, Bond et al., 2004, Sherratt & Church, 1994). Larval fitness is affected by many of the same abiotic and biotic factors that influence adult oviposition site selection including habitat size and permanence (Minakawa et al., 2005b), temperature (Lyimo et al., 1992, Tuno et al., 2005), inter- and intra-specific competition (Renshaw et al., 1993, Schneider et al., 2000, Barrera, 1996, Gimnig et al., 2002, Koenraadt & Takken, 2003), food availability (Bond et al., 2005, Bond et al., 2004, Takken et al., 1998) and the presence of predators (Blaustein et al., 2004, Sherratt & Tikasingh, 1989, Kesavaraju & Juliano, 2008).

In this study, we use game theory to explore the evolution of co-adapted trait complexes associated with exploitation of a range of larval habitats and how environmental factors might change the habitats selected. By considering the traits of mosquito adult and larva together, we may gain new insights into the mechanisms that drive the evolution of oviposition site exploitation in different mosquito species. We ask two questions – (1) What traits are associated with oviposition site selection and these traits become linked? (2) How do those traits change with changes in the environment?

Much like how the phenotype of an organism is the interaction between the genotype and environment, the *expression* of a strategy is the result of the interactions between an individual's strategy with other individuals and with the environment. We examine these interactions between the strategy and the environment using a genetic algorithm (GA) model where adult and larval traits are considered concurrently. In a GA, traits are allowed to evolve independently, but often traits will coalesce (i.e., become linked) as a function of processes analogous to natural selection. To our knowledge, this is the first attempt at linking larval and adult fitness together and allowing them to coalesce in the context of different environments. Using a GA allows us to play frequency-dependent games between a large number of different (and complex) strategies which, in our case, represent more than one type of behaviour. Furthermore, they allow us to compete these strategies against one another in a density-dependent manner. Together, the frequency- and density-dependent game theoretic processes are best done with a GA, and are difficult to implement with simpler analytical game theoretic approaches. To this effect, we consider four traits (two adult and two larval traits) to characterize habitat exploitation performance and focus on two environmental factors: food availability and predation risk.

We characterize the adult life history stage using two traits, both of which modulate preference for ovipositing in different habitat types. We only used two adult traits because we felt that it was the fewest number of traits that we could use to accurately describe the key components of oviposition site selection. The first trait considers the oviposition habitat preference. The second trait reflects the strength of that preference, which modifies how closely the actual egg-laying behaviour follows the oviposition site ranking (i.e., behavioural flexibility). In modeling oviposition site preference using two independent traits, we can capture a wide breadth of oviposition site preferences, ranging from habitat specialists and habitat generalists. For instance, in a generalist strategy (where the strength of preference is zero), the ranking of different habitats does not matter. Mosquitoes of different species tend to lay their eggs in specific habitats (Clements, 1999), but the breadth of oviposition habitats that are acceptable to a particular species of mosquitoes may vary. For instance, *Anopheles gambiae* mosquitoes prefer small temporary habitats such as small hoof prints and puddles, but can also be found in ponds, sheltered lakes, stream edges and marshes (Munga et al., 2007, Gimnig et al., 2001). The way in which we model our adult traits assumes mosquitoes base their decisions on the absolute quality of a habitat and not in relation to other habitats (Kiflawi et al., 2003).

We characterize the larval life history stage using larval growth flexibility and aggression. These two traits represent what we feel is the bare minimum number of traits that were necessary to influence the outcome in interactions between an individual and its environment. Larval growth flexibility is described as the maintenance of behavioural or physiological flexibility that allows an individual to cope with varying environmental conditions (Levins, 1968), and we therefore assume that there is a tradeoff between increased flexibility and reduced performance at the optimum. Thus, habitats that have less environmental stochasticity should harbour larvae that perform well at the optimum. For example, under thermodynamic constraints, enzymes should be temperature specific and not function well over a broad range of temperatures (Somero, 1978); therefore, any enzymes associated with larval growth should be negatively affected by deviation away from an optimal temperature (*but see* (Paaijmans et al., 2008). Larval aggression is described as the innate ability of a larva to attack others and defend itself, and will be an important determinant of the outcome of density-dependent interactions such as cannibalism and competition for resources. Each habitat type has a different degree of density-dependence based on the number of individuals as well as the surface area available; therefore, aggression should play a larger role in habitats with a smaller surface area.

The environmental context where individuals interact is predicted to play an important role in the evolution of traits because it will lead to differences in both density-dependent and density-independent processes. For instance, the size of a body of water should play a significant role in the evolution of strategies. For instance, Service (1977) demonstrated that larger bodies of water tend to have more predators. Conversely, when the surface area of a larval habitat is smaller, larval interactions tend to increase via increased density (Koenraadt et al., 2004) and water temperature tends to be have higher variability (Paaijmans et al., 2008), both of which can lead to decreases in the rate of development of larvae (Lyimo et al., 1992). Furthermore, cannibalism is known to occur when larval densities are high, with larger 3rd and 4th larval instars feeding on smaller instars (Koenraadt & Takken, 2003; Sherrat & Church, 1994). Given how the environment may influence interactions between individuals (i.e., density-dependent interactions), it is necessary to model both larval interactions as they are mediated by a mother's selection of larval habitat. This sets up this problem nicely as a frequency and density dependent game model. The Model

We develop an individual-based game-theory simulation model called a genetic algorithm (GA) in which strategies compete with each other in a manner analogous to natural selection. Our goal is to converge on optimized solutions to ecological problems (and not the genes *per se*). In these models, strategies are comprised of different trait combinations, have differential reproduction associated with variation, and create new variants via processes similar to crossover and mutation (Forrest, 1993). Generally, GAs lack the mathematical elegance of analytical models but have the benefit of making the underlying assumptions explicit (Ruxton & Beauchamp, 2008). However, they also run the risk of becoming over-parameterized and complete exploration of parameter space is difficult; therefore, our exploration of parameter space was not exhaustive. By using a game theoretic approach, we allow for frequency dependence in female adult oviposition decisions and the co-evolution of adult and larval traits.

We employ a GA model using 'a mosquito' without a particular mosquito species in mind. Each individual is described by a strategy which contains the traits for (1) the adult female mosquito's preference ranking of different larval habitats (a_1), (2) the strength of that habitat ranking (a_2), (3) the aggressiveness of a larva when it encounters another larva (l_1), and (4) larval growth flexibility (l_2). Each strategy consists of a string of binary code (i.e., 0 or 1), where trait a_1 is 1 bit long, which allows for two (2^1) possible trait values, and the remaining three traits are 3 bits long, which allows for 8 (2^3) possible trait values. The trait values are shown in Table 4.1. Thus, each strategy is represented using a string that is 10 bits long for a total of 1024 (2^{10}) unique strategies.

In each simulation, we consider two habitat types (h). Our virtual world is not spatially explicit but has a spatial component; i.e., it is divided into different larval habitats that occur in defined numbers. Each habitat type is characterized by the number of habitats of each type, usable surface area (SA_h), a per unit time predation risk (μ_h), and the availability of food resources (g_h). Additionally, each habitat type is characterized by the degree of environmental stochasticity in growth conditions. We attribute this stochasticity to changes in water temperature such that smaller bodies of water are more responsive to changes in ambient temperature than larger bodies of water (Paaijmans *et al.*, 2008). Initially, we assume that the two habitat types differ in only one characteristic, but later we focus on scenarios where mosquitoes are presented with pots and ponds.

There are several limiting assumptions to our model. We assume that females deposit their entire egg load into a single habitat. Thus, this theory only applies to individuals that oviposit in batches (e.g., *Anopheles gambiae;* W. Takken *pers. obs.*) and will not apply to skip ovipositors (e.g. *Aedes aegypti*). Furthermore, we do not use an explicit spatial topology. Spatial distribution of offspring has been used as a measure linking oviposition preference and offspring performance; however, other processes might also account for observed offspring distribution like dispersal limitations or spatially heterogeneous mortality rates (Ellis, 2008). Oviposition decisions have also been shown to be linked to the spatial proximity to hosts in *A. gambiae s.s.* and *Anopheles arabiensis* (Minakawa et al., 1999). By tracking only a single cohort, we assume that larvae have sufficient time to develop such that habitat permanence does not play an important role (Minakawa et al., 2005b, Mokany & Mokany, 2006). It has been shown that decreased habitat permanence can lead to decreases in pupal occurrence for *A. gambiae s.l.*, a proxy for adult vector production (Minakawa et al., 2005b). Furthermore, we assume that the environment is fixed; i.e., that the number of ponds and pots does not change through time or across generations. This may be an interesting parameter to study, as we predict that any changes in this will likely select for a more general preference for oviposition sites, which would likely change values for the larval traits as well such that larvae will likely be more suited for utilizing both pots and ponds. Finally, we recognize that there are other factors that may affect the ability of females to produce offspring such as nutritional effects (Grech et al., 2007) and body size (Lyimo & Takken, 1993). However, the focus of this study was to determine how habitat preference affects offspring life history and we therefore kept these other factors fixed.

4.2.1 Model initialization

At the beginning of a simulation, we start with $N_{\max}$ individuals that are each assigned a random strategy and habitat; thus, the simulations begin with larvae in their aquatic habitats. In subsequent generations, the placement of individuals is a function of the adult female's decisions. We track this cohort of individuals through $G_{\max}$ generations. Each generation is subject to (1) within generation dynamics and (2) between generation dynamics.

4.2.2 Within generation dynamics

Each copy of a strategy is treated as an individual. Individuals within a certain habitat interact with one another, eat, and develop over a maximum of $T_{\max}$ time steps. In a given time step and habitat, larvae interact as a function of larval density (N). We make a simplifying assumption and assume that larval density (N) is a simple function of the number of individuals per unit of surface area (i.e., we ignore landscape features of the water bodies). The smaller surface area will increase the likelihood of individuals encountering one another as a function of increased larval density. If there is more than one individual, individuals are paired up randomly, and the probability of encounter ($P_{encounter}$) is calculated. We assume that an individual can only interact with one other individual at a given time, and therefore $P_{encounter}$ js expected to saturate as the density of individuals increases, yielding the equation:

$$P_{encounter} = \frac{N}{N + b} \qquad \text{eq. (1)}$$

where b is the density at which there is a 50% chance of an interaction occurring. If an encounter does occur, the outcome is a simple probabilistic function based on the relative aggression trait (l_1) of the two larvae mediated by their larval instar stages, where the probability of larva 1 (the individual of higher stage) winning (P_{win}) is defined as

$$P_{win} = \frac{l_{1,larva1} s_{larva1}}{l_{1,larva1} s_{larva1} + l_{1,larva2} s_{larva2}} \qquad \text{eq. (2)}$$

where we assume that larva 1 is always the larva of higher stage. If the two larvae are of the same stage, then the assignment of larva 1 and 2 is arbitrary. $l_{1,larva\_i}$ is the aggression trait value of individual i and s_i is the stage on individual i. We draw a random number between 0 and 1 and compare it to P_{win}. If the larva with the lower aggression trait value wins an interaction, we assume that they successfully escape. If the larva with the higher aggression trait value wins the interaction, there are two possible outcomes that depend on the difference in instar classes between the two individuals. If the difference in larval stages is < 2, then the winner gains access to a fixed reward of α energy units; if the difference is ≥ 2, then the loser is eaten (Koenraadt & Takken, 2003, Sherratt & Church, 1994) and the larger larva takes all of the smaller larva's energy as well as the α energy units. This sets up a scenario where both exploitative and scramble competition are present in a situation where resources are inexhaustible. In all cases, when an interaction occurs, each individual pays a metabolic cost: the winner pays a small metabolic cost β_{win}; the loser pays a larger metabolic cost β_{lose}. In the case of a successful escape both individuals pay a metabolic cost of β_{win}.

In addition to interacting with one another, individuals also eat food in their larval habitat. The amount of energy that each individual consumes is a function of environmental stochasticity and the ability of the larva to cope with this variability (l_2). Individuals with low l_2 trait values exhibit behavioural plasticity that allows them to cope with changes in temperature more readily than individuals with high l_2 trait values. We also assume that individuals with a low l_2 (temperature generalist) trait do worse than individuals with a high l_2 (temperature specialist) trait at optimal conditions. Each habitat type (h) has a food density associated with it, g_h, which is modified by the ability of the larva to take it in, $\gamma(l_2)$, where

$$\gamma(l_2) = \frac{(x/3)^{l_2}}{2(1/3)^{l_2} + 2(2/3)^{l_2} + (3/3)^{l_2}} \qquad \text{eq. (3)}$$

and x is a rank score from 3 for optimal conditions to 1 for ± 2 S.E. $\gamma(l_2)$ is normalized to be bound between 0 and 1. This value discounts the food available in the habitat such that food from habitat type $h = g_h \gamma(l_2)$.

To track the total energy through a generation for each individual ($\varepsilon(t)$), we include the energy gained and lost through interactions, as well as energy gained from organic matter present in the environment. The energy in the next time step by the larger of the two individuals if it wins the interaction is

$$\varepsilon(t+1) = \left\{ \begin{matrix} \varepsilon(t) + \alpha + g_h \gamma(l_2) - \beta_{win} & \text{if } s_{larva1} - s_{larva2} < 2 \\ \varepsilon(t) + \alpha + \varepsilon_{loser} + g_h \gamma(l_2) - \beta_{win} & \text{if } s_{larva1} - s_{larva2} \geq 2 \end{matrix} \right\},$$

eq. (4a)

where ε_{loser} represents the energy of the loser. For the smaller individual, the energy in the next time step is

$$\varepsilon(t+1) = \left\{ \begin{matrix} \varepsilon(t) + \alpha + g_h \gamma(l_2) - \beta_{loss} & \text{if } s_{larva1} - s_{larva2} < 2 \\ 0 & \text{if } s_{larva1} - s_{larva2} \geq 2 \end{matrix} \right\}.$$

eq. (4b)

If the small larva escapes the interaction, the energy in the next time step for both individuals is

$$\varepsilon(t+1) = \varepsilon(t) + g_h \gamma(l_2) - \beta_{win}, \qquad \text{eq. (4c)}$$

and if no interaction occurs, the energy in the next time step for both individuals is

$$\varepsilon(t+1) = \varepsilon(t) + g_h \gamma(l_2). \qquad \text{eq. (4d)}$$

If an individual has gained enough energy to reach some critical energy threshold ε_{thresh}, then the larva will increase in instar stage (s_i) (from larval stage 1 to 4). Once a larva has reached ε_{thresh} a fourth time (i.e. is in instar 4), it will pupate. The pupa then has a 50:50 chance of emerging as an adult on either the first or second day.

Empirical evidence shows that in organisms with distinct life history transitions, individuals that emerge as adults sooner also emerge with a larger body size (assuming that larval density and water temperature are held constant) (Gimnig et al., 2002, Wilbur & Collins, 1973, Plaistow et al., 2004, Day & Rowe, 2002, Lyimo et al., 1992). Furthermore, a larger body size is related to higher fitness in mosquitoes (Takken et al., 1998, Briegel, 1990, Andersson, 1992, Lyimo & Takken, 1993). Given this relationship between size and time to emergence, our measure of fitness (F) is a linear function of the time to emergence (T_{emerge}).

$$F = T_{\max} - T_{emerge}. \qquad \text{eq. (5)}$$

4.2.3 Between generation dynamics

At the end of a generation, we take the ratio of the fitness summed across all of the individuals of a given strategy over the total fitness of all individuals that survive and pupate. This ratio gives us the proportional or relative fitness that a particular strategy represents in the 'mating pool'. Higher fitness strategies therefore make up a higher proportion of the N_{max} individuals that comprise the mating pool for the next generation. From these $N_{\max}$ individuals, mating pairs are then randomly drawn together to 'mate' and produce two offspring.

Upon mating, new strategies are generated through mutation- and crossover-like processes. The chance that a mutation occurs at random at each byte along the 12-byte string is 1%, and the chance that a crossover, whereby the parental chromosomes are spliced at a random location along the string, occurs is 10%. These high rates are used to converge on an optimal solution more quickly (Browning & Colman, 2004). If mutation or crossover does not occur, the parental strategies are passed unchanged to the next generation.

The habitat that larvae of the new generation are laid in is decided by the female adults's habitat preference (a_1), the strength of that preference (a_2), and the habitats encountered. Habitats are encountered probabilistically ($P_{habitat}$) based on the proportion of the number of that habitat type h (N_h) to the total number of habitats available; i.e.,

$$P_{habitat} = \frac{N_h}{\sum_{h=1}^{h=2} N_h}. \qquad \text{eq. (6)}$$

Upon encounter, the probability of accepting a habitat is ranked either 2 or 1 according to the trait value for a_1, where rank 2 is the most profitable (Table 4.1). This score is a relative measure of rank and is normalized into a probability of acceptance. The probability of accepting habitat h of rank x upon encounter ($P_{accept}(h)$) is described using a function describing the relative strength of accepting a habitat based on the preference rank (a_1) of the habitat encountered, and modified as a power function by the preference strength (a_2), normalized between zero and one by including all possible outcomes on the denominator.

$$P_{accept}(h) = \frac{(x/2)^{a_2}}{(1/2)^{a_2} + (2/2)^{a_2}}, \qquad \text{eq. (7)}$$

such that a low value of a_2 is associated with a generalist egg-laying strategy; i.e., where the strength of the habitat ranking is weak. As the value of a_2 increases, the strength of the habitat ranking increases towards an egg-laying strategy that specializes on the preferred habitat type. If a habitat is rejected, a new habitat is randomly drawn and accepted or rejected following eqs. (6) and (7).

Once a habitat is accepted by the mother, she lays her entire egg load into an oviposition site of that habitat type. The specific site is drawn randomly from the number of available sites of that type. For simplicity, we assume that each generation is discrete (i.e., non-overlapping), but to incorporate a continuous element into the timing of egg laying behaviour, individuals that reject habitats experience a delay in oviposition directly proportional to the number of rejections. After oviposition, larvae emerge and the generational cycle is repeated beginning with the ‘within generation dynamics’ of larval interactions. This cycle is repeated until the simulation is terminated.

4.2.4 Simulation termination

There are three possible outcomes to the simulations: (1) the simulation converges on a dominant strategy, (2) the simulation converges on multiple strategies, or (3) the model does not converge on a solution. Convergence is achieved when the total population fitness across generations asymptotes to a maximum.

4.3 Methods

All simulations were run using $N_{\text{max}} = 1000$ individuals for $G_{\text{max}} = 1000$ generations in a world with five each of two habitat types. Individuals within a generation were given $T_{\text{max}} = 100$ time steps to emerge as adults. In all simulations, total population fitness increased to an asymptote at ~100 generations. Each simulation for a novel parameter set (i.e., a different environment) was replicated three times. The individuals (and their strategies) that remained at the endpoint of a simulation were optimized for the environment described by the parameter set. Thus, we performed all analyses on these individuals, allowing for the final generation of individuals to complete all within generation processes. For each simulation, we recorded the average trait values, the number of individuals, the number of interactions, and the proportion of deaths in each habitat.

We considered a world that is described by five pots and five ponds. The innate difference between pots and ponds is that pots have a smaller surface area, which leads to an increased chance for larval interactions (eq. 1) (Koenraadt et al., 2004), and a higher variability in water temperature (eq. 3) (Paaijmans et al., 2008) to occur. The parameter values used are found in Table 4.2.

It is necessary to apply statistical techniques to the simulation results for two reasons. Firstly, there is variation in the results between each run of a simulation. Mating is a random event, with pairs assigned randomly, with the chance of cross-over or point mutations occurring for each individual when mating occurs. Within a larval generation, the placement of larvae in a particular habitat of a given habitat type is random. Furthermore, interactions between individuals have a stochastic component, where the probabilities of encounter another individual and winning an interaction are based on randomly drawn probabilities compared against trait values. Secondly, although a high number of simulation runs will reduce variability, the computational time associated with simulation runs was significant (~10h per run) making it unfeasible to perform so many runs as to make statistical analysis unnecessary.

To test for emergent links between the four traits (i.e., the formation of trait complexes) we used principal components analysis (PCA) using the data for average trait values across the entire range of combinations for environmental parameters explored. We used a correlation analysis to determine the correlation between trait values. In general, PCAs are used to account for as much of the variability as possible into what are called ‘principal component axes’. These axes, ranked by the proportion of variability in the variables they account for, can then be used to look for emergent links between traits. To satisfy the assumption of linearity in the data, we used an extended logit transformation on the average trait values using the maximum and minimum trait values (Table 4.1).

To determine the strategies adopted by individuals in a population, we examined two metrics. First we determined habitat preference of adult females as expressed by the number of larvae in a particular habitat type using an ANOVA. Second, to determine if there was a difference in the strategies employed between individuals in each habitat, we examined the traits associated with the exploitation of a given habitat by comparing the average trait values of individuals in pots and ponds using an ANOVA.

We examined the effects of varying environmental parameters in two parts. First we isolated the effects of a single environmental parameter by performing simulations using two habitat types that were equal in all ways and independently varied the parameter of interest. The baseline parameter values we used are found in Table 4.2. The parameters we examined were predation risk (μ_h), and food availability (g_h). Second, we returned to our environment that consists of pots and ponds, and focused our analysis on the environmental parameters describing food availability (g_h) and the per-unit-time predation risk (μ_h). We increased the predation risk in the pond habitat (μ_{ponds}) because studies have shown that larger bodies of water tend to harbor more predators (Service, 1977). We also focused on the mean food availability in pots (g_{pots}) which is thought to increase more quickly in smaller than larger habitats (C. Phelan, *unpubl results*).

Determining the effect of the environment on the strategies adopted by individuals in each habitat was done in two parts. First, we measured the number of larvae in each habitat using a logistic regression of the number of larvae in habitat type against the environmental parameter of interest. Second, we measured the average trait values of individuals in each of the two habitats using an analysis of covariance (ANCOVA) treating habitat type (h), background predation risk (μ_h), and food availability (g_h) as covariates. In general, ANCOVAs are used to compare the difference in means between two or more treatment groups across a range of one or more covariates, which is a continuous variable across which the treatment groups were measured. In this case, the ANCOVAs were used to examine how the mean trait values for the two types of larval habitat (pots or ponds) differ as the environment varies. The model was weighted by the number of individuals in each habitat type because in some instances (e.g., when predation risk was high) there were fewer individuals contributing to the average trait values. Data was linearized using an extended logit transformation because of the sigmoidal relationship in the data. To find the generalized linear model that best explained the simulation data we used the model with the lowest Akaike Information Criterion (AIC) score. AIC scores are used as a model selection tool, where models that are overparameterized are penalized (Johnson and Omland 2004). We also reported the adjusted r^2 value, which represents the proportion of variability in a data set accounted for by the statistical model where the covariates that are not of interest are fixed at their mean values. We reported higher order terms and interactions in the statistical model when they were significant. We used the mean for each habitat type adjusted for covariates to compare trait values between habitats. Additionally, in this set of simulations, we measured the difference in the number of interactions and also the proportion of individuals that died between habitats using ANCOVAs with habitat type as the treatment and the environmental parameters (i.e., background risk of death and food availability) as the covariates.

All statistical analyses were performed using SAS (v9.1.3).

4.4 Results

Are trait complexes formed?

The PCA demonstrated that 76.6% of the variation in the four traits is described by the first two PC axes which had eigenvalues > 1 (eigenvalues = 2.060 and 1.004 for Axis 1 and Axis 2 respectively) (Table 4.3). Axis 1 of the PCA demonstrated an interconnection between adult preference rank, adult preference strength, and larval growth specificity indicating the formation of a trait complex between these traits. Axis 2 was dominated by larval aggression with a weaker loading of residuals from the other three traits. There was a negative correlation between adult preference rank and adult preference strength ($r_p = -0.328$; $p < 0.0001$) suggesting that the preference for ponds was weaker than preference for pots. There was a strong positive correlation between adult preference rank and larval growth specificity ($r_p = 0.749$; $p < 0.0001$) suggesting that the preference for ponds was correlated with a larval strategy that performs well under optimal conditions but performs poorly under sub-optimal conditions. There was also a negative correlation between adult preference strength and larval growth specificity ($r_p =$

-0.410; $p < 0.0001$) indicating that strongly preferred habitats are associated with larvae that are robust against environmental variability.

What traits are associated with habitat exploitation?

In the environment characterized by pots and ponds, when predation risk and food availability are equal between habitats, 74.0 ± 1.8% of individuals were found in the larger pond habitat (ANOVA: $F_{1,4} = 366.66$, $P < 0.0001$). The average preference rank for individuals found in ponds was higher than for individuals found in pots but an overall preference for ponds was displayed regardless of habitat (ponds: 0.903 ± 0.014; pots: 0.784 ± 0.014; $F_{1,4} = 36.26$, $p < 0.01$) (Figure 1a). The preference strength was greater in ponds than pots, with individuals in ponds adhering to the ranking of habitats more strictly, and individuals in pots adopting a more general oviposition site strategy (ponds: 1.025 ± 0.021; pots: 0.879 ± 0.021; $F_{1,4} = 23.87$, $p < 0.01$) (Figure 4.1b). There was no difference in either of the larval characteristics. Larval aggression trait values were low and independent of habitats (ponds: 1.330 ± 0.012; pots: 1.320 ± 0.012; $F_{1,4} = 0.34$, $p > 0.5$) (Figure 4.1c). Similarly, larval growth specificity (Figure 4.1d) was independent of habitat (ponds: 0.479 ± 0.014; pots: 0.463 ± 0.014; $F_{1,4} = 0.67$, $p > 0.4$).

How does the environment change habitat preference and the corresponding trait values?

When we isolated the effect of a single environmental parameter, the preferred habitat had lower predation risk (μ_h) (logistic regression: $\chi^2 = 6462.37$, df=1, $p < 0.0001$), and higher food availability (g_h) ($\chi^2 = 2212.04$, df=1, $p < 0.0001$). In the world characterized by pots and ponds, when we varied predation risk and food availability, we found that habitat choice (as reflected the number of individuals in a habitat) changed, and also that the traits associated with individuals in each habitat changed. As food availability increased in pots (relative to ponds) more individuals were found in pots (logistic regression: $\chi^2 = 2016.195$, df=1, $p < 0.0001$), and as the predation risk in ponds increased more individuals were found in pots ($\chi^2 = 3410.64$, df=1, $p < 0.0001$).

The statistical model that best described the change in trait values associated with changing environmental parameters was selected using the AIC. The AIC scores for several models were within 2 units, suggesting that there is a family of statistical models that describe the data equally well; however, we present the model with the lowest AIC score (Table 4.3). In the full model, we included habitat (h), food availability in pots (g_{pots}), predation risk in ponds (μ_{ponds}), as well as quadratic and cubic terms for food availability and predation risk were also included. We used only first-order interactions when considering the best model. The analysis is performed over both covariates, and therefore the results for each trait value represent a three-dimensional response surface. However, when we graphically represent the responses, a three-dimensional surface obscures the most relevant results and we therefore present the results in two dimensions where one of the covariates is fixed at its average value while the other is allowed to vary (Figure 4.2).

With respect to adult traits, the adult trait for larval habitat preference rank changed significantly with changes in food availability and predation risk ($r^2 = 0.846$) (Table 4.4). Larval habitat preference rank decreased sigmoidally from preference for ponds to preference to pots as food availability in pots increased relative to food availability in ponds (Table 4.4) (Figure 4.2a). The switch in preference is shown by the higher adult preference rank (a_1) for individuals in ponds than in ponds (lsmeans; ponds:

0.342 ± 0.078; pots: 0.137 ± 0.037, $p < 0.0001$). This sigmoidal relationship reflects the switch in the preferred habitat as food availability increased, and this switch to a lower preference rank occurs sooner for higher predation risk (Table 4.4) (Figure 4.2b). Figure 4.2b shows a seemingly bimodal distribution of trait values, but this is because of the sigmoidal relationship across food availability. The data is normalized for the adjusted mean value for the covariate not examined – food availability, and therefore the seemingly bimodal relationship does not play a factor in the ANCOVA result. The strength of larval habitat preference by adults was relatively high in all cases and increased with changes in food availability and predation risk ($r^2 = 0.370$), and adults that laid their eggs in ponds tended to have weaker habitat preference strength than adults that laid their eggs in pots (ponds: 1.273 ± 0.068; pots: 1.052 ± 0.068; $p < 0.0001$) (Table 4.4) (Figure 4.2c, d). Preference strength increased sigmoidally with an increase in food availability, but at a slower rate in ponds than pots because of an interaction between h and g_{pots}. There was a decelerating increase in preference strength as predation risk increased.

With respect to larval traits, there was a significant effect of food availability and predation risk on larval aggression ($r^2 = 0.012$) (Table 4.4), but there was no difference between habitats (ponds: 0.500 ± 0.022; pots: 0.502 ± 0.011; $p > 0.05$) (Figure 4.2e, f). Although there was a significant effect of the covariates on mortality and larval aggression, there was very little change in larval aggression as food availability and mortality change. There was a significant impact of food availability and predation risk on larval growth specificity ($r^2 = 0.683$) (Table 4.4). Larval growth specificity was higher for individuals found in pots than those found in ponds and are therefore better able to cope with environmental fluctuations (ponds: 1.320 ± 0.056; pots: 1.422 ± 0.064; $p < 0.01$), but there was a significant three-way interaction between habitat, g_{pots} and μ_{ponds}. There was an approximately sigmoidal decrease in larval growth specificity in both habitats as food availability increased (Figure 4.2g, h). There was also a sigmoidal decrease in larval growth specificity in pots as predation risk in ponds increased, but there was a decelerating increase in larval growth specificity in ponds.

There were significantly more interactions per individual in pots than ponds (ANCOVA lsmeans: ponds = 0.0050 ± 0.0132; pots = 1.189 ± 0.013; $p < 0.0001$), with interactions decreasing as food availability in pots increased (Figure 4.3a), and increasing as the predation risk in ponds increased (Figure 4.3b). Furthermore, interactions seldom led to cannibalism (<5%). There was also a higher proportion of survivors in pots as the predation risk in ponds increased (ANCOVA lsmeans: ponds = 0.284 ± 0.004; pots 0.816 ± 0.004; $p < 0.0001$).

4.5 Discussion

When considering egg-laying habitat decisions in organisms with two distinct life stages, the characteristics of a mother and her offspring should be considered together. Using mosquitoes as our model organism, we used an evolutionary game simulation to ask how adult and larval traits might change to exploit different habitats under different environmental conditions.

The theory presented here suggests that egg-laying habitat exploitation is modulated by a mother’s behavioural traits as well as the ability of her larvae to cope with environmental conditions (cf. Perry and Roitberg’s (2005) study on the evolution of maternal mitigation by ladybird beetles). Two general strategies: (1) individuals that adopted a pot-loving strategy had a strong preference for pots and had larvae that were robust against environmental variability, and (2) individuals that adopted a pond-loving strategy had a marginally weaker preference strength and had larva that performed well under optimal conditions (i.e. temperature specialists). These traits formed a co-evolving trait complex of a mother's egg-laying habitat preference, the strength of that preference, and the ability of larvae to handle environmental stochasticity (in our case, the increased temperature fluctuations experienced in small larval habitats). However, there was no directional selection for larval aggression.

We expected that density-dependence in larval habitats would have a large effect on the coalesced trait complex that emerged from the simulations, but this was not the case as indicated by the lack of directional selection for larval aggression and <5% of interactions resulting in cannibalism. This suggested that interactions were usually non-lethal and occurred between individuals of similar instars. Our simulations accounted for differences in the timing of decisions by assuming that mothers that reject habitats must delay oviposition; we did not allow for overlapping generations. However, in a series of post-hoc simulations, an increase larval aggression evolved when we forced half of the individuals to emerge on day 1 and the other half on day 20. This led in a wider variation in the size of larvae which in turn increased the frequency of cannibalism, suggesting that the payoff that older instars gained from cannibalizing younger instars drove the larval aggression trait to high values. Thus, the model presented here predicts that the cost of laying eggs in an environment late is strongly selected against. This is supported by empirical evidence. *An. gambiae s.l.* females tend to avoid ovipositing in sites with older instar larvae (McCrae 1984). Sherratt and Church (1994) performed a similar manipulation to ours (but in an empirical study using *Trichoprospon digitatum*) and found that larger larvae would cannibalize smaller larvae, and therefore mothers tended to choose habitats without larger conspecifics.

Our results suggest there were no ‘generalists’ *per se*, although our model was designed to allow generalist strategies to evolve. However, preference strength was never fixed at a pure specialist strategy suggesting that a small degree of ‘generality’ was evolutionarily stable. This result is consistent with empirical evidence that suggests that the females of given species of mosquito lay their eggs in particular habitats (e.g., *Anopheles gambiae s.s.*, *Anopheles arabiensis*, *Aedes aegypti*, and *Aedes albopictus*) (Clements, 1999). However, our results also suggest that a small degree of generality in habitat choice by mosquitoes is optimal provided the cost of laying eggs in the ‘wrong’ habitat type is not too high.

As the food availability in the smaller habitats (pots) increased, more larvae were found in those habitats. This was correlated with a strong (i.e., high l_2-value) preference for pots. There is evidence that food availability in the larval habitat should play a major role in determining oviposition site preference by adults. Studies have shown that an important source of food for many mosquito species is algae (Bond et al., 2005, Kaufman et al., 2006) and the presence of algae is positively correlated with the presence of larvae (Bond et al., 2004, Gimnig et al., 2001). Female adult *Anopheles funestus*, when given a choice, prefer habitat with algae present over clean fresh water (Gimnig et al., 2001). *Trichoprosopon digitatum* females chose pots with more food (bread crumbs) than pots with less food (Sherratt & Church, 1994). Increased food for larvae allows them to emerge more quickly and at a larger size (Gimnig et al., 2002, Wilbur & Collins, 1973, Takken et al., 1998, Koenraadt et al., 2004), thereby emerging with higher relative fitness and escaping other risks associated with prolonged exposure to the aquatic larval habitat such as predators.

Furthermore, preference strength increased as mortality increased, which was due to the increasing cost of females laying eggs in the sub-optimal habitat, as evidenced by fewer survivors in habitats with higher mortality. This was coupled with an increase in larval growth specificity as mortality increased. When mortality was low in both pots and ponds, there was little difference in the ability of larvae to handle changes in environmental conditions whereby individuals on average adopted intermediately high larval growth specificity, suggesting that they performed well under optimal growth conditions but were capable of dealing with some environmental stochasticity. However, as mortality in the ponds increased, growth specificity increased for individuals in ponds but decreased for individuals in pots. These results suggest that there were two ways of dealing with the risk of death – either have larval characteristics that allowed them to grow quickly to escape the aquatic environment (Koenraadt *et al.*, 2004) or have adult characteristics where a different (less risky) habitat is chosen (Munga et al., 2006, Kiflawi et al., 2003).

Understanding egg laying behaviour can have implications on population control. For instance, Gu *et al.* (2006) argue that a reduction in the proportion of suitable larval habitats will increase the gonotrophic cycle length because females will spend more time searching for a suitable egg-laying habitat. This subsequently reduces the ability of a mosquito population to transmit parasites. Thus, a greater understanding of the mechanisms leading to differences in life histories of different mosquito species will give us a better understanding of the preferred habitats of a given species, and potentially account for the degree of generality in their habitat preferences. This is especially important for mosquito species that are disease vectors because decreasing the ability of adult mosquitoes to oviposit successfully and decreasing larval performance will have an impact on disease transmission (Gu & Novak, 2005, Gu et al., 2006).

Although the work presented here is on mosquitoes, our model predictions should be applicable to other organisms with life history stages that utilize different habitats. For instance, in frogs the selection of oviposition sites can greatly affect the life history characteristics of tadpoles (Resitaritis 1996). Resitaritis (1996) links adult behaviour, in particular oviposition site preference, to life history evolution in frogs. Also, in insect parasitoid systems, the environment (i.e. host) that a parasitoid larva experiences is contingent on their mother’s selection of hosts (Henry et al., 2006, Henry et al., 2009), and mothers select hosts based on their perceived quality (Mackauer et al., 1996).

4.6 Acknowledgements

We would like to thank R.W. Stein for help with statistics and useful comments on a previous draft, G. Baker for help with the code (bit manipulation), C. Phelan for his extensive knowledge on mosquito larvae, and the Roitberg lab for discussions at various points along the way. This work was funded by NIH and NSERC grants held by B.D. Roitberg and a traveling grant from Wageningen University to W. Takken.

4.7 Tables

Table 4.1: Trait values for each of the four traits

	Trait value							
Trait	0	1	2	3	4	5	6	7
Adult Preference Rank (a_1)	0, 1	1, 0						
Adult Preference Strength (a_2)	0	0.25	0.50	0.75	1	1.25	1.5	1.75
Larval Aggression (l_1)	0.01	0.15	0.29	0.43	0.57	0.71	0.85	0.99
Larval Growth Specificity (l_2)	0	0.25	0.50	0.75	1	1.25	1.5	1.75

Table 4.2: Parameter values for evolutionary simulations

Parameter	Value	Description
h_{pot}	5	Number of pots in environment
h_{pond}	5	Number of ponds in environment
g_{pot}	3	Organic matter concentration in pots
g_{pond}	3	Organic matter concentration in pond
$å_{thresh}$	10	Critical energy threshold to molt
$ì_{pot}$	0.005	Hourly mortality in pot
$ì_{pond}$	0.005	Hourly mortality in pond
$â_{win}$	1	Metabolic cost of winning an interaction
$â_{lose}$	1	Metabolic cost of losing an interaction
$å$	1	Energetic gain from winning an interaction
b	500	Density at which there is a 50% probability of interaction occurring
SA_{pot}	0.01*	Useable surface area of pot
SA_{pond}	100.0*	Useable surface area of pond

* When habitat types were set to be equal, we used a value of 0.01 for both SA_{pot} and SA_{pond}.

Table 4.3: Principal components analysis (PCA) for the four trait values across a range of environmental conditions.

	Comp.1	Comp.2	Comp.3	Comp.4
Eigenvalues	**2.060**	**1.004**	0.691	0.245
Importance of Components:				
Standard Deviation	40.498	9.546	12.647	4.736
Proportion Variation	0.515	0.251	0.173	0.061
Cumulative Proportion	0.515	0.766	0.939	1.000
Loadings:				
Adult preference rank	0.600	-0.258	0.337	-0.678
Adult preference strength	-0.463	-0.254	0.843	0.106
Larval aggression	0.198	0.904	0.378	0.019
Larval growth specificity	0.621	-0.227	0.182	0.727
SS loadings	1.00	1.00	1.00	1.00
Proportion Variation	0.25	0.25	0.25	0.25
Cumulative Proportion	0.25	0.50	0.75	1.00

Table 4.4: Parameter estimates of linear model for habitat type (*h*) of individual mosquitoes for each of the four traits as a function of the food availability in the pots (g_{pot}) and mortality in ponds (μ_{pond}).

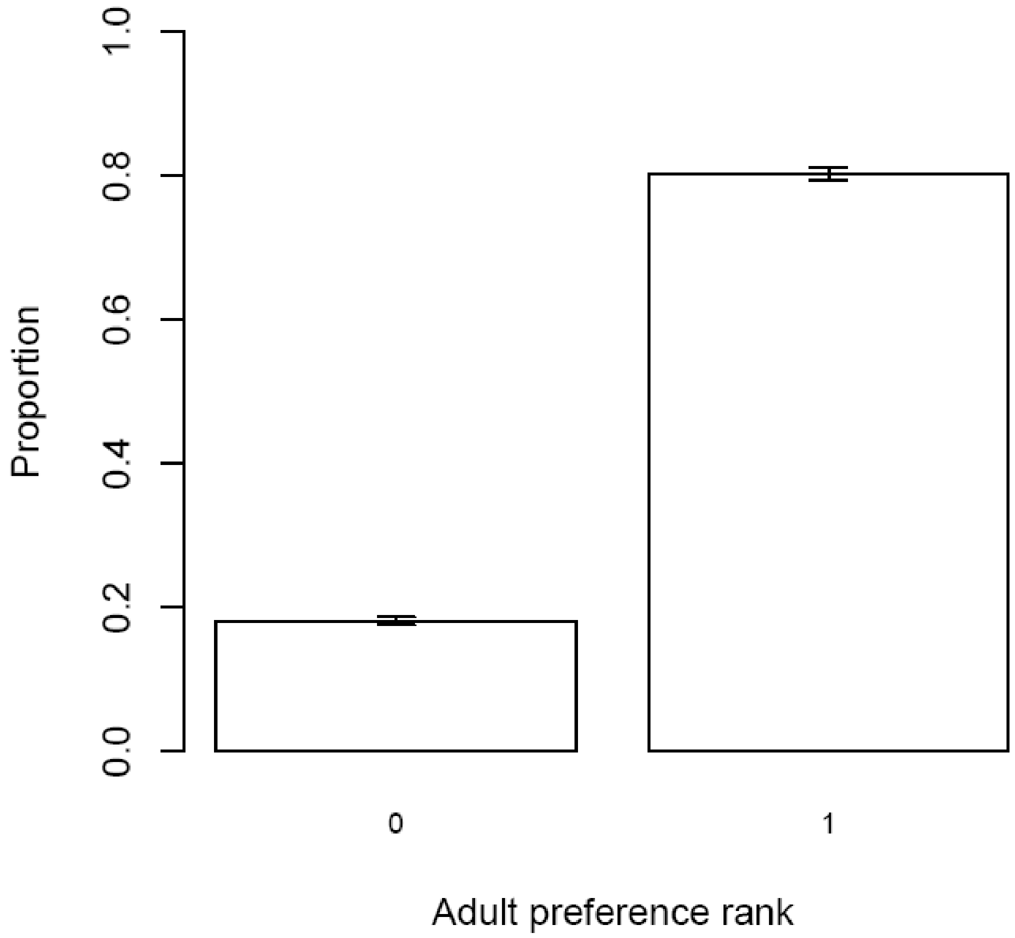
Trait	Estimates	SE	df	t value	Pr > |t|
Adult Preference Rank (a_1)			8,762	528.77*	<0.0001
intercept	4.488	0.184	1	24.40	<0.0001
h	1.188	0.090	1	13.25	<0.0001
g_{pot}	-1.771	0.079	1	-22.34	<0.0001
g_{pot}^{2}	0.160	0.015	1	10.75	<0.0001
g_{pot}^{3}	-0.00469	0.00079	1	-5.92	<0.0001
μ_{pond}	-82.930	10.478	1	-7.91	<0.0001
μ_{pond}^{2}	1078.241	240.351	1	4.49	<0.0001
μ_{pond}^{3}	-5092.333	1546.410	1	-3.29	0.0010
$g_{pot} \times \mu_{pond}$	0.995	0.285	1	3.49	0.0005
Adult Preference Strength (a_2)			11,829	45.80*	<0.0001
intercept	0.143	0.103	1	1.38	0.168
h	0.542	0.111	1	5.13	<0.0001
g_{pot}	-0.0827	0.038	1	-2.17	0.030
g_{pot}^{2}	0.0185	0.068	1	2.70	0.0071
g_{pot}^{3}	-0.00101	0.00036	1	-2.79	0.0055
μ_{pond}	27.552	5.100	1	5.40	<0.0001
μ_{pond}^{2}	-381.472	112.288	1	-3.40	0.0007
μ_{pond}^{3}	1251.123	714.100	1	1.75	0.0801
$h \times g_{pot}$	-0.0972	0.0156	1	-6.25	<0.0001
$h \times \mu_{pond}$	-7.515	3.368	1	-2.23	0.026
$g_{pot} \times \mu_{pond}$	0.783	0.153	1	5.11	<0.0001
$h \times g_{pot} \times \mu_{pond}$	0.978	0.486	1	2.01	0.045
Larval Aggression (l_1)			3,837	4.40*	0.0044
intercept	0.0402	0.0163	1	2.46	0.0139
h	-0.00854	0.02484	1	-0.34	0.7309
μ_{pond}^{3}	111.418	41.924	1	2.66	0.0080
$g_{pot} \times \mu_{pond}$	-0.164	0.046	1	-3.60	0.0003
Larval Growth Specificity (l_2)			11,824	164.67*	<0.0001
intercept	2.174	0.098	1	22.21	<0.0001
h	-0.344	0.105	1	-3.27	0.0011
g_{pot}	-0.190	0.036	1	-5.27	<0.0001
g_{pot}^{2}	-0.0108	0.0065	1	-1.67	0.095
g_{pot}^{3}	0.00165	0.00034	1	4.81	<0.0001
μ_{pond}	13.982	4.826	1	2.90	0.004
μ_{pond}^{2}	-365.968	106.267	1	-3.44	0.0006
μ_{pond}^{3}	2282.227	675.795	1	3.38	0.0008
$h \times g_{pot}$	0.0400	0.0147	1	2.72	0.0067
$h \times \mu_{pond}$	15.896	3.189	1	4.98	<0.0001
$g_{pot} \times \mu_{pond}$	-0.779	0.145	1	-5.37	<0.0001
$h \times g_{pot} \times \mu_{pond}$	-0.713	0.460	1	-1.55	0.1220

* F statistic and P-value for whole model

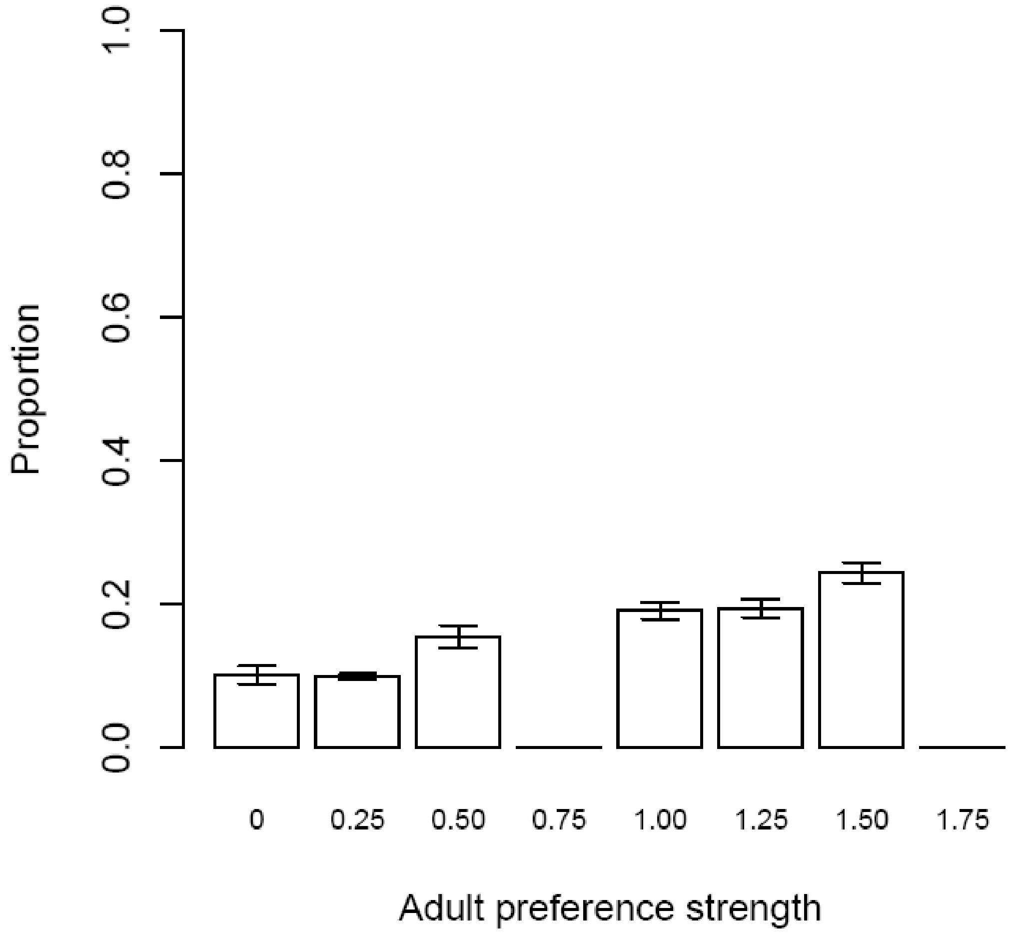
4.8 Figures

Figure 4.1: Trait values in a world with five pots and five ponds using the baseline parameter set (Table 2). Panel a shows the female adult mosquito's preference rank (a_1), where '1' represents a preference for pond habitats and '2' represents a preference for pot habitats. Panel b shows the female adult mosquito's strength of preference (a_2), where a high value represents a stronger preference strength. The two traits are combined following (eq. (7)). Panel c shows larval aggression (l_1), where a larva with a high value of aggression will tend to act aggressive to another larva, and a larva with a low value will tend to avoid interactions. Panel d shows larval growth flexibility (l_2), where a higher value represents a smaller degree of flexibility.

a)



b)


Proportion
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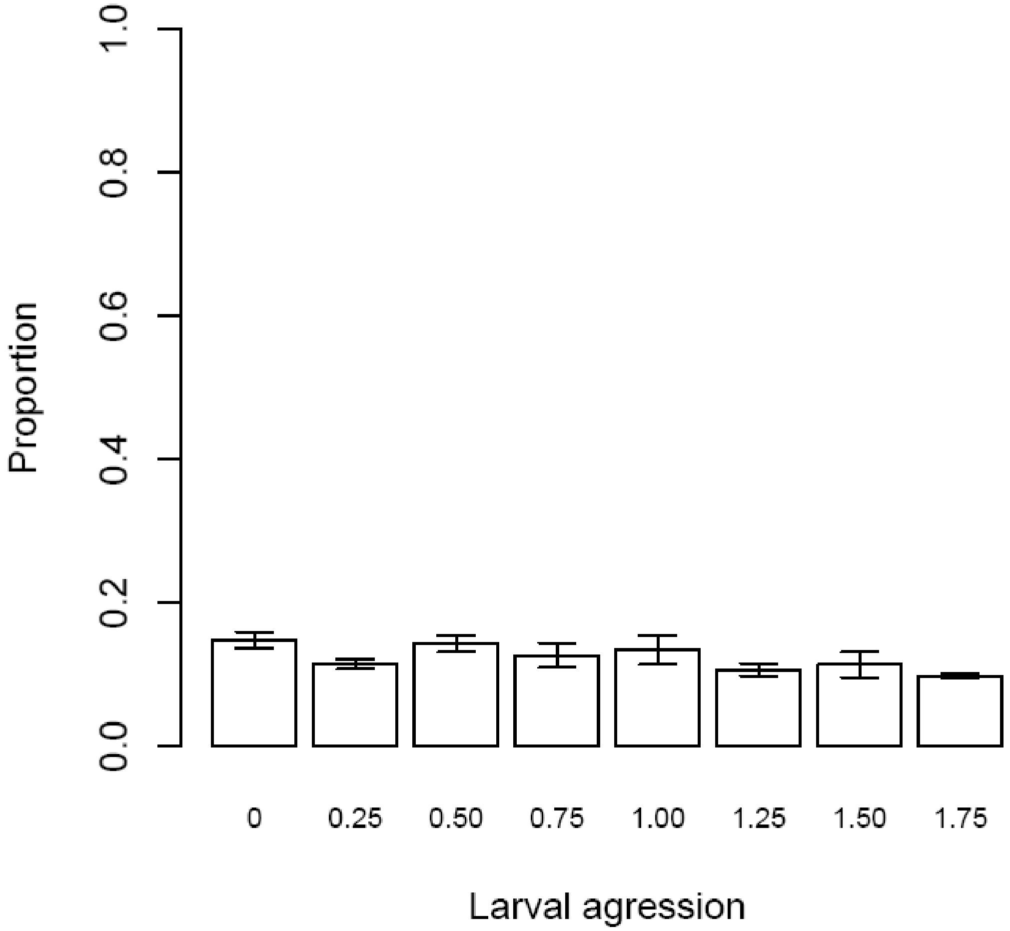
0.2
0.4
0.6
0.8
1.0
0
0.25
0.50
0.75
1.00
1.25
1.50
1.75
Adult preference strength

c)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0.2
0.4
0.6
0.8
1.0
0
0.25
0.50
0.75
1.00
1.25
1.50
1.75
Larval agression

d)

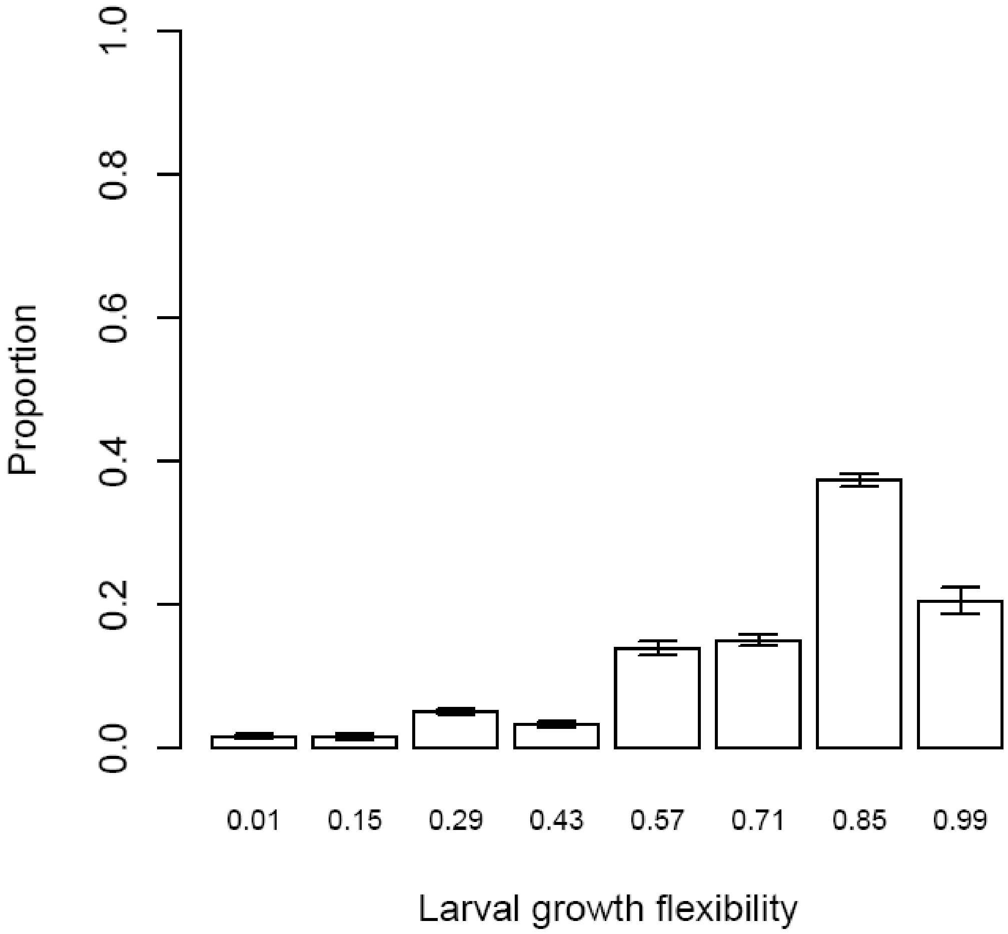
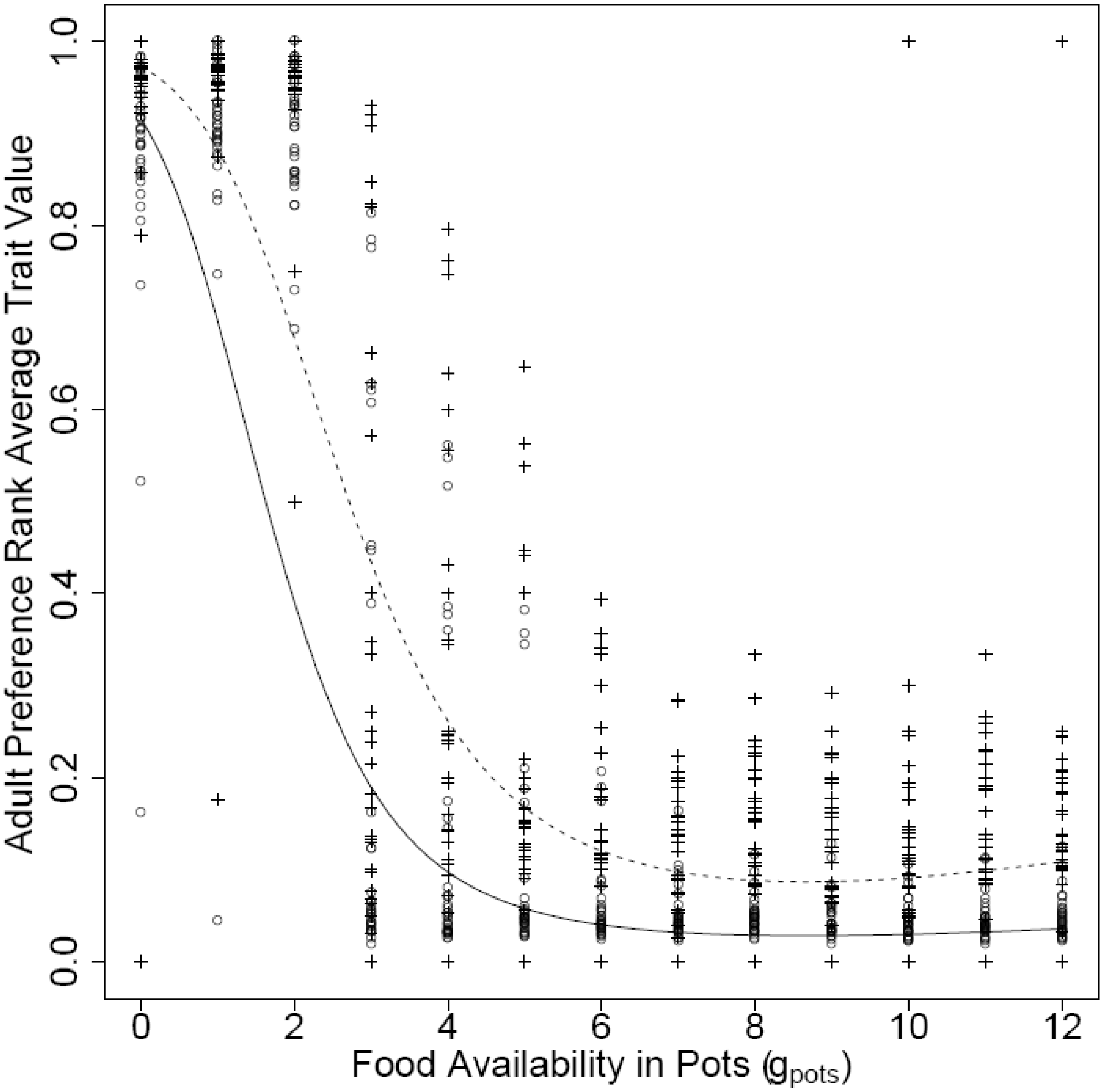
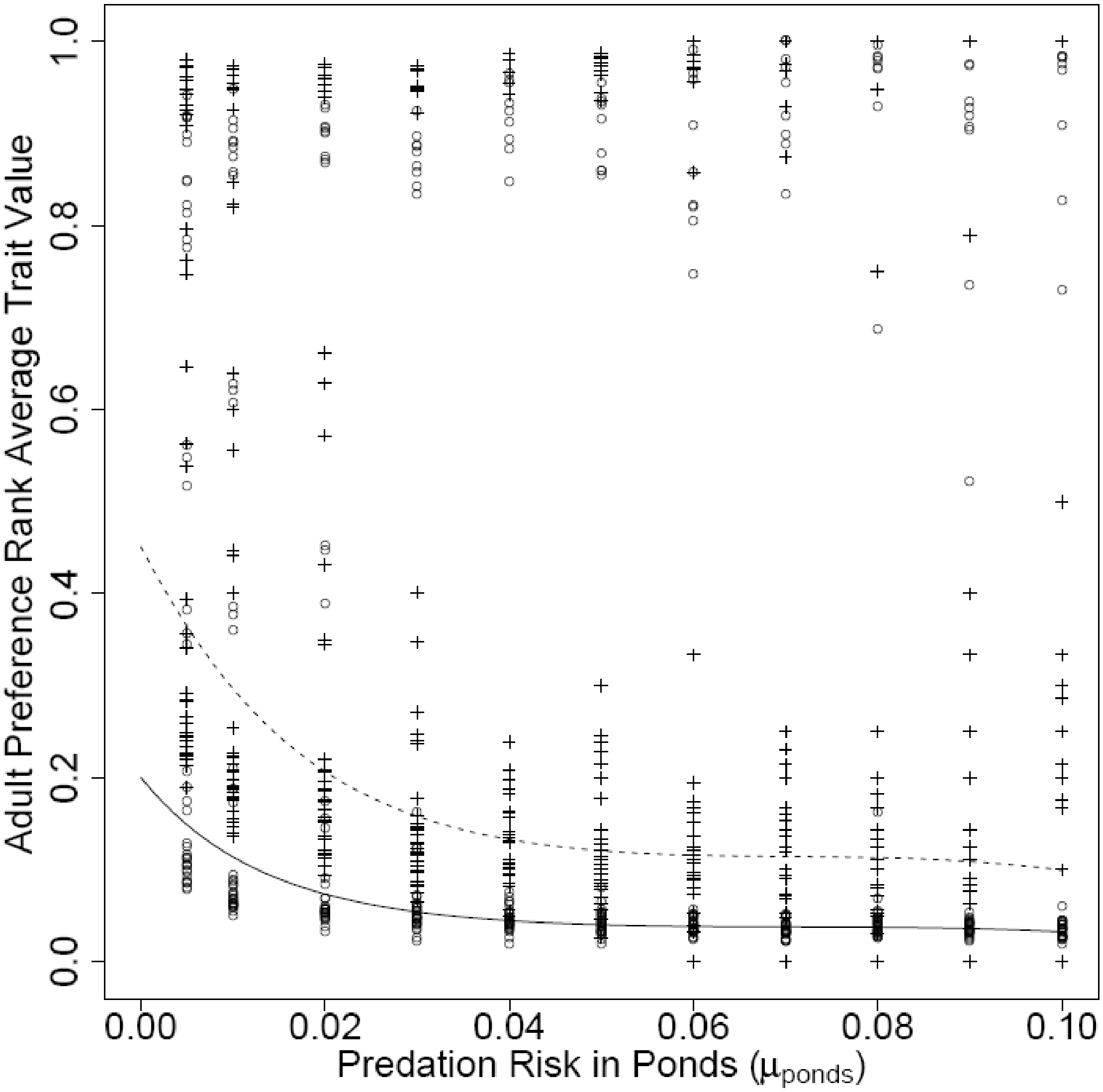

Proportion
1.0
0.8
0.6
0.4
0.2
0.0
0.01
0.15
0.29
0.43
0.57
0.71
0.85
0.99
Larval growth flexibility

Figure 4.2: The effect of food availability in pots (g_{pots}) and predation risk in ponds (μ_{ponds}) on the average trait values. When one environmental parameter is varied, the other is fixed at its average value. Data for individuals in ponds are represented by open circles ('o') while data for individuals in pots are represented by pluses ('+'). The solid line represents the best fit line for individuals in pots, while the dashed line represents the best fit line for individuals in ponds, and the equations for each is described in Table 4. Panels a and b show adult preference rank (a_1), panels c and d show adult preference strength (a_2), panels e and f show larval aggression (l_1), and panels g and h show larval growth specificity (l_2). The baseline parameters used in the simulations are shown in Table 2.

a)


Adult Preference Rank Average Trait Value
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
12
Food Availability in Pots (gpots)

b)


Adult Preference Rank Average Trait Value
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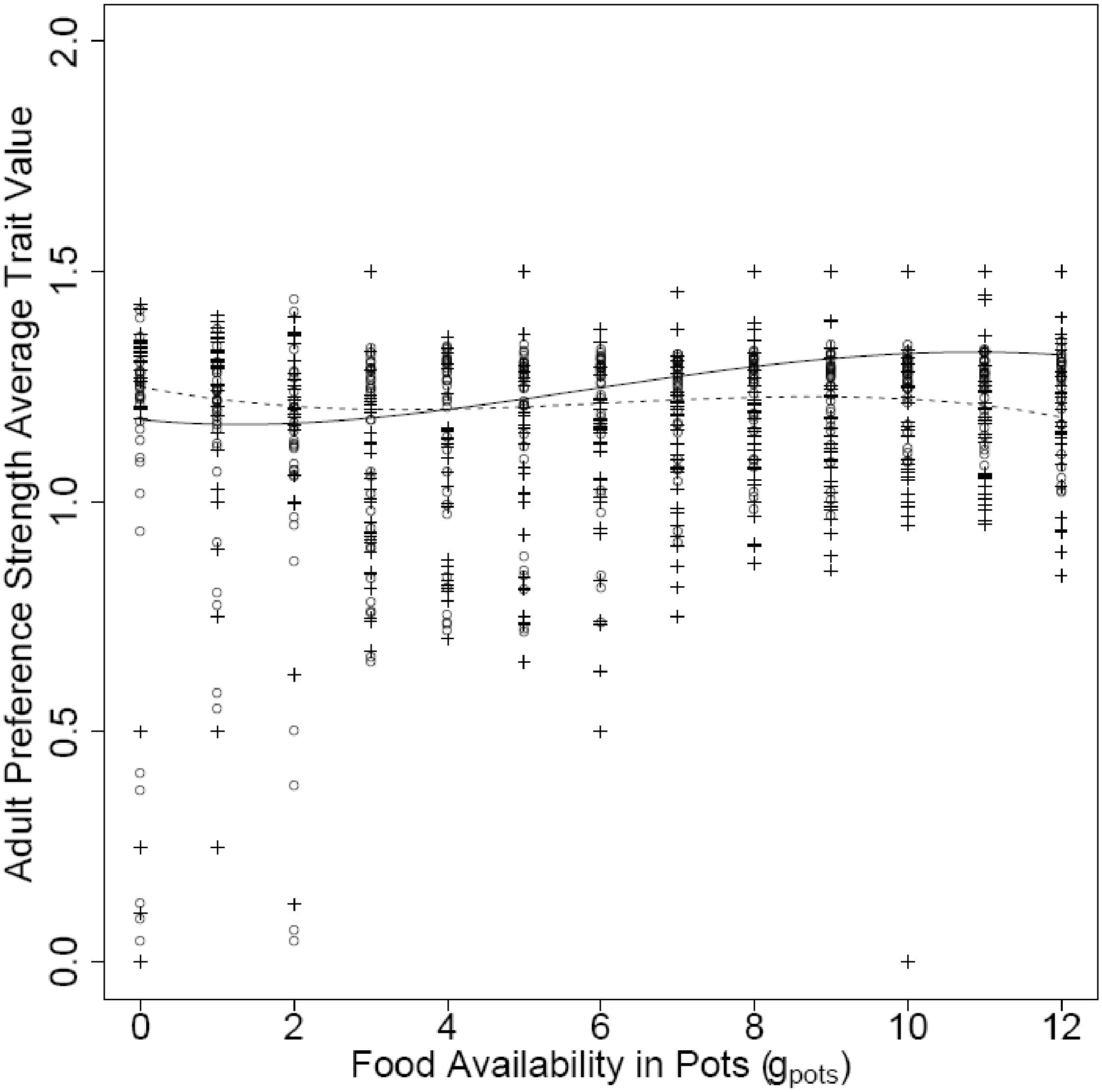
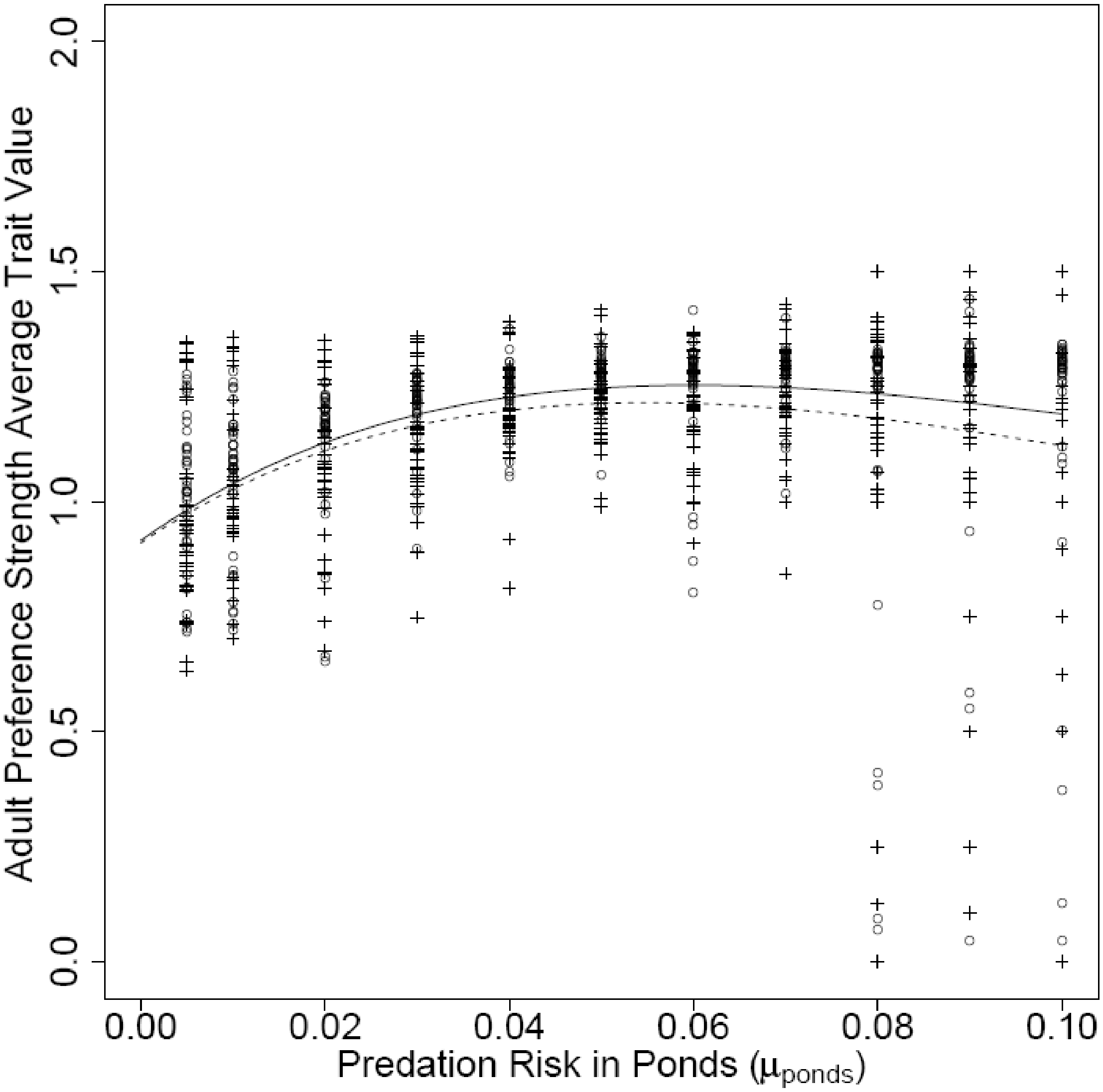
0.2
0.4
0.6
0.8
1.0
0.00
0.02
0.04
0.06
0.08
0.10
Predation Risk in Ponds (μ_{ponds})

c)

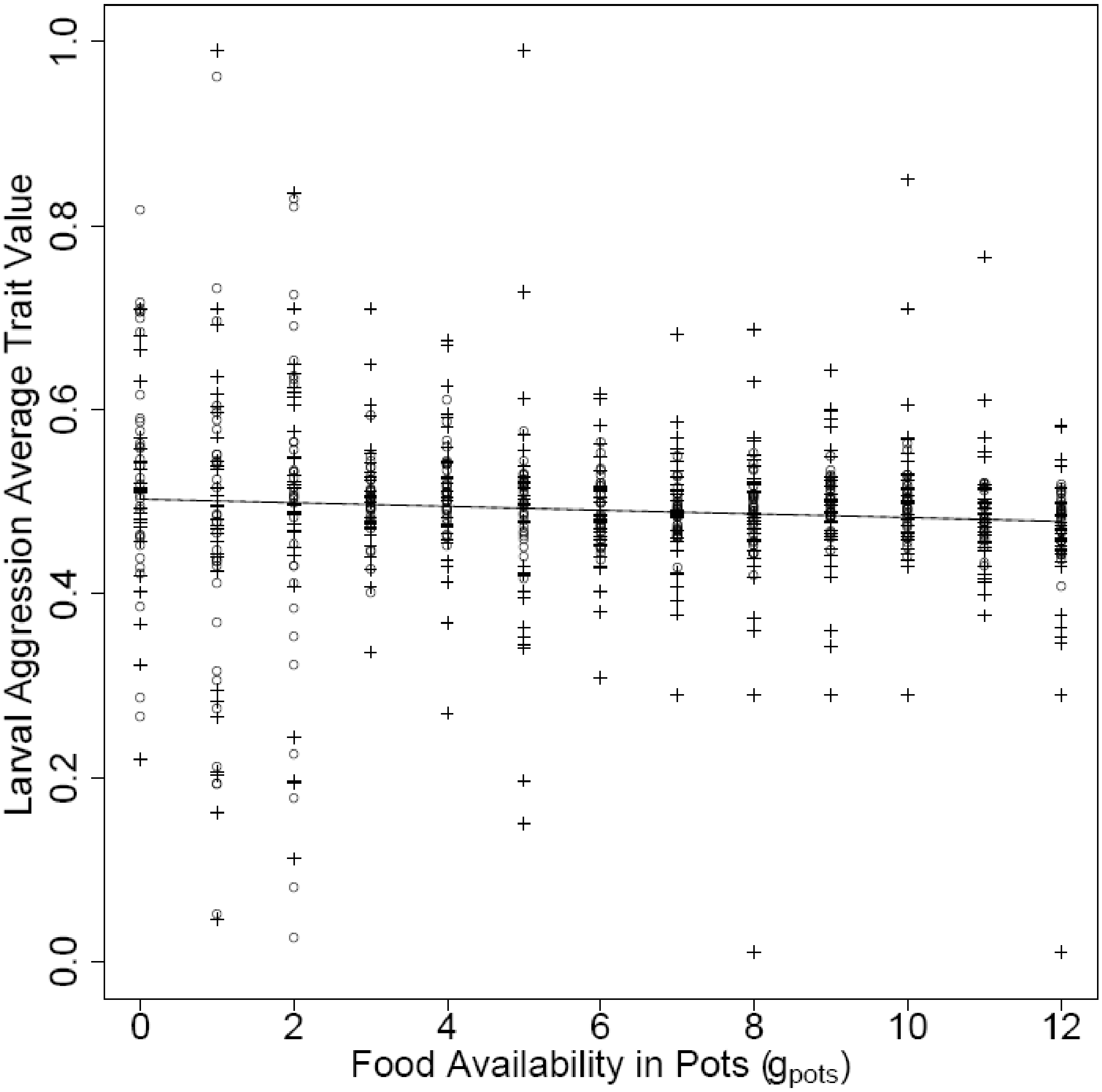


Adult Preference Strength Average Trait Value
0.0
0.5
1.0
1.5
2.0
0
2
4
6
8
10
12
Food Availability in Pots (g_pots)

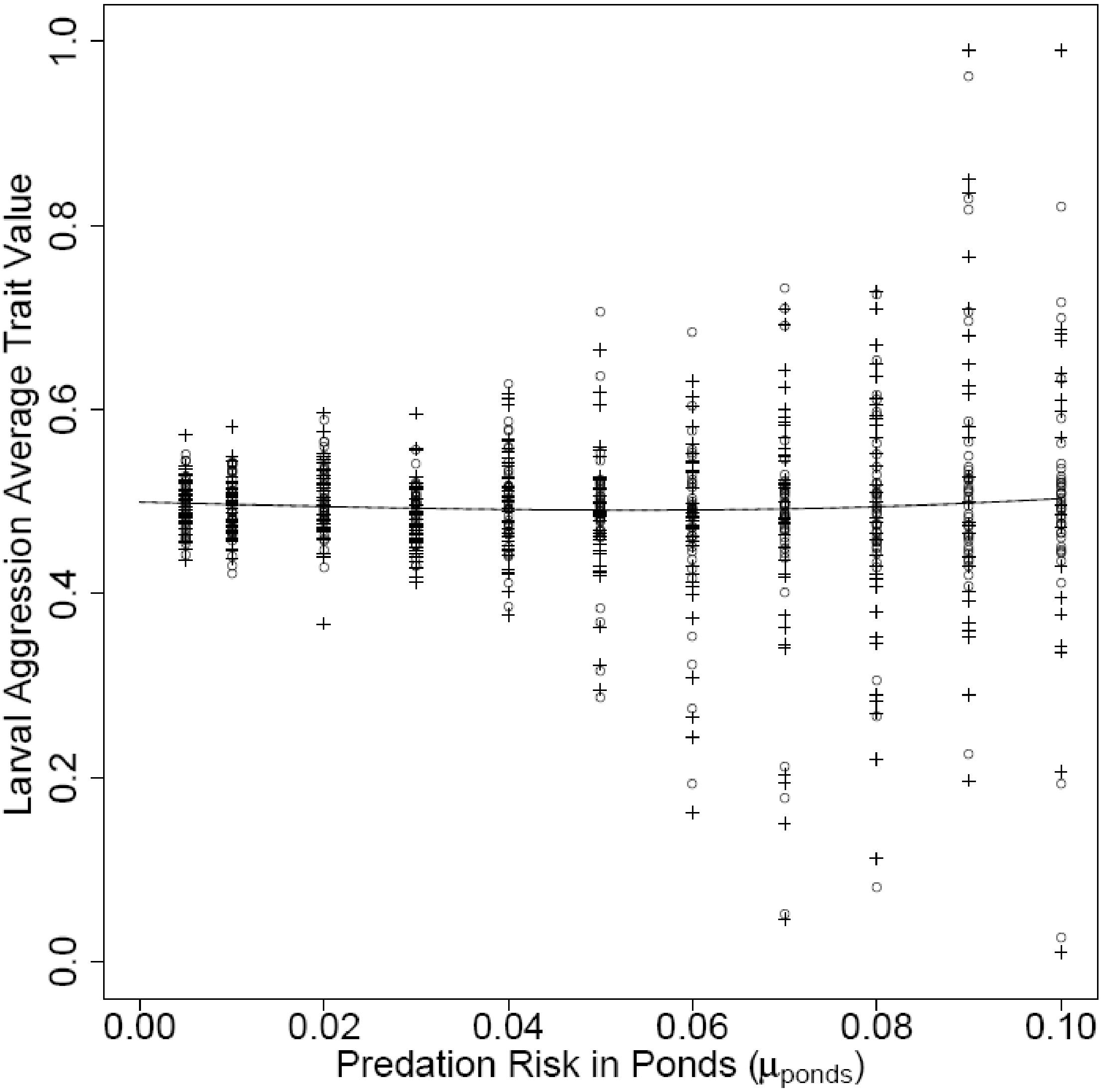
d)


Adult Preference Strength Average Trait Value
0.0
0.5
1.0
1.5
2.0
0.00
0.02
0.04
0.06
0.08
0.10
Predation Risk in Ponds (μ_{ponds})

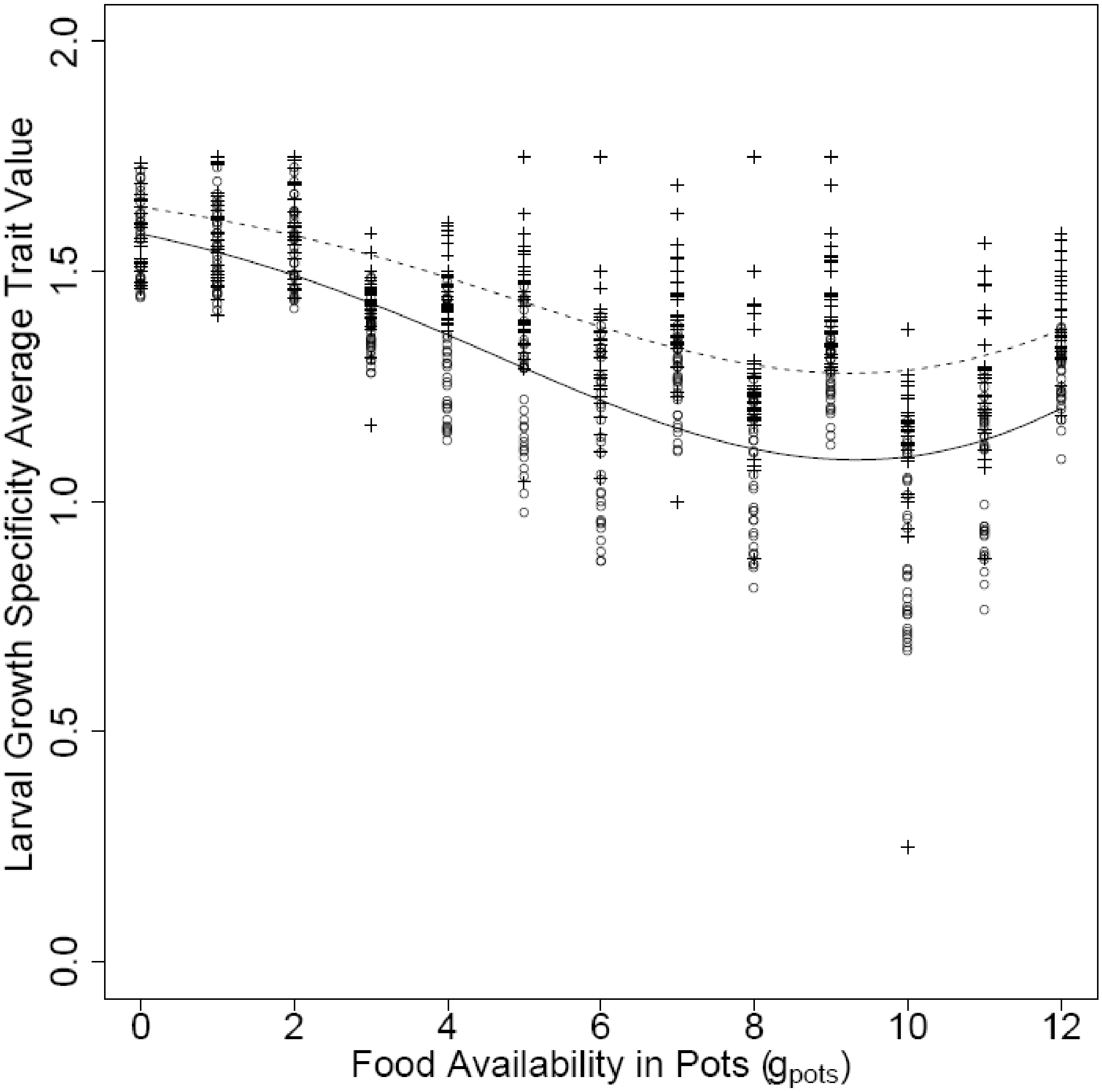
e)


Larval Aggression Average Trait Value
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
12
Food Availability in Pots (g$_{pots}$)

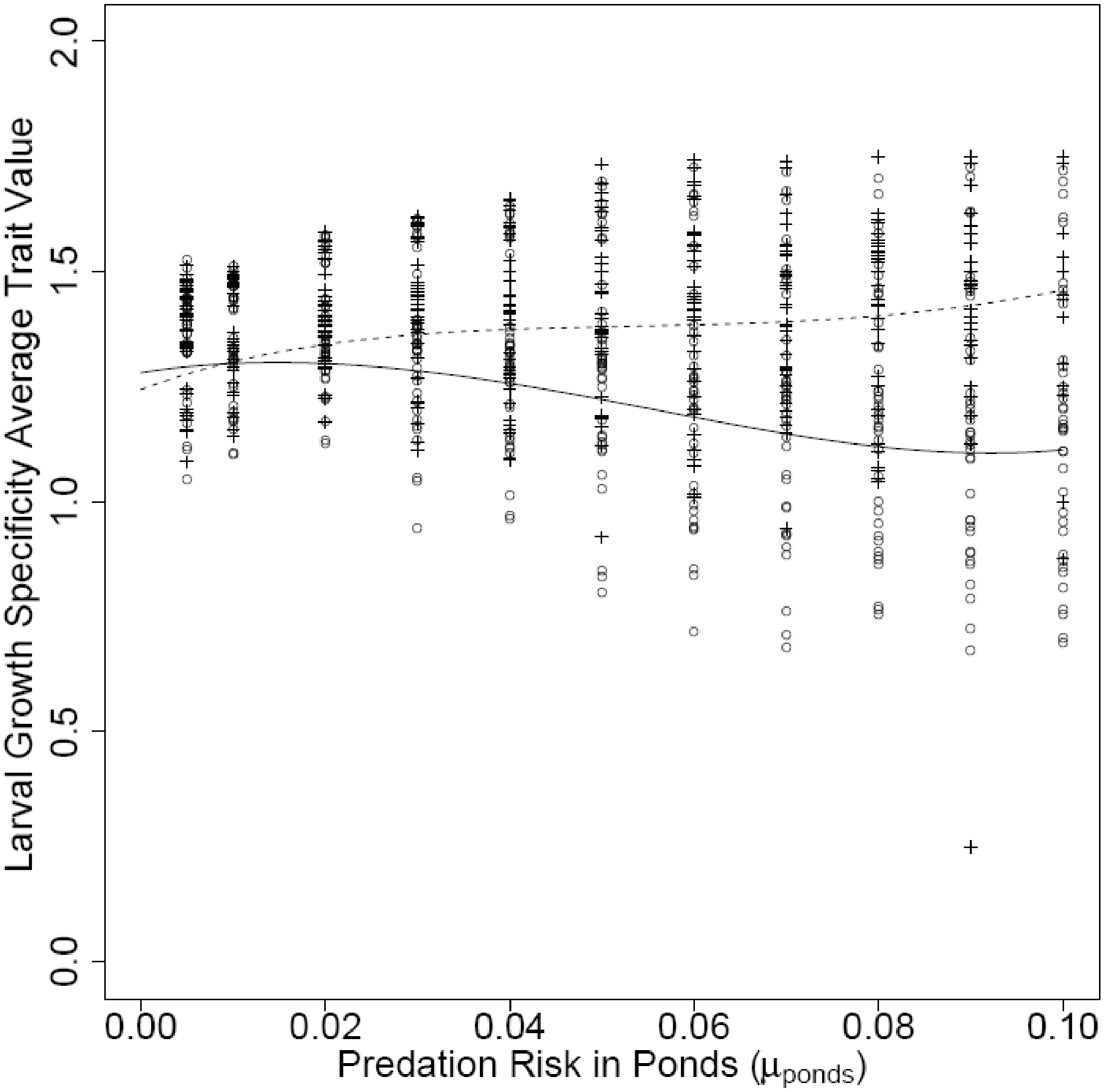
f)


Larval Aggression Average Trait Value
0.0
0.2
0.4
0.6
0.8
1.0
0.00
0.02
0.04
0.06
0.08
0.10
Predation Risk in Ponds (μ_{ponds})

g)


Larval Growth Specificity Average Trait Value
0.0
0.5
1.0
1.5
2.0
0
2
4
6
8
10
12
Food Availability in Pots (gpots)

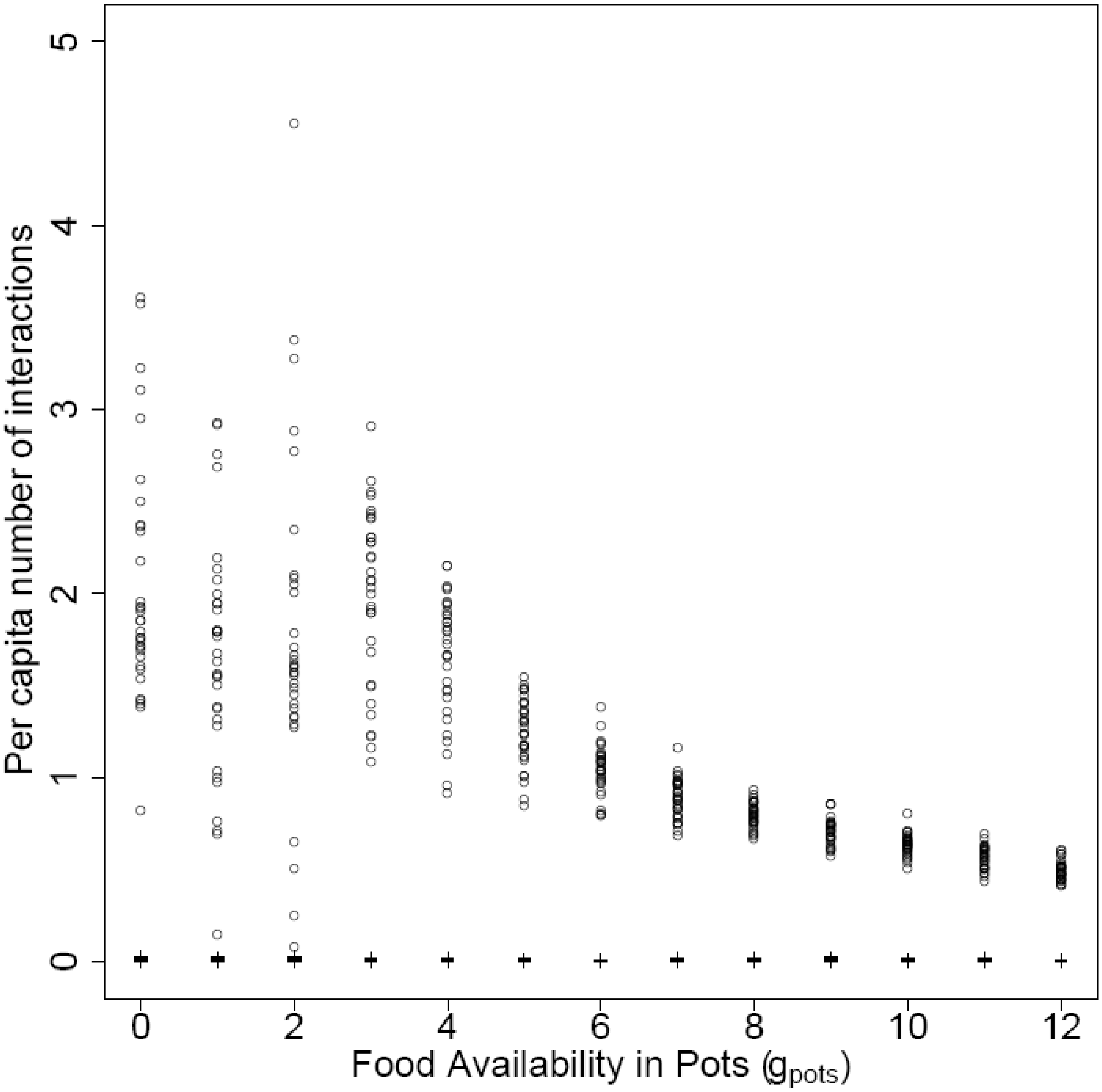
h)



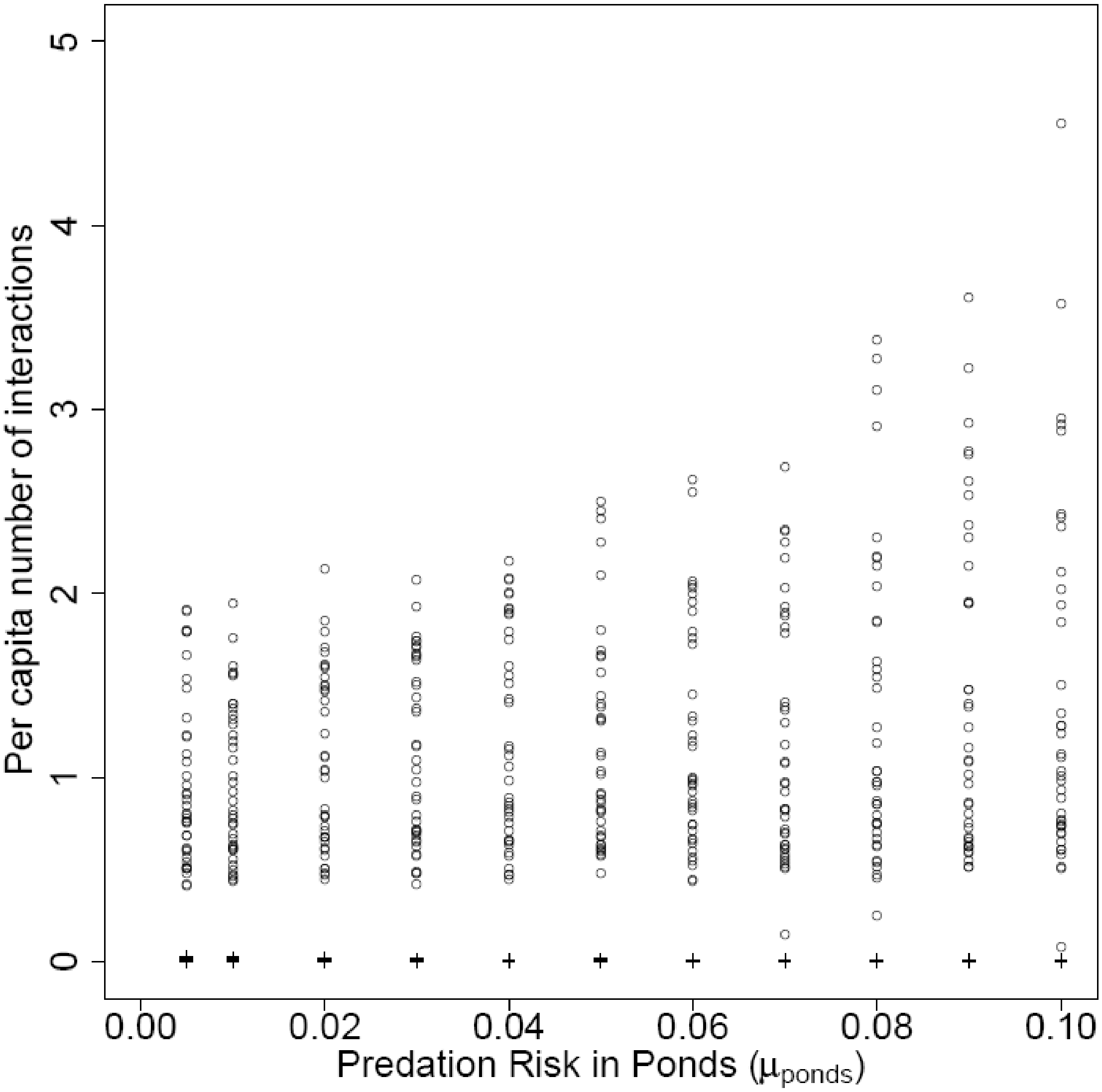
Larval Growth Specificity Average Trait Value
0.0
0.5
1.0
1.5
2.0
0.00
0.02
0.04
0.06
0.08
0.10
Predation Risk in Ponds (μ_{ponds})

Figure 4.3: The effect of environmental conditions on the number of interactions within the final generation of a simulation as a function of food availability in pots (g_{pots}) (panel a) and predation risk in ponds (μ_{ponds}) (panel b). Circles (‘o’) represent average trait values in pot habitats, and pluses (‘+’) represent average trait values in pond habitats

a)


Per capita number of interactions
0
1
2
3
4
5
0
2
4
6
8
10
12
Food Availability in Pots (g_pots)

b)


Per capita number of interactions
0
1
2
3
4
5
0.00
0.02
0.04
0.06
0.08
0.10
Predation Risk in Ponds (μ_{ponds})

4.9 List of references

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4.10 Connecting statement

In the following chapter, I expand on individual decision making strategies of female mosquitoes to a population level. Specifically, I explore how resource availability (both sugar and blood) can change the ability of a mosquito to transmit parasites, and how this is mitigated by mosquito behaviour. To do this, I use conventional measures of parasite transmission ability such as the vectorial capacity and the basic reproductive ratio.

5: Resource availability, mosquito behaviour, and malaria control

5.1 Abstract

I explore the role of the availability of sugar and blood hosts on a female mosquito's ability to transmit parasites. I argue that the role of resource availability on parasite transmission will be mitigated by mosquito behaviour. My results are presented in terms of how they affect metrics and dynamics of classic metrics of parasite transmission, including the vectorial capacity, the entomological inoculation rate, and the basic reproductive ratio as calculated from the classic Ross-Macdonald model. The results demonstrate that the availability of both sugar- and blood-hosts have a large impact on parasite transmission. Furthermore, I highlight the importance of the access to sugar on parasite transmission. I discuss the results in light of current malaria prevention programs.

Keywords: malaria, mosquitoes, parasite transmission, resource availability, mosquito behaviour, bed nets, environmental management.

5.2 Introduction

Human malaria is a disease caused by the parasite, *Plasmodium* spp., which is transmitted between human hosts by mosquitoes. Malaria continues to have significant medical and economic implications. From a health perspective, malaria remains a high cause of mortality, with 247 million cases of malaria leading to nearly one million deaths in 2006 (WHO, 2008).

Vector control has been the most effective means of reducing the incidence of disease and much of the practical efforts have been guided by simple models and metrics. Dynamic models of parasite transmission generally deal with the prevalence of infection, which describes the proportion or number of humans and mosquitoes harbouring parasites; these are often referred to as compartmental models. These models have provided insights into how to best control malaria (WHO, 2008). These insights include (1) malaria can exist in a population only if mosquito density exceeds a critical threshold, (2) endemicity of malaria is most sensitive to changes in mosquito survival rate (Macdonald, 1957), (3) variability in transmission can considerably bias the predictions of the impact of control measures and affect their outcome (Dobson, 1988; Kingsolver, 1987; Smith *et al.*, 2007), and (4) acquired immunity (and vaccination) may increase disease prevalence in adults (Aron, 1983; Aron, 1988a; Aron, 1988b; Koella, 1991).

Although these models have provided us some very good insight into how specific factors may affect disease transmission, they often ignore the importance of mosquito behaviour. These epidemiological models hinge on several simplifying assumptions. One such assumption is that mosquito biting rate is homogeneous, and therefore, incorporating behaviour into these models generally leads to incorporating behaviour as the ‘average’ behaviour of mosquitoes (Mangel & Roitberg, 1994). Although many models have downplayed the significance of mosquito behaviour, there are extensive studies on how different factors can influence a mosquito’s feeding decisions. Given the current understanding of mosquito foraging behaviour, why are most models so simple in their inception? The immediate answer is tractability (Ngwa, 2001). Classic models of mosquito-human dynamics are attractive because they are easy to understand, but this comes as a tradeoff as they make many restrictive assumptions. How might the predictions from models change if we consider mosquito behaviour?

Two general issues may affect the success of vector control, the variation in mosquito biting behaviour, and the possibility that it will change in the face of anti-mosquito measures (Feachem, 2009). Thus, mosquito feeding behaviour can influence parasite transmission dynamics in several ways. First, mosquitoes may exhibit preference for certain hosts, leading to heterogeneous bites. For instance, there is some evidence that mosquitoes can exhibit preference for infected hosts over uninfected hosts (Day *et al.*, 1983) because they may give off more attractive odour and temperature profiles (Lacroix *et al.*, 2005). This has a direct influence on compartmental based models of parasite transmission because the effects are between compartments. These influences on population level consequences have been explored by Kingsolver (1987) in a phenomonological manner (i.e., without any particular mechanism in mind). However, there is still conflicting evidence as to whether this preference exists or not (Burkot *et al.*, 1989; Moore, 1993). Regardless, heterogeneity in the bites per human can also arise from differences in attractiveness of hosts unrelated to parasite burden (Dye & Hasibeder, 1986; Lindsay *et al.*, 1993). Second, mosquitoes may exhibit differences in feeding behaviour as a result of changes in their physiological state. For instance, the human biting rate can change as a result of the mosquito's stage in parasite infection (Anderson *et al.*, 2000; Koella & Packer, 1996) or a mosquito's energy state (Chapters 2 & 3). Environmental factors should influence mosquito feeding behaviour. One such factor that can affect feeding behaviour is the availability blood hosts and/or sugar hosts (Ma & Roitberg, 2008), where resource availability is defined as the probability of acquiring a resource when sought. The role of resource availability is more difficult to account for in classic deterministic (compartmental) models of host-vector dynamics, such as the Ross-Macdonald model, because the environment is assumed to be homogeneous and individual mosquitoes are considered 'average' mosquitoes. In this situation, mosquito behaviour will not play a direct role in changing parasite transmission dynamics because it does not directly influence the proportion of infected and uninfected hosts and/or vectors. In this study, I focus on the latter of these two issues.

The relationship between resource availability and parasite transmission should be mitigated by mosquito behaviour (Ma & Roitberg, 2008). Thus, changes in resource availability can lead to changes in biting rate and survival (Beier, 1996; Bowen & Romo, 1995; Impoinvil *et al.*, 2004; Okech *et al.*, 2003; Straif & Beier, 1996). A change in the availability of resources should influence mosquito biting rates and survivorship, but the shapes of these relationships are poorly understood. As an example, as the number of blood hosts available decreases, one should not expect a linear decrease in the biting rates of mosquitoes because they will likely make up for a decrease in the number of blood hosts by becoming biting the remaining hosts more often (Lines *et al.*, 1987). Although there are many experiments that describe the general relationship (i.e., whether there is a positive or negative correlation) between sugar and blood feeding (e.g., (Straif & Beier, 1996), the functional relationship between resource availability and mosquito foraging behaviour is poorly understood.

Recently, Ma and Roitberg (2008) developed a theory on the effect of resource availability on mosquito behaviour, survivorship, and reproduction. The simple premise behind this model was that female mosquitoes can utilize two disparate resources: blood and sugar. Despite growing evidence that sugar plays an integral role in a mosquito's life (Foster, 1995), the role of alternate food resources (i.e., non-blood hosts) on foraging decisions is seldom considered in the ability of mosquitoes to transmit parasites (but see Roitberg and Mangel 2010). Ma and Roitberg (2008) considered the role of an omnivorous lifestyle and examined the feeding strategy of mosquitoes when faced with varying availability of blood and sugar resources. Resource availability is defined as the probability of finding a particular resource when it is sought. Through a dynamic state variable model, which considers the role of physiological condition on foraging decisions, their model presents relationships, sometimes nonlinear, describing the effect of sugar and blood availability on feeding behaviour. Not surprisingly, resource availability plays an important role in mosquito behaviour, survivorship, and reproduction, but more importantly, this study explored these aspects in unison. Therefore, this approach provides a useful framework to make comparisons on the relative impact of mosquito foraging behaviour and survivorship on parasite transmission.

In this study, I expand on the theory derived by Ma and Roitberg (2008) to determine how changing mosquito foraging behaviour with changes in resource availability affects parasite transmission. I argue that even within the simple context of existing models and parasite transmission measurements we can gain valuable insights into the effect of environment on parasite transmission. In particular, this approach allows us to understand the importance of both blood and sugar resources on parasite transmission. The approach I take is to formalize the behaviour of mosquitoes using simulation data from Ma and Roitberg (2008) and then incorporate the understanding of the functions into traditional measures of parasite transmission whereby I relax several of the restrictive assumptions associated with those measures. Specifically, I make the daily biting rate and daily survivorship probability functions of blood and sugar availability.

5.3 Methods and results

I begin with a brief review of the metrics and models used to describe parasite transmission dynamics, and the assumptions associated with them. Throughout the paper, the measure of parasite infection is described by the prevalence of infection (or parasitism rate), described as the proportion of humans and mosquitoes harbouring the parasites. I then explored the role of mosquito foraging decisions, including foraging for sugar, on parasite transmission dynamics. Specifically, I examined the role of resource availability on the life history of a female mosquito with respect to survivorship and biting rate.

5.3.1 Understanding classic measures of parasite transmission

Models of parasite transmission dynamics can be measured in two general classes, static and dynamic measures (Anderson & May, 1991; Smith & McKenzie, 2004). Static measures of parasite transmission dynamics include the vectorial capacity and the entomological inoculation rate. These measures hinge on the assumption that the proportion of hosts and vectors that are infected remains constant. The vectorial capacity (C) describes the transmission potential of a mosquito *population* when *Plasmodium* infection is virtually non-existent. The entomological inoculation rate (*EIR*) is the number of infectious bites received per day by a human. Both of these metrics are contingent on several simplifying assumptions which are outlined below. Dynamic models, such as the Ross-Macdonald model, examine the population dynamics of vectors and hosts, which give us an understanding of the temporal dynamics of the host-vector system as well as the stability of that system. Results usually focus around the basic reproductive ratio (R_0), which describes the number of secondary infections that arise from a single infection in a population of susceptible hosts. Static and dynamic models of parasite transmission share several simplifying assumptions. From the perspective of mosquitoes the assumptions are that they bite at random, have a constant daily probability of survival, and that the population is homogeneous and the population size is constant. From the perspective of humans, the assumptions are that there is no lifelong or acquired immunity, there is no difference in death rates between the infected and uninfected humans, and the population size is constant.

5.3.1.1 Static measures parasite transmission

All of the measures below are based on a world in equilibrium (i.e., where only proportions change, but the number of individuals does not). These are referred to in the paper as “statics”. I begin by assuming that there is a constant per capita death rate (g) such that survivorship to age A is described by the exponential decay function:

$$\lambda(A) = e^{-gA}. \qquad \text{(eq. 1)}$$

This is sometimes expressed in terms of a daily survival probability (p)

$$p = e^{-g}. \qquad \text{(eq. 2)}$$

The proportion of mosquitoes that bite a human by age A ($\eta(A)$) given an expected number of bites on humans per mosquito, per day of a can be described by the equation:

$$\eta(A) = 1 - e^{-aA} \qquad \text{(eq. 3)}$$

This is based on the Poisson distribution, where e^{-aA} would represent the probability of not biting a single human by age A. The proportion of mosquitoes that become infected at age A ($v(A)$) given a proportion of infected humans X and the probability that an uninfected mosquito becomes infected when biting on infectious humans (c), can be expressed as

$$v(A) = 1 - e^{-acXA}, \qquad \text{(eq. 4)}$$

Once a mosquito becomes infected, a proportion then become infectious $\mu(A)$ after surviving the incubation period, in days, of the parasite (n). This is expressed as

$$\mu(A) = \begin{cases} 0 & \text{if } A <= n \\ 1 - e^{-acX(A-n)} & \text{if } A > n \end{cases} \qquad \text{(eq. 5)}$$

Finally, by combining the proportion of infectious mosquitoes (eq. 5) and the survivorship to age A (eq. 1), the proportion of infectious mosquitoes in a cohort (Z) can be calculated as

$$Z = \int_0^{\text{inf}} \mu(A)\lambda(A)\,dA \Bigg/ \int_0^{\text{inf}} \lambda(A)\,dA = \frac{acX}{g + acX} e^{-gn} \qquad \text{(eq. 6)}$$

Entomological inoculation rate (EIR)

The entomological inoculation rate (*EIR*) is the number of infectious bites received per day by a human, and is subsequently a function of the proportion of infectious mosquitoes in a cohort. *EIR* is expressed as

$$EIR = maZ = \frac{ma^2cXe^{-gn}}{g + acX} . \qquad \text{(eq. 7)}$$

Where m represents the ratio of mosquitoes to humans in the environment.

Vectorial capacity (*C*)

The vectorial capacity (C) describes the transmission potential of a mosquito *population* when *Plasmodium* infection is virtually non-existent. In other words, C describes the incidence of malaria inoculations per case per day of a mosquito population (Garrett-Jones, 1964; Macdonald, 1957). It follows the equation:

$$C = \frac{ma^2e^{-gn}}{g} = \frac{ma^2p^n}{-\ln p} \qquad \text{(eq. 8)}$$

This implicitly assumes that the probability of an uninfected mosquito becoming infected after biting an infectious human (b) and the probability of an infectious bite by a mosquito leads to human infection (c) are equal to one (i.e., $b = c = 1$). Equation 8 suggests that when daily survivorship (p) is initially high, a decrease in daily survivorship drastically decreases the vectorial capacity (C) of a mosquito population. Furthermore, another term that has a large effect on vectorial capacity is the expected number of bites on humans per mosquito, per day (a). This is a quadratic term because the mosquito must first bite an infectious human and then bite again (~12 days later) in order to pass along the infection. Thus, changes in biting rate will have a greater than linear effect on C.

C is closely related to *EIR* because it represents the slope of the relationship between *EIR* and the prevalence of infection in humans when malaria is virtually absent. Substituting *C* into *EIR* while including the term *c*, which is how vectorial capacity is defined as sometimes, yields

$$EIR = cCX \frac{1}{1 + acX/g} \qquad \text{(eq. 9)}$$

$$cC = \frac{\partial EIR(X)}{\partial X}\Big|_{X=0} \qquad \text{(eq. 10)}$$

It is worth noting that *C* does not require an independent estimate of the proportion of infected humans (*X*) unlike *EIR* (Smith & McKenzie, 2004).

5.3.1.2 Host-Vector Dynamics

When considering the epidemiology of the dynamics of parasite transmission, I consider a simple system describing the proportions of mosquitoes and humans, which are infected. As a whole, these SIR (susceptible-infected-recovered) models generally ignore the time lag introduced by incubation of gametocytes to sporozoites. Other key assumptions are that the sizes of the mosquito and human populations are constant, mosquito populations are homogeneous (e.g., there is no difference in the biting rates of infected and uninfected mosquitoes), and that transmission of parasites between hosts and vectors occur via mass action (i.e., random mixing of the two host types). Generally, these assumptions are made to make the deterministic model more tractable (Ngwa, 2001).

The simple model describes the change in the proportion of infected humans (*X*) and proportion of infectious mosquitoes (*Z*) over time. Following the notation of Smith and McKenzie (2004),

$$\frac{dZ}{dt} = aX(1-Z) - gZ \tag{eq. 11a}$$

$$\frac{dX}{dt} = maZ(1-X) - rX \tag{eq. 11b}$$

Where *Z* is the proportion of mosquitoes that are infectious, *m* is the number of mosquitoes per human host, *a* is the biting rate of mosquitoes, *r* is the recovery rate of humans (or the replacement of infected humans with susceptible humans), and *g* is the mortality of infected mosquitoes.

MacDonald (1957) added to this model the probability that mosquitoes survive the infectious period (e^{-gn}), such that

$$\frac{dZ}{dt} = aX(e^{-gn} - Z) - gZ \tag{eq. 12a}$$

$$\frac{dX}{dt} = maZ(1-X) - rX \tag{eq. 12b}$$

Further inclusions into the model are the proportion of infectious bites that lead to human infection (*b*), and the proportion of bites on infectious humans that lead to infectious mosquitoes (*c*), yielding the equations:

$$\frac{dZ}{dt} = acX(e^{-gn} - Z) - gZ \tag{eq. 13a}$$

$$\frac{dX}{dt} = mabZ(1-X) - rX \tag{eq. 13b}$$

At equilibrium X^* and Z^* represent the equilibrium proportion of infected humans and mosquitoes respectively. There are two equilibria: a zero equilibrium where the infection does not persist (i.e., $X^* = Z^* = 0$), and an endemic equilibrium where X^* and Z^* have non-zero values. The endemic equilibrium is:

$$X^* = \frac{ma^2bc - e^{gn}gr}{a^2bcm + ace^{gn}r} \tag{eq. 14a}$$

$$Z^* = \frac{ma^2bce^{-gn} - gr}{a^2bcm + abgm} \tag{eq. 14b}$$

Basic reproductive ratio (R_0)

The basic reproductive rate (R_0) is described as the number of secondary infections that arise from a single infection in a population of susceptible hosts. It can be shown that for the Ross-Macdonald model, the endemic equilibrium is stable when infection growth rate outweighs infection loss rate; i.e., when

$$ma^2bce^{-gn} - gr > 0 \tag{eq. 15}$$

This is equivalent to a positive determinant of the Jacobian matrix for the equations describing host and vector population dynamics. This is one of the Routh-Hurwitz conditions for a two-dimensional system, (and the other is a negative trace, which is always true for this system assuming that all parameters are positive). Rearranging the inequality, the basic reproductive ratio (R_0), is

$$R_0 = \frac{m\,a^2 bce^{-gn}}{rg} , \qquad \text{(eq. 16)}$$

where $R_0 > 1$ for malaria to persist. This is related to the vectorial capacity (C) such that $R_0 = bcC/r$. Thus, the basic reproductive ratio can be thought of as a proportional metric to the vectorial capacity (C) that incorporates the probability of a mosquito becoming infected (b), the probability of infecting a human (c). If, as is true for vectorial capacity, a bite is assumed to always lead to infection (i.e., c=b=1), then the basic reproductive ratio is only inversely proportional to the intrinsic growth rate of a mosquito population. Therefore, for a fixed vectorial capacity, if the intrinsic growth rate of a mosquito population increases, the basic reproductive ratio will decrease.

5.3.2 Resource availability

I explored the role of resource availability on parasite transmission dynamics, with the understanding that the effect of resource availability on parasite transmission dynamics is mitigated by mosquito behaviour. The findings are based on simulation results from Ma and Roitberg (2008). Briefly, the premise of the study was to determine the behavioural decisions that a mosquito makes to maximize her reproductive success as sugar and blood hosts vary. Individual mosquitoes searched for a blood meal, searched for a sugar meal, oviposited, or rested. The environment was separated into two distinct areas: near human blood hosts, and near oviposition sites. As an outcome of the model, sugar host availability near oviposition sites (λ_{sug}) and blood host availability (λ_{blo}) affected survivorship and biting rate, which are examined in greater detail below.

Survivorship

Based on the predictions from the model derived by Ma and Roitberg (2008), survivorship is strongly impacted by the availability of sugar near oviposition sites, and decreases only slightly with a decrease in blood availability. Using the raw data in Ma and Roitberg (2008), I calculated the total lifespan of a mosquito as a function of blood and sugar availability. For each unique combination of λ_{sug} and λ_{blo}, I calculated the average lifespan of each mosquito (Figure 5.1). As sugar availability decreased, survivorship also decreased. The measures of parasite transmission presented above assume a constant per-capita daily death rate. Adhering to this assumption, the daily survival probability (p) becomes a function of mosquito age in days (A) described by an exponential decay function (eq. 1). This was calculated using a linear regression on log-transformed life table data from Ma and Roitberg (2008) (Figure 5.2). Daily survival probability decreased exponentially as sugar availability decreases such that only at low sugar availability was there a marked difference in survival. Sugar plays an important role in fuelling activity and somatic maintenance and blood plays a lesser role. However, when blood becomes very difficult to acquire, an individual will spend a large portion of their time and energy searching for a blood meal and, therefore, risk death due to starvation or host defensive behaviour. Interestingly, when blood was not available (i.e., λ_{blo}=0.0), there was a slight rise in survivorship, which was likely due to a lack of mortality due to host defensive behaviour and mitigated by the availability of sugar near blood hosts.

Biting rate

The frequency of blood feeding over the lifetime of a mosquito was calculated from the raw data from the Ma and Roitberg (2008) study. This was done by taking the average number of successful bites per mosquito over her lifespan (Figure 5.3). However, most metrics of parasite transmission use daily rates; thus, I converted survivorship into a daily biting rate for a mosquito (a), corrected for the difference in lifespan and for the 15 minute time steps used in the model over a 8 hour activity period of mosquitoes, such that

$$a = \frac{number\ of\ bites\ over\ lifetime}{day} \times \frac{day}{8\,h\ active} \times \frac{h}{4\,timesteps} \qquad \text{(eq. 17)}$$

This is shown as a function of blood and sugar availability in Figure 5.4. I assumed that mosquitoes only bite humans; i.e., the Human Blood Index, HBI = 1. As sugar availability decreased, the daily biting rate decreased monotonically from ~2.0 to ~0.5 bites per day. The decreased biting rate as sugar availability decreased was because the mosquitoes tended to spend more time outdoors searching for sugar when it was scarce. However, once a mosquito acquires a sugar meal and replenishes its energy reserves, any additional search for sugar has little benefit. On the other hand, blood is a necessary resource for egg production and therefore as blood availability increased, there is initially a steep acceleration towards an asymptote in the daily biting rate. This response was non-linear because mosquito feeding behaviour dictated that in the presence of a blood host, unless severely energy deficient, a mosquito should always search for a blood meal (Ma & Roitberg, 2008). Thus, mosquitoes were more persistent as blood hosts became more difficult to access, but when blood is very difficult to acquire, the daily biting rate decreased significantly because of a lack of hosts.

5.3.3 Mosquito behaviour and parasite transmission

I described the effect of blood host availability (λ_{blo}) and sugar host availability (λ_{sug}) on the biting rate (a) and daily survivorship probability (p). By doing so, I relaxed the assumption that the biting rate (a) and daily survivorship probability (p) are constant and make them functions of resource availability (i.e., $a(\lambda_{blo}, \lambda_{sug})$, and $p(\lambda_{blo}, \lambda_{sug})$) . I then explored the impact of resource availability on parasite transmission metrics.

To gain a better insight into the effects of the change in survivorship and biting rate on parasite transmission, I considered their effect on the vectorial capacity (C) separately (Figure 5.5a – only the effect change in survivorship on C, and Figure 5.5b – only the effect of changes in daily biting rate on C). C follows the equation 8, and therefore a non-linear change in C as survival and daily biting rate change. As both blood and sugar availability increased, when only changes in daily survival probability are considered, λ_{sug} had a larger effect on C than λ_{blo}, and that λ_{blo} has only marginal impact on C when it is low (0.0 – 0.3) (Figure 5.5a). In Figure 5.5b, as λ_{blo} and λ_{sug} decrease, C decreases. Note here that at $\lambda_{blo} = 0$, C does not reach zero because of the way that the daily survival probability is estimated. We assume a constant daily survival probability, and therefore fit the simulation data to an exponential decay function, leading to survival probabilities that are very low but never reach zero. This inaccuracy is magnified in the vectorial capacity because the effect of changes in p is raised to the power of n, the incubation period of the parasite in the mosquito. However, the shape of the relationship is different between the two environmental factors. An increase in λ_{sug} led to an asymptotic change in C while an increase in λ_{blo} led to an initial lag then linear change in C. Thus, a small reduction in blood availability led to immediate changes in C

through all values λ_{blo}. On the other hand, a small reduction in sugar availability has only a small effect when λ_{sug} is initially high but a large effect when λ_{sug} is low.

I then considered the effects of resource availability on these two factors together on the vectorial capacity (Figure 5.6a) and the basic reproductive ratio (Figure 5.6b). These results show the non-linear functions that describe the effect of resource availability on several (related) parasite transmission metrics. Both the vectorial capacity and the basic reproductive ratio change in a similar way because of the similarity in their equations (eq. 9 and eq. 17 respectively). The theory predicts that decreasing λ_{blo} only marginally decreased C and R_0 until λ_{blo} becomes < 0.5. On the other hand, decreasing λ_{sug} immediately reduced C and R_0. For instance, when $\lambda_{sug} = 1.0$, a ~40% reduction in C occurred at $\lambda_{blo} = 0.3$; when $\lambda_{blo}= 1.0$, a ~40% reduction in C occurred at $\lambda_{sug} = 0.8$.

5.4 Discussion

I used classic measures of parasite transmission such as the vectorial capacity (C) and the basic reproductive ratio (R_0), and relaxed the assumption that daily survival probability (p) and daily biting rate (a) are constants. Instead, these parameters are treated as functions of sugar and blood availability. These functions were characterized based on the results from a previous study (Chapter 2; Ma and Roitberg, 2008) that considered how mosquito behaviour would change under different environmental conditions. I demonstrated that resource availability, mitigated through changes in mosquito behaviour, could have a significant affect the ability of mosquitoes to transmit parasites between humans. Importantly, based on the simulation results from Ma and Roitberg (2008), predictions are generated on the shape of the functions describing the effect of both sugar and blood availability on vectorial capacity.

Blood availability is positively correlated with the ability of a mosquito population to transmit parasites. The results suggest that limiting the availability of blood hosts (for instance, by covering a proportion of individuals, usually children under the age of five, using bednets) will only lead to marginal reductions in parasite transmission unless much of the population is covered (i.e., biting rate does not significantly decrease until blood availability is low). This result is linked to the reduction in the daily biting rate of mosquitoes as blood meals become more difficult to find. These predictions will only apply to untreated bednets because I do not consider additional mortality or behavioural changes from unsuccessful searches for blood meals. Of course, the use of (treated or untreated) bednets will lead to reductions on biting rates at an individual level (Mwangi et al., 2003). Insecticide treated bednets (ITNs) can lead to additional changes in mosquito behaviour and survivorship (Roitberg & Mangel, 2010; Takken, 2002). Furthermore, they require re-impregnation with insecticides to retain their efficacy, and in practice this often does not occur (Clarke et al., 2001). Thus, the evaluation of the efficacy of untreated bednets is of practical importance. Some studies indicate that the vectorial capacity of mosquito vectors may not be lowered with untreated bednets (Burkot et al., 1990) nor ITNs (Somboon et al., 1995). My study suggests that this may be partly to do with the biting behaviour of mosquitoes and the low to intermediate proportion of humans using bednets.

Sugar availability is also positively correlated with the ability of a mosquito population to transmit parasites. The results suggest that limiting the availability of sugar sources should have an immediate effect on vectorial capacity. This is largely driven by the strong link between survivorship and sugar availability because of the reliance of mosquitoes on sugar sources as a means to fuel flight activity and avoid starvation (Bowen & Romo, 1995; Foster, 1995; Okech et al., 2003), and enhancing reproduction over the lifetime of the mosquito (Ma and Roitberg, 2008). Thus, I have shown that the role of sugar sources is likely more important than traditionally thought, and I suggest that environmental management of sugar sources will provide tangible benefits to reduce parasite transmission. This result is supported by a real-world example. Utzinger et al. (2001) performed a detailed analysis on the efficacy of a control program undertaken in the Roan Antelope copper mine of Zambia located in the Ndola Rural district, near the town of Luanshya – an area identified as highly endemic for malaria, with *Anopheles gambiae* as the most abundant mosquito. The control program included environmental management, including vegetation clearance along the river, modification of river boundaries and draining of flooded areas and swamps. Weekly adult mosquito catches and monthly maria incidence rates were monitored, and the authors found that the programme was highly successful. Monthly malaria rates, estimated from records from the mining company on Europeans and Africans, dropped substantially (70-95%) within 3 – 5 years after the programme start (Utzinger et al., 2001). I speculate here that this was at least in some part due to the diminished availability of vegetation – the source of sugar meals.

Models have played a central role in the understanding of vector-borne disease transmission and dynamics. They have been useful tools that allow us to identify critical gaps in understanding and uncertainties in data and comparing alternate control strategies (McKenzie & Samba, 2004). The emphasis of these models is on qualitative (and not quantitative) results. In most cases, simple models have provided the best insights, where models have simple and explicit assumptions (Koella, 1991). More complex models may be able to generate quantitative predictions, but tend to make a larger number of assumptions about interactions, which may lead a higher probability of making a mistake on a critical assumption (Koella, 1991). I am not arguing that the common metrics for evaluating the efficacy of control programs such as vectorial capacity should be discarded – these metrics remain important tools in the fight against malaria. As with most model results, the aim is to make qualitative predictions, and as such the results should not be misinterpreted as quantitative predictions (McKenzie & Samba, 2004). However, researchers must be cautious when performing comparisons between mosquito populations where environmental conditions are different between habitats (or have changed over time) without first considering the impact of the environment and mosquito behaviour.

5.5 Figures

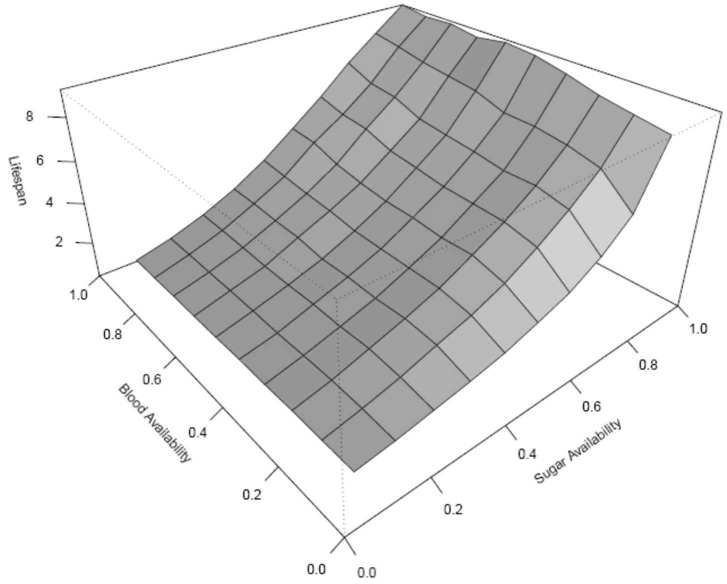

Figure 5.1: The total lifespan, in days, of a mosquito as a function of the probability of finding a sugar host (λ_{sug}) or blood host (λ_{blo}). The results were generated from raw data from Ma and Roitberg (2008).

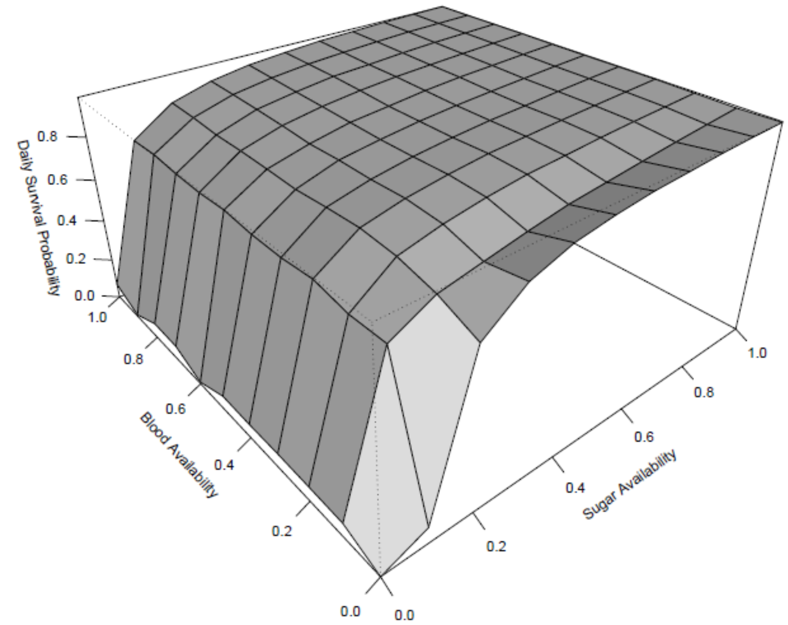

Figure 5.2: Daily survival probability (p) of mosquitoes as a function of the probability of finding a sugar host (λ_{sug}) or blood host (λ_{blo}). This was calculated using a linear regression on the log-transformed life table data from Ma and Roitberg (2008).

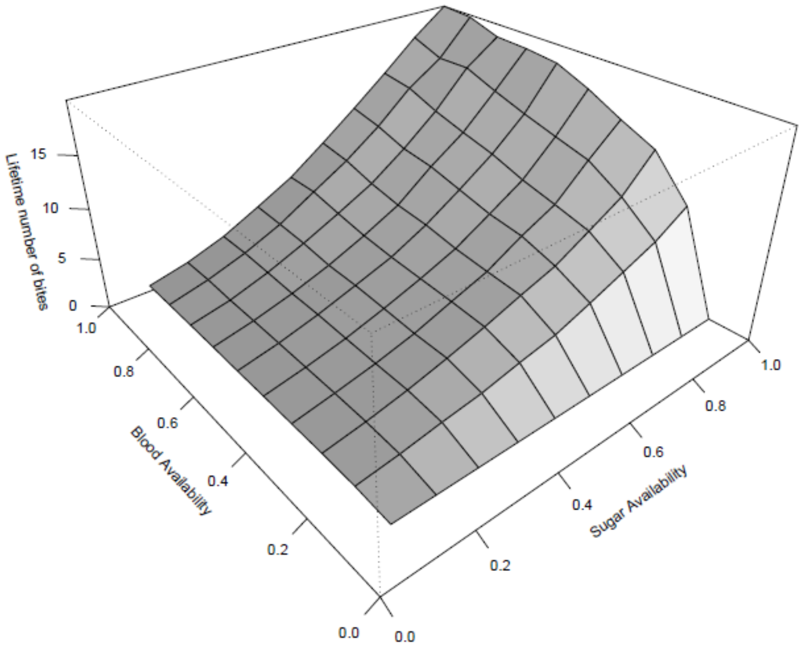

Figure 5.3: Number of bite on humans over a mosquito's lifetime as a function of the probability of finding a sugar host (λ_{sug}) or blood host (λ_{blo}).

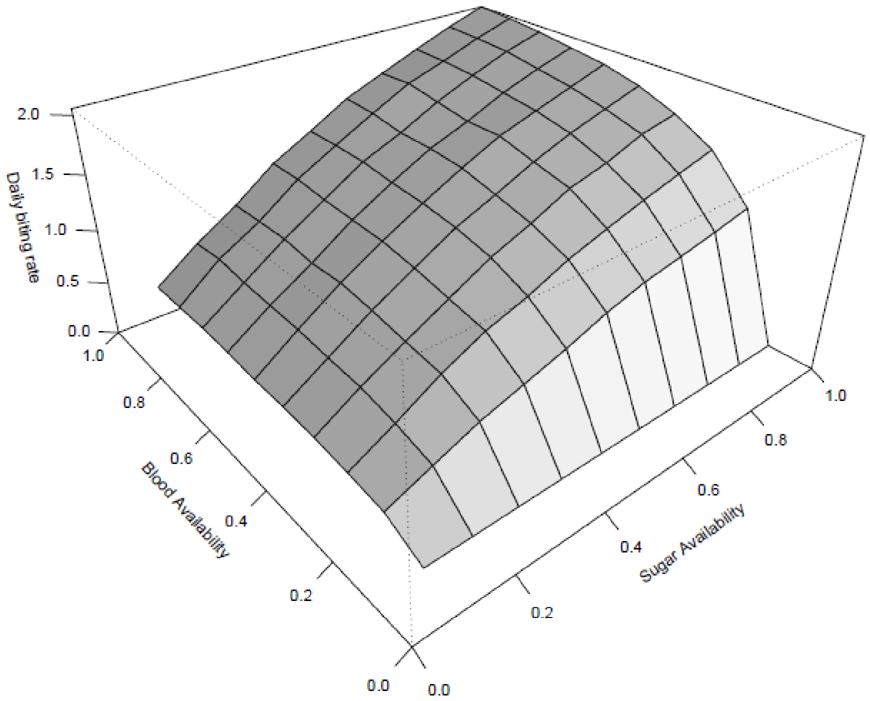

Figure 5.4: The daily biting rate (a) of mosquitoes as a function of the probability of finding a sugar host (λ_{sug}) or blood host (λ_{blo}).

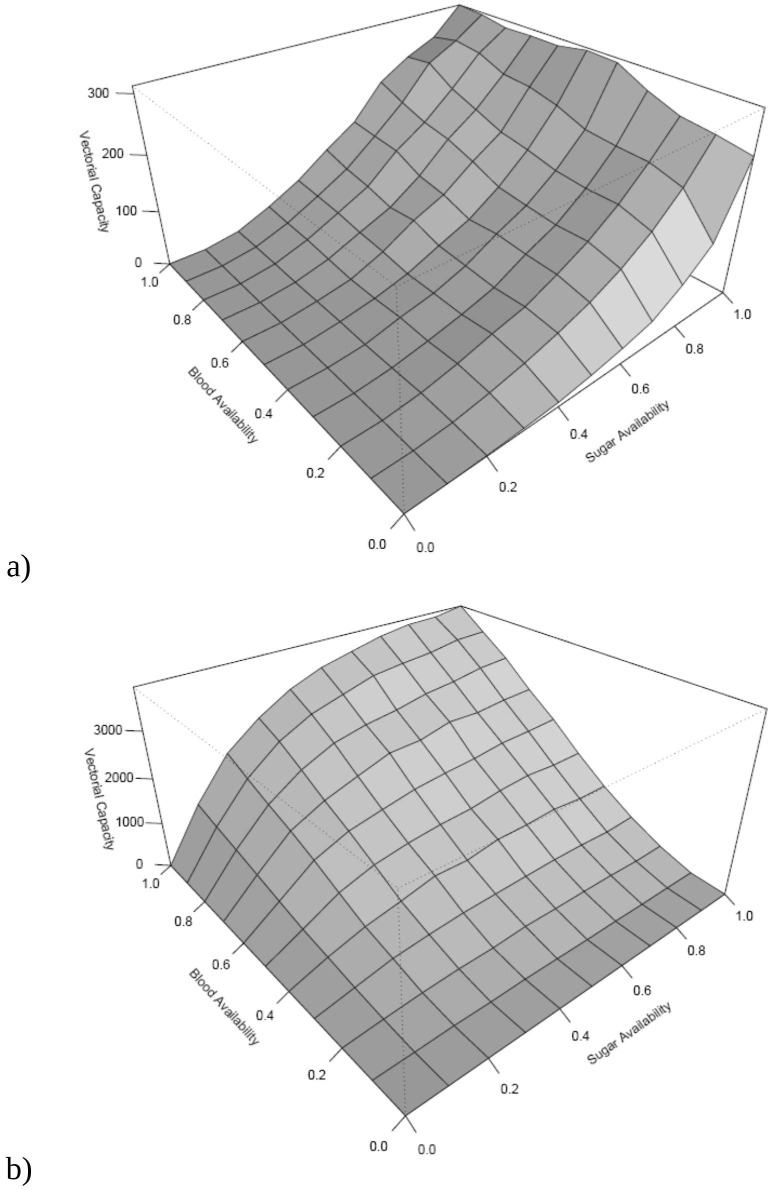

Figure 5.5: Vectorial capacity (C) where the effect of changes in the probability of finding a sugar host (λ_{sug}) and blood host (λ_{blo}) on a single parameter are considered. Panel a shows the change in C with changes in daily survival probability (p); panel b shows the change in C with changes in the daily biting rate (a). C is described by eq. 9. The fixed parameter values are m = 10, a = 1, n = 10.

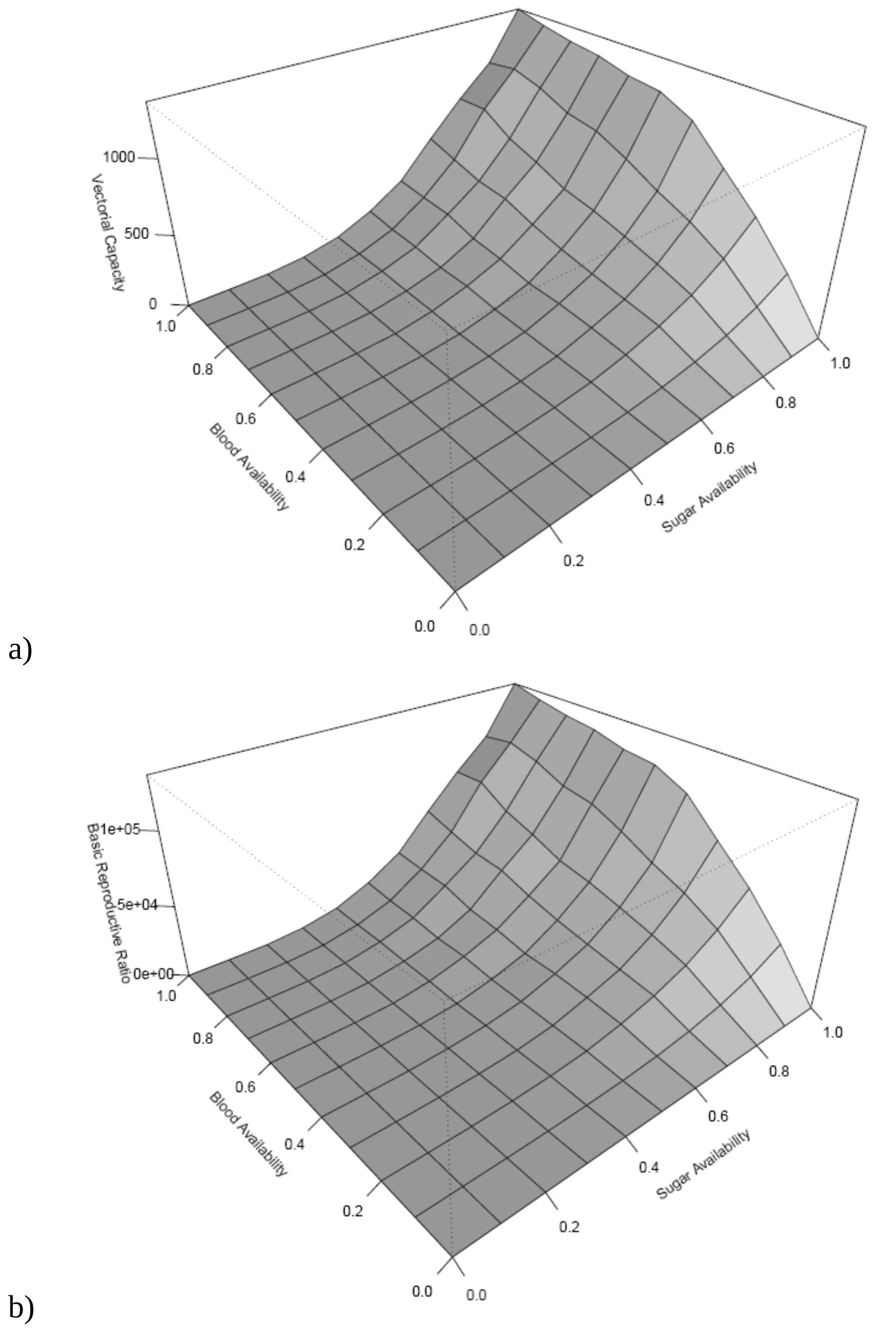

Figure 5.6: The vectorial capacity (C) (panel a) and basic reproductive ratio (panel b) as a function of the probability of finding a sugar host (λ_{sug}) and blood host (λ_{blo}). C is described by eq. 9. R_0 is described by eq. 17. The fixed parameter values are m = 10, b = 1, c = 1, n = 10, r = 0.01.

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6: Conclusion: Blood, sweat and sugar

6.1 Blood, sweat and sugar

Malaria continues to be a problem throughout the world, with many organizations making concerted efforts to reduce the malaria burden. Generally, an integrated approach malaria reduction is implemented that includes antimalarial drugs, vector control, and personal protection (bednets) (McKenzie et al. 2007). In 1998, the World Health Organization (WHO) began the Roll Back Malaria (RBM) campaign with the goal of halving deaths caused by malaria by 2010. Progress towards this goal has been limited, with the WHO acknowledging that the RBM program is operating under a background of increasing malaria burden (WHO 2003). In light of the increasing malaria burden, we must re-evaluate the next steps to reduce malaria worldwide.

One approach to controlling malaria is the use of antimalarial drugs. These drugs fall under two broad categories: (1) asexual stage drugs which target the stages of *P. falciparum* that cause clinical malaria and death in humans, and (2) transmission-blocking drugs, that target the gametocyte stage of *P. falciparum*, and subsequently block the further transmission of the parasite to other vectors. Asexual stage drugs such as chloroquine and sulfadoxine-pyrimetamine have been the most common form of drug, but there is increasing resistance to these drugs (Trape 2001; Sa et al 2009), and this is thought to be a key reason of increasing deaths because of malaria (Attaran et al. 2004).

Recently, there has been a shift towards transmission-blocking drugs (Butler 2009), and the latest drug therapy – artemisinin-based combination therapy (ACT) has been found to be effective in treating the symptoms of malaria and also as a means to reduce gametocytemia (i.e., block transmission) (Bousema et al. 2005). However, even with these latest artemisinin-based drugs, there is growing evidence that *P. falciparum* is becoming resistant (Noedl et al. 2008; Dondorp et al. 2009; Sanderson 2009). This is in part because drug makers are using artemisinin alone (monotherapies), which fails to comply to the demands of the World Health Organization (WHO) to only provide these drug therapies in combination with other drugs (Butler 2009).

There is substantial theory on the efficacy of vaccines on disease transmission, and much of this theory is directly applicable to anti-malarial drug application. Drug use can be thought of as similar to vaccine use if drugs are readministered before the duration of their effect is over. Several interesting conclusions arise from this theory. For instance, the theory states that for either asexual stage or transmission blocking vaccines to significantly lower the basic reproductive ratio, the duration of effect of the drugs must be relatively long (50-100% of a human lifespan) (Koella 1991). Furthermore, asexual stage vaccines are predicted to have a larger impact on disease prevalence than transmission-blocking vaccines. An individual treated with an asexual stage vaccines cannot become infected for the duration of efficacy of the vaccine. On the other hand, individuals with transmission-blocking vaccines can still succumb to the disease and the theory predicts that disease prevalence will only go down if the vast majority of individuals are protected (Koella 1991).

With the increasing threat of resistance to antimalarial and transmission-blocking drugs and theory that states that antimalarial drugs must be administered to a large proportion of a population and be efficacious for long durations, we should turn towards the other methods at our disposal to combat malaria: vector control and personal protection (bednets) (McKenzie et al. 2007). These approaches operate as preventative measures against malaria; i.e., they limit contact between infectious mosquito vectors and human hosts. The work presented in this thesis focuses on vector control and the ability to limit contact between infectious mosquito vectors and human hosts via manipulating mosquito behaviour, survivorship, and access to resources. The theory presented here is general to most mosquito-host systems but where possible, I provide direct reference to the specific pairing of *Anopheles gambiae* and *Plasmodium falciparum*.

The first two chapters (Chapter 2 and 3) build upon the basic premise that an adult female mosquito is an omnivore that feeds on both blood and sugar and therefore must have some sort of diet choice rule that dictates when to feed on each. I demonstrate the mosquito feeding decisions are energy-dependent. When a female mosquito is relatively starved, it is more likely to search for a sugar meal rather than a blood meal because sugar meals are better for fueling somatic maintenance and less risky to acquire. The theory also predicts that mosquitoes will largely ignore sugar meals when in the surroundings close to blood hosts, but will choose sugar when blood hosts are not nearby. The theory also predicts that there are non-linear relationships between blood and sugar availability and mosquito biting rate and survivorship. Not surprisingly, increasing the probability of finding a sugar meal increases a mosquito's lifespan, but the ability to find blood hosts has little effect on lifespan. On the other hand, the daily biting rate is only reduced when blood availability is reduced substantially (>50%).

I also explore the coalescence of larval and adult traits involved in ovipositional site exploitation (Chapter 4). The major finding of this study is that when adult females prefer pots there is a strong correlation for robust larval development strategies, but when ponds are preferred, larval development is good under optimal conditions but poor in other conditions. There was no selection for aggressive larvae, suggesting that mothers mitigate larval interactions through strong preference leading to greater synchrony in egg-laying.

The final chapter expands on the role of individual behaviour to a population level. By using classic measures of a population of mosquitoes ability to transmit parasites, I ensure that any changes in the predictions of these measures are because of changes in the behaviour of the mosquito as predicted by the theoretical model presented in the thesis and not because of the metrics themselves. The theory suggests that reducing the availability of sugar hosts in the environment may have a larger effect on the ability of mosquitoes to transmit parasites than would a reduction in the availability of blood hosts, in part because of the mitigating effects of mosquito behaviour.

The findings in this thesis suggest that vector control should continue to be a primary focus of integrated approaches to malaria control (van den Berg and Takken 2008; McKenzie et al 2007). This thesis highlights the importance of mosquito behaviour in mitigating the effects of changes in the environment from blood feeding frequency and survivorship (Chapters 2 & 5), as well as oviposition site selection (Chapter 4). All of these factors have the capacity to play a large role in the ability of mosquitoes to transmit parasites.

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