NUTRITIONAL ECOLOGY OF THE MALARIA VECTOR *ANOPHELES GAMBIAE*

by

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ABSTRACT

Vector survival rate is a key factor when estimating the potential of a vector population to transmit a parasite and as such, a small change in vector survival can have a significant effect on vectorial capacity. Using laboratory experiments, I investigated the possibility that *Anopheles gambiae*, a vector of *Plasmodium*, can alter the proportional allocation of resources between somatic and reproductive functions in response to limited nutritional intake. I also conducted a field survey to document how the nutritional status of *An. gambiae* varies within a population. Lastly, I used laboratory experiments to examine the effect of *Plasmodium* on vector fitness. The results of these investigations show that *An. gambiae* does have some control over its longevity, can mediate the effects of limited nutrition, that nutritional status of mosquitoes varies within a population and can alter the effect of *Plasmodium* on its vector.

Keywords: *Anopheles gambiae*; nutritional ecology; life history tradeoffs; *Plasmodium*; vectorial capacity

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GLOSSARY

Gonotrophic cycle — The time between blood feeding and oviposition in female mosquitoes.

Minimum irreducible amounts — The portion of somatic energy reserves that a mosquito cannot mobilize to maintain somatic functions such as structural components and reproductive tissues. This value is obtained by measuring the reserves remaining in a mosquito that has been starved to death after eclosion.

Nulliparous — A description of the reproductive status of female mosquitoes used to estimate physiological age in *Anopheles gambiae*. Nulliparous females have not produced or oviposited any eggs and are on average, younger than parous females.

Parous — A description of the reproductive status of female mosquitoes used to estimate physiological age in *Anopheles gambiae*. Parous females have produced eggs and oviposited at least once and are on average, older than nulliparous females.

Somatic energy reserves — The lipid, protein, sugar, and glycogen stores in the somatic tissue of a mosquito.

Teneral — A short maturation period following emergence from the pupal casing, usually lasting less than 12 hours. Physiological and behavioural changes such as hardening of the cuticle, degeneration of larval tissues, increase in size of flight muscles, sexual maturation of males, and development of female preference for human odours.

Vectorial capacity — The potential of a mosquito population to transmit *Plasmodium*. It can be described mathematically by the equation: $C = \frac{ma^2 p^n}{-\log p}$, where *m* is the number of female mosquitoes per human, *a* is the number of bites per female mosquito per night, *n* is the incubation period of *Plasmodium* in the mosquito, and *p* is the daily survival rate of mosquitoes.

CHAPTER 1: INTRODUCTION

1.1 Mosquitoes as vectors and vectorial capacity

Many insects are vectors of blood-borne diseases such as yellow fever, dengue fever, sleeping sickness, and malaria. Of these, malaria arguably has the greatest impact on human health, resulting in the death of approximately 1 million people each year (World Health Organization 2005). Malaria is caused by parasites in the genus *Plasmodium* and is transmitted to humans through the bite of female mosquitoes in the genus *Anopheles*. Consequently, an understanding of mosquito biology is a key component of developing effective malaria control strategies.

The concept of vectorial capacity is often used to describe the role of an insect population in the transmission of vector-borne diseases. In the context of malaria, vectorial capacity is a measure of the potential of a mosquito population to transmit human malaria parasites (Dye 1986). It can be described mathematically by the equation: $C = \frac{ma^2 p^n}{-\log p}$,

where *m* is the number of female mosquitoes per human, *a* is the number of bites per female mosquito per night, *n* is the incubation period of the parasite in the mosquito, and *p* is the daily survival rate of mosquitoes (Snow and Gilles 2002). Factors that impact mosquito fecundity, feeding behaviours, and especially longevity have the potential to affect vectorial capacity. Because mosquito

survival rate is an exponential function in the numerator of this equation, a small change in survivorship has a large impact on vectorial capacity.

Constant values are typically assumed for terms in the vectorial capacity equation, although there is a growing body of evidence to suggest that this is not a valid assumption (Gu *et al*. 2006). Mosquito survivorship can be altered by changes in environmental conditions such as temperature and humidity, food availability (Gary and Foster 2004, Lyimo *et al*. 1992), and by changes in the physiological condition of the mosquito (Briegel 2003). There is also some evidence to suggest that individuals may have indirect control over their own longevity via altering the allocation of resources between somatic and reproductive functions (Ahmed *et al*. 2002, Blanckenhorn *et al*. 2007, Roitberg 1989). Due to the potential for small changes in survivorship to have a large impact on vectorial capacity, it is important to increase our understanding of what factors impact survivorship and how survivorship changes within a population. There is increasing evidence to suggest that nutrition in particular can significantly affect mosquito longevity, in addition to affecting fecundity and feeding behaviours (Beier 1996, Fernandes and Briegel 2005, Gary and Foster 2001, 2004, Straif and Beier 1996). This thesis describes a series of studies investigating various aspects of nutritional ecology in the malaria vector *Anopheles gambiae sensu stricto*. In particular, I examine the role of nutrition in determining mosquito longevity and fecundity and investigate the interaction between nutrition and the effects of a malaria parasite on a mosquito vector.

1.2 Study organism

Anopheles gambiae sensu stricto was chosen as the study organism for this work because of its status as a medically important vector. *Anopheles gambiae sensu stricto* is a member of the *Anopheles gambiae* species complex (also referred to as *Anopheles gambiae sensu lato*), a group of 7 species that accounts for the vast majority of malaria transmission in sub-Saharan Africa (Levine *et al*. 2004). Within this species complex, *An. gambiae s.s*. and *Anopheles arabiensis* are the most efficient vectors of *Plasmodium falciparum*, the most virulent of the human malaria parasites (Levine *et al*. 2004). The high efficiency of *P. falciparum* transmission by *An. gambiae s.s*. is due to its capability to support development of the parasite, its tendency to rest and feed indoors (endophilly and endophagy), and its strong preference for feeding on humans (anthropophilly) (Alavi *et al*. 2003).

Anopheles gambiae sensu stricto is a holometabolous insect and passes through 4 different life stages, embryo (egg), larval, pupal, and adult. Female mosquitoes lay their eggs in aquatic habitats that range from hoof prints, small shallow puddles and man-made containers to sheltered lake and stream edges and marshes (Munga *et al.* 2007). Larvae hatch from their eggs approximately 3 days after oviposition and feed on plant and animal matter suspended near the surface of the water (Briegel 2003). Conditions in the larval habitat, including temperature, humidity, larval density, presence of conspecifics and predators, and particularly food availability contribute to time to pupation and final body size of larvae and adult body size and condition (Briegel 2003, Lyimo *et al*. 1992).

Larval nutrition also affects the size and health of adults. Larvae reared on restricted diets emerge as smaller adults with fewer energy reserves (Briegel 2003). As adults, males feed exclusively on plant sugars while females feed on plant sugars and blood (Foster 1995). *Anopheles gambiae s. s.* is an anautogenous species, meaning females cannot reproduce without first obtaining a blood meal (Clements 1992).

Anopheles gambiae can store nutrients acquired through feeding in the form of lipid, protein, sugar and glycogen. Lipid is stored in the fat body and in addition to protein, is essential for oogenesis in females (Foster 1995). In addition, males and females utilize lipid as an energy source during flight (Kaufmann and Briegel 2004, Kaufmann and Brown 2006). Glycogen and sugar are also stored in the fat body and used to power flight (Foster 1995). High levels of all 4 types of reserves are correlated with increased longevity and fecundity in females (Briegel 2003). The experiments described in this thesis focus on the adult stage of the mosquito lifecycle.

1.3 Overview of thesis

The goals of this thesis were three-fold. Firstly, I tested the ability of *An. gambiae* females to reallocate nutritional resources between somatic and reproductive functions. This was done in the context of life history theory, which suggests that organisms experience tradeoffs between life history components due to physiological constraints (Krebs and Davies 1993). I used laboratory manipulations to detect changes in the proportional allocation of energy between longevity and reproduction in response to changes in sugar and blood

availability. Secondly, I examined another limitation of the standard model of vectorial capacity, which does not account for variation in the physiological condition of mosquitoes within a population. In this study, the amount of somatic energy reserves (lipid, protein, sugar, and glycogen) was measured as an indicator of physiological condition. I conducted a survey of a field population of *An. gambiae s.l.* and manipulations of a laboratory colony of *An. gambiae s.s.*, and described patterns of variation in energy reserves within these populations. Lastly, I used a rodent malaria model to investigate the effect of a malaria parasite on vector fitness.

This chapter presents a brief summary of the thesis. Chapter 2 provides a review of feeding habits of female *An. gambiae* and the role of sugar and blood in determining female physiological condition, and describes a laboratory study designed to determine if *An. gambiae* females can alter the proportional allocation of resources between somatic and reproductive functions when under nutritive stress. In other words, can *An. gambiae* females alter their own longevity indirectly by changing the allocation of resources between somatic and reproductive functions?

Chapter 3 includes a discussion of the role of lipid, protein, sugar, and glycogen as energy reserves and their use towards somatic and reproductive functions. The primary goal of Chapter 3 is to answer the question: how does the somatic energy reserve status of *An. gambiae* vary within a population? This goal was accomplished through surveys of a field population and manipulation of a laboratory colony. Variation in somatic energy reserves within each population

and between populations is discussed in relation to physiological factors such as age and sex, and environmental factors such as habitat.

In Chapter 4, the effects of *Plasmodium berghei* on the fitness of an anopheline vector are investigated. Chapter 4 also explores the possibility that the effect of *P. berghei* on the mosquito is affected by the nutritional intake. The final chapter summarizes the results of these 3 studies in the context of vectorial capacity and suggests directions for future work.

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CHAPTER 2: LIFE HISTORY TRADEOFFS IN *ANOPHELES GAMBIAE*

2.1 Introduction

A central tenet of behavioural ecology is that tradeoffs between life history components such as longevity and reproduction occur due to physiological constraints (Krebs and Davies 1993). Allocating energy and resources towards current reproductive effort may decrease the resources available for somatic functions such as growth and maintenance of somatic tissues. This may decrease the probability of survival and therefore decrease future reproductive success (Bell 1980, Sheldon 2000). Selective processes should maximize the lifetime reproductive success by optimizing the proportional input into survival and current reproductive effort. Tradeoffs between life history components can be mediated through behaviours (Krebs and Davies 1993). For example, to balance increased risk of predation during periods of foraging activity with the benefit of greater food intake, many prey species decrease foraging activity in response to the presence of predators (McPeek 2004, Urban 2007). In other cases, tradeoffs may be mediated through physiological processes. For example, an individual may increase its reproductive output to take advantage of an abundance of resources or increase reproductive output using somatic reserves to compensate for a shortage (Briegel 1990). However, the ability to shift allocation of resources in response to changes in their physiological status or the environment may be limited and is expected to vary both within and

between species (Krebs and Davies 1993). Organisms that live in unpredictable environments may be expected to be especially flexible in their proportional allocation of energetic resources between survival and current reproductive effort. *Anopheles gambiae* is just such an organism. Due to its role as the primary vector of *Plasmodium falciparum* in sub-Saharan Africa, it is especially important to understand the allocation of resources between somatic and reproductive functions in *An. gambiae*. Any factor that increases the longevity or fecundity of a female has the potential to increase the transmission of *Plasmodium* spp. (Beier 1996).

Anopheles gambiae lives in an environment that varies drastically in its characteristics throughout the year. During the rainy season, larval habitats are numerous and the population density of adults increases. The abundance and quality of larval habitats decreases during the long dry season, resulting in a reduction in adult population size and density (Minakawa *et al*. 2001). In addition to the unpredictable environment in which they live, life history tradeoffs are important in this mosquito species due to its typically physiologically poor condition. *Anopheles gambiae* tend to emerge in metabolically poor condition, with limited reserves carried over from their larval state (Chapter 3, Briegel 1990). As a result, some females require more than one blood meal to produce an initial batch of eggs (Gillies 1954, 1955). This suggests that nutrition and metabolic reserves are of particular importance to this species and directly impact female reproductive success.

As adults, *An. gambiae* females feed primarily on blood, but can take sugar meals from floral and extra-floral nectaries (Foster 1995). Females cannot reproduce without obtaining a blood meal, from which they acquire protein and lipids (Briegel 1990). These nutrients are used to synthesize eggs as well as to increase maternal somatic reserves (Fernandes and Briegel 2005). The frequency and relative importance of sugar feeding in field populations is not well understood, although there is a growing body of evidence that suggests sugar feeding is important for females of this species (Gary and Foster 2001, 2004, Manda *et al*. 2007, Okech *et al*. 2003). Laboratory studies show that sugar meals are used to synthesize glycogen and lipid stores in somatic tissues (Briegel 1990, Fernandes and Briegel 2005). Provision of sugar meals can increase lifespan (Gary and Foster 2001), egg quality (Fernandes and Briegel 2005) and egg batch size, and reduce the amount of blood required to produce eggs (Foster 1995). Data also indicate that females have the ability to change the proportional allocation of nutrient types between reproductive and somatic tissues, depending on diet (Fernandes and Briegel 2005). Therefore, despite the inference made in early studies that sugar does not play an important role in the fitness of *An. gambiae* females (Muirhead-Thomson 1951), recent evidence suggests that both sugar and blood contribute to female fitness and are both important components of the female diet. There is also evidence that interactions between physiological condition (as measured by body size) and diet composition (sugar vs. blood) affect tradeoffs between somatic and reproductive functions (Briegel 1990).

In this chapter, I describe a study that was designed to determine if *An. gambiae* females are able to change the proportional allocation of nutritional resources between life history traits in response to variation in their diet. The nutritional quality of the mosquito diet was altered by varying the concentration of sucrose solution provided and by varying the number of blood meals provided in the first gonotrophic cycle. The life history traits measured in this experiment were mosquito longevity and fecundity (as a measure of current reproductive effort). Additionally, this study was used as a preliminary investigation to determine the ability of the experimental design to detect the effects of nutritional limitation on *An. gambiae*. A similar design is employed in Chapter 4, where the effect of *Plasmodium berghei* on the longevity and reproduction of anophelines is investigated.

Predictions

If experimental conditions are not limiting or if *An. gambiae* females are not able to change the way they allocate resources between somatic and reproductive functions, mosquitoes on restricted diets will have a shorter lifespan and reduced reproductive output compared to mosquitoes provided with greater amounts of nutritional resources (Figure 2-1A).

If experimental conditions are limiting and *An. gambiae* females are capable of changing the proportional allocation of resources between somatic and reproductive functions, mosquitoes on restricted diets may be able to prevent a reduction in one life history component at the expense of a competing function. Individuals experiencing limited resources may maintain reproductive

output at the expense of longevity (Figure 2-1B), or decrease reproductive output and thereby maintain their lifespan (Figure 2-1C). Mosquitoes can be described as *r*-selected organisms, with short life spans, reproduction at a young age, and many offspring per individual. Therefore, it is most likely that *An. gambiae* females on a restricted diet will employ the 1st strategy described, maintenance of reproductive output at the expense of longevity. It is also possible that females on restricted diets may be able to increase their reproductive output relative to individuals on higher quality diets, as depicted in Figure 2-1D. This would indicate a relatively high degree of flexibility in the proportional allocation of resources between somatic and reproductive functions.

Some of the predictions described above assume that *An. gambiae* is capable of reallocating resources in response to changes in factors such as environmental conditions, resource availability, or physiological stress. An alternative hypothesis is that this species does not possess this ability. However, previous studies have indicated that *An. gambiae* does possess the ability to shift allocation of resources in response to stressors. For example, activation of the immune system decreases egg production (Ahmed *et al*. 2002). Therefore, the assumption of flexible allocation of resources is reasonable for the purposes of this study.

2.2 Materials and methods

Rearing conditions

Mosquitoes were reared in a growth chamber at 28°C (± 2°C) and 75% RH (± 10%). Larvae were raised under conditions determined by Lyimo *et al*. (1992) to produce small-bodied adults. This was done in an effort to produce females with limited somatic reserves at emergence, reflecting the relatively poor condition of *An. gambiae* in field populations (Briegel 1990). Briefly, 1000 1st instar larvae were placed into a 32 x 46 x 6 cm plastic tray containing 3 litres of distilled water and fed Nutrafin® Basix Staple Tropical Fish Food *ad libitum* daily. Food that had accumulated on the bottom of the tray was siphoned off periodically and distilled water was added as needed to maintain the 3-litre volume in the tray. Disposable plastic pipettes were used to transfer pupae daily to glass bowls that were then placed into 30 x 30 x 30 cm Plexiglas™ cages with mesh sides and a cotton sleeve for access. Cages contained approximately 200 adult mosquitoes. Each cage of adults was provided with either a 2.5% w/v or 10% w/v sucrose solution.

Experimental design

I employed a 2 x 2 factorial design for this study where the concentration of sucrose solution and the number of blood meals provided in the first gonotrophic cycle differed between treatment groups, and longevity and fecundity were recorded as response variables. A factorial design was required for this study due to the omnivorous diet of *An. gambiae* females. Sugar and blood are both important components of diet, and there is likely an interaction between

these 2 components (see Introduction). The literature on this species indicates that when access to one component of the diet is limited, females can alter the proportional allocation of nutritional resources between somatic and reproductive functions (Fernandes and Briegel 2005). For example, if sugar sources are not available, females can use the energy acquired from blood meals for maintenance of somatic functions rather than for reproduction (Briegel 1990). Therefore, proportional allocation of resources appears to be dependent on the presence of nutritional stress (Briegel 1990, Fernandes and Briegel 2005, Gary and Foster 2001). A factorial design allows for detection of interactions between the 2 diet components, sugar and blood, as well as interactions between the 2 response variables, longevity and fecundity.

The 4 treatment groups generated by the 2 x 2 factorial design are as follows: 2.5% sucrose and 1 blood meal, 2.5% sucrose and 2 blood meals, 10% sucrose and 1 blood meal, 10% sucrose and 2 blood meals. The sucrose solution was removed from all treatment groups on the 3rd day post-emergence and mosquitoes were provided with distilled water for 24 hours. A blood meal was provided to all treatment groups at 4 days post-emergence, allowing females to initiate their 1st gonotrophic cycle. The sucrose solution was returned to all cages following the blood meal. Any females that did not take a full blood meal were removed from the cage at this point and were excluded from the study. Since blood meal volume was not measured, removal of these females was necessary to avoid blood meal size as a confounding factor. Two treatment groups (2.5% sucrose and 2 blood meals, 10% sucrose and 2 blood meals) were

provided with a second blood meal at 5 days post-emergence. The sucrose solution was replaced with distilled water for these treatment groups 4 hours prior to blood feeding. Any females that did not take a blood meal were removed from the cage and excluded from the study. Remaining females in all treatment groups were transferred to individual housing that limited flight activity. The individual housing was constructed from polystyrene narrow diameter tubes, 25 x 95 mm (Thermo Fisher Scientific Inc.), and covered with cotton mesh. A 1.5 cm piece of braided cotton roll moistened with distilled water was placed inside each of these units to provide an oviposition site, and a second piece of braided cotton roll was placed on top of the mesh and moistened with sucrose solution. The oviposition site was removed 72 hours after transfer to individual housing and the number of eggs oviposited by each individual was recorded. This marked the end of the 1st gonotrophic cycle.

All females were provided with another blood meal on day 9 post-emergence and allowed to initiate a 2nd gonotrophic cycle. The sucrose solution was replaced by distilled water 24 hours prior to blood feeding. Half of the mosquitoes in each treatment group were kept on distilled water following this blood meal and were thereby starved to death. The purpose of removing access to food at this point in the experiment is to prevent nutritional compensation for any metabolic stress that may be induced by the treatments and thereby enhance any potential effects of the various treatments. In essence this protocol allows the nutritional condition of each mosquito to be fixed at the time of the final blood meal. Any allocation of resources towards reproductive or somatic

functions must be based on the nutritional condition of the mosquito at this point (Moret and Schmid-Hempel 2000).

The other half of each treatment group was provided with sucrose for the remainder of the study and acted as an internal control. Internal control groups are used to confirm the induction of nutritional stress in the corresponding treatment groups. The longevity (days survived past last blood meal) and number of eggs oviposited were recorded for each individual. The total fecundity of each individual over both gonotrophic cycles was used in statistical analyses. The term 'starvation resistance' has been used to describe longevity following removal of food (Blanckenhorn *et al*. 2007). There were between 11 and 20 individuals in each treatment and internal control group and the experiment was conducted twice to increase sample sizes (Trial 1 and Trial 2).

Statistical analyses

The mean longevity and fecundity ± standard error are reported for all treatment and internal control groups. Analysis of variance (ANOVA) was used to test the effects of continued access to sucrose (i.e. internal control groups), sucrose diet, the number of blood meals in the 1st gonotrophic cycle, and trial (1st or 2nd trial) on longevity and fecundity. The sum of squares, F ratios, and p values are reported. Tukey's Honestly Significant Difference (HSD) test was used to determine which sucrose diets resulted in significant differences in longevity and fecundity. Student's t tests were used to determine the differences in longevity and fecundity between mosquitoes provided with one and two blood

meals in the 1st gonotrophic cycle. The least squares mean values are reported for HSD and Student's t tests.

Statistical analyses were conducted using JMP 6.0 (SAS Institute Inc. 2005) and graphs were generated using GraphPad Prism 4.00 (GraphPad Software Inc. 2005).

2.3 Results

Longevity

There was no significant difference in results between Trial 1 and 2 (F = 0.9961; p = 0.4638), so the factor 'Trial' was excluded from the statistical model. Continued access to sucrose had a significant effect on longevity (Table 2-3). Females allowed continued access to sucrose survived longer than those that were starved to death (Table 2-1, Figure 2-2). There was a significant interaction between the type of sucrose diet and number of blood meals (Table 2-3). The effect of sucrose diet on longevity was greater when only 1 blood meal was given in the 1st gonotrophic cycle (compared to 2 blood meals), and the effect of number of blood meals was greater when mosquitoes were reared on 2.5% sucrose (compared to 10% sucrose) (Figure 2-2). Due to the significant interaction between sucrose diet and number of blood meals, a separate ANOVA was conducted to determine the effects of each factor separately (Zar 1999). The number of blood meals in the 1st gonotrophic cycle had a significant effect on longevity for mosquitoes reared on both 2.5% and 10% sucrose ($p < 0.0001$ in both cases). Mosquitoes provided with two blood meals survived longer than

those provided with only one blood meal (Table 2-1). The type of sucrose diet had a significant effect on longevity when mosquitoes were given either 1 or 2 blood meals in the 1st gonotrophic cycle ($p < 0.0001$ in both cases). Mosquitoes reared on 2.5% sucrose solution had a shorter lifespan than those reared on 10% sucrose solution, when either 1 or 2 blood meals were provided in the 1st gonotrophic cycle (Table 2-1, Figures 2-2, 2-4, 2-5).

Fecundity

Results did not differ significantly between Trial 1 and 2 ($F = 0.5085$; $p = 0.4766$), so the factor 'Trial' was excluded from the statistical model. Continued access to food did not significantly affect fecundity ($F = 0.4779$, $p = 0.4902$), therefore it was also excluded from the statistical model. There was a significant interaction between the type of sucrose diet and number of blood meals (Table 2-4). Due to the significant interaction between sucrose diet and number of blood meals, a separate ANOVA was conducted to determine the effects of each factor separately (Zar 1999). The number of blood meals in the 1st gonotrophic cycle had a significant effect on fecundity for mosquitoes reared on both 2.5% and 10% sucrose ($p < 0.0001$ in both cases). Mosquitoes provided with 2 blood meals in the 1st gonotrophic cycle had greater fecundity than those provided with only 1 blood meal when reared on 10% sucrose (Table 2-1, Figures 2-3). In contrast, when reared on 2.5% sucrose, mosquitoes provided with 2 blood meals in the 1st gonotrophic cycle had reduced fecundity compared to those provided with 1 blood meal (Table 2-1, Figures 2-3). The type of sucrose diet had a significant effect on fecundity when mosquitoes were given either 1 or 2 blood

meals in the 1st gonotrophic cycle ($p < 0.0001$ in both cases). Mosquitoes reared on 10% sucrose had greater fecundity than those reared on 2.5% sucrose when provided with 2 blood meals in the 1st gonotrophic cycle (Table 2-1, Figures 2-2, 2-4, 2-5). However, when provided with only 1 blood meal in the 1st gonotrophic cycle, mosquitoes reared on 10% sucrose had reduced fecundity compared to those reared on 2.5% sucrose (Table 2-1, Figures 2-2, 2-4, 2-5). Starvation tended to reduce fecundity, although this trend was not statistically significant (Table 2-2, Figure 2-3).

2.4 Discussion

Tradeoffs between life history components are common across various taxa (Krebs and Davies 1993, Pianka and Parker 1975). This study provides further evidence of resource-based tradeoffs in *An. gambiae* and clearly demonstrates that variation in nutrient intake impacts resource allocation between fitness components.

Individuals reared on a low sugar diet and only provided with one blood meal in their 1st gonotrophic cycle exhibit a tradeoff between starvation resistance and reproductive output, as shown in Figure 2-4. Females on this restricted diet allocate an increased proportion of their metabolic reserves towards their current reproductive output and reduce their starvation resistance in doing so. It is interesting to note that this tradeoff is also seen in the control groups. Even when the females on the restricted diet of 2.5% sucrose and 1 blood meal were allowed continued access to food, they still exhibited greater fecundity over the experimental period and reduced longevity compared to

individuals reared on 10% sucrose and 1 blood meal (Figures 2-2, 2-3). This suggests that sucrose concentration in the 1st gonotrophic cycle is critically important for *An. gambiae* females. Continued access to food during and following the 2nd gonotrophic cycle did not mitigate the effects of restricted nutritional input earlier in life. A 2004 study by Foster and Takken showed that *An. gambiae* females exhibit a behavioural preference towards sugar over blood during the 1st 36 hours of their adult life. Results presented in this chapter indicate that this early behavioural preference for sugar could benefit the mosquito by increasing fecundity in the 1st gonotrophic cycle. An examination of the control groups also suggests that the physiological or behavioural processes that determine allocation of resources during a gonotrophic cycle are based on conditions in the early part of the cycle and are not based on expectations of future conditions. This inference is based on the similar patterns in longevity and fecundity observed in mosquitoes that were allowed continued access to food (control groups). Even when access to food was continued, females that experienced restricted diets in the 1st gonotrophic cycle employed the life history strategy of increased current reproductive effort at the expense of longevity.

When one component of female diet was limited, an increase in the other component of female diet compensated for this limiting effect. For example, females reared on a low sucrose diet and only provided with 1 blood meal in the 1st gonotrophic cycle diverted nutritional resources away from somatic functions and towards reproduction (Figure 2-4). Such a pattern indicates that this particular diet is limiting for the mosquitoes (Figure 2-1B). In contrast, females

reared on a low sucrose diet but provided with an additional blood meal did not exhibit this pattern and had reduced longevity and fecundity compared to their high sucrose counterparts (Figure 2-5). These results further highlight the importance of diet for females early in their adult life, and are consistent with work conducted by other authors that shows blood-meal derived nutrients may be used for somatic functions rather than reproduction when sugar sources are limited (Briegel 1990). Other laboratory and field studies have shown that small females emerge with fewer metabolic reserves than larger females (Briegel 1990), and that small females are likely to take more than 1 blood meal during their 1st gonotrophic cycle (Briegel and Hörler 1993, Fernandes and Briegel 2005, Lyimo and Takken 1993). Females that emerge in poor condition or have reduced access to sugar may be under strong evolutionary pressure to take multiple blood meals very rapidly after emergence (Takken *et al*. 1998). The propensity to take multiple blood meals, especially early in life, increases the probability of acquiring *Plasmodium* spp. and may partially explain why this particular species is such an efficient vector of malaria parasites (Gary and Foster 2001, Takken *et al*. 1998).

Data presented in this study indicate that both blood and sugar are important for *An. gambiae* during the 1st gonotrophic cycle, and highlights the complexity of the relationship between these 2 components of the female diet. For females reared on 10% sucrose, those provided with 2 blood meals in the 1st gonotrophic cycle oviposited more eggs than those provided with only 1 blood meal. This is consistent with studies showing the low efficiency of conversion of

blood into eggs (Briegel 1990) and the propensity for this species to take multiple blood meals within a gonotrophic cycle (Beier 1996, Gillies 1954). When females have low levels of reserves, their initial blood meal is used to synthesize somatic reserves prior to egg development (Briegel 1990, Fernandes and Briegel 2005). An interesting deviation from this result occurred in the 2.5% sucrose treatment groups. In these treatments the opposite result was obtained, mosquitoes provided with only one blood meal in the 1st gonotrophic cycle laid more eggs than mosquitoes provided with 2 blood meals. This result was unexpected. Based on current understanding of *An. gambiae* reproductive physiology, one would predict that an increase in blood intake would increase fecundity. However the results of this study indicate that this may not always occur and that utilization of blood towards egg production might be altered by the availability of carbohydrates. This study did not include an investigation into the mechanisms governing the allocation of resources between somatic and reproductive functions. Therefore, it is not possible to determine what processes resulted in this unusual outcome. If this result were upheld in future experiments, it would indicate that there are other physiological factors that determine resource allocation in *An. gambiae* which are not yet understood.

During this investigation, larvae were reared under conditions known to produce small-bodied adults (Lyimo *et al*. 1992). This was done in an effort to produce females with limited somatic reserves at emergence, reflecting the relatively poor condition of *An. gambiae* in field populations (Briegel 1990, Chapter 3) and to increase the probability that limiting the adult diet resulted in

nutritional stress. Larger females emerge with greater teneral reserves and tend to survive longer and oviposit more eggs than smaller females (Takken *et al*. 1998). It can therefore be predicted that body size would affect the relationship between sugar feeding, blood feeding, and fitness components described in this investigation. Since larger females emerge with more somatic reserves than small females, they would likely allocate a greater proportion of the nutrients acquired in their 1st blood meal towards egg production and exhibit greater fecundity, particularly in the 1st gonotrophic cycle. Increased somatic reserves at emergence in large-bodied mosquitoes might compensate for the reduced nutrient intake of mosquitoes in the 2.5% treatment groups. If this were the case, one would no longer expect the expression of a tradeoff between starvation resistance and current reproductive effort by females subjected to restrictive diet treatments. Including body size as a 3rd factor in the experimental design could test these predictions.

The interaction between sucrose diet and number of blood meals had a statistically significant effect on both the longevity and fecundity of females in this study (Tables 2-3, 2-4). These results suggest that although *An. gambiae* can alter the proportional allocation of resources between soma and reproduction, this flexibility is limited. For example, females reared on a low sucrose diet did not increase their fecundity when provided with an additional blood meal in the 1st gonotrophic cycle. In other words, there is an upper limit on the proportion of a blood meal that can be allocated away from egg production and towards somatic maintenance and/or somatic reserve accumulation.

Under the conditions of this study, small-bodied females reared on a low quality diet allocate more resources towards current reproductive effort at the expense of longevity and therefore future reproductive effort. This strategy may be advantageous in an unpredictable or resource-poor environment. In this type of environment, a strategy that favours immediate use of resources towards reproduction as they become available might evolve (Krebs and Davies 1993). Evidence of resource-based tradeoff between life history components has been described for many species (Pianka and Parker 1975), and has been particularly well studied in insect parasitoids. Insect parasitoids face a choice between a current reproductive opportunity and potential future reproductive opportunities when they encounter a host. Laying an egg in the host provides an opportunity for immediate reproductive success. Alternatively, feeding on the host provides an opportunity to increase somatic reserve tissue, increasing longevity and potential future reproductive success (Casas *et al*. 2005). A tradeoff between diet and life history components is expressed by *Euphelmus vuilletti*, a Hymenopteran parasitoid. When non-host carbohydrate sources are unavailable, this parasitoid tends to feed on an available host rather than oviposit (Casas *et al.* 2005). This represents an increased investment in somatic maintenance and potential future reproduction at the expense of current reproductive output (Casas *et al*. 2005) and is an example of energy-dependent flexibility in resource allocation similar to the one described in this Chapter.

This investigation confirms that *An. gambiae* is capable of shifting allocation of resources between life history traits when nutritional intake is limited.

To some extent, females can reallocate nutritional resources to mitigate the effects of limited nutritional resources, presumably to reduce fitness costs associated with such stressors. This result is consistent with other work indicating the ability of *An. gambiae* to alter resource allocation. Ahmed *et al*. (2002) demonstrated that *An. gambiae* reduces fecundity when the immune system is activated in response to wounding and inoculation with bacteria. Fernandes and Briegel (2005) also demonstrated plasticity in resource allocation in *An. gambiae*. Females deposited greater amounts of lipid per egg when sugar meals were provided in addition to blood.

Such plasticity can greatly affect the fitness cost of any stress *An. gambiae* may experience and should be incorporated into any vector control program based on genetic modification for this medically important insect. There is great interest in developing genetically modified *An. gambiae* that have reduced susceptibility to malaria parasites. For example, transgenic *Anopheles stephensi* have been produced that express salivary gland and midgut binding peptide 1 (SM1) (Ito *et al*. 2002). Expression of this peptide reduces the ability of *Plasmodium berghei* to invade the midgut epithelium and salivary glands of the mosquito, decreasing the proportion of mosquitoes that become infected with and transmit the malaria parasite (Ito *et al*. 2002). SM1-expressing mosquitoes also exhibited higher fecundity and lower mortality following a *P. berghei*-infected blood meal than non-transgenic mosquitoes (Marrelli *et al*. 2007). Data presented in this Chapter suggest that the relationship between expression of any such peptide and fitness components will likely be altered by the quality of

diet available to transgenic mosquitoes. How this relationship would function under field conditions is not clear.

The relationship demonstrated here between nutritional stresses, allocation of resources, and life history tradeoffs also has the potential to impact the malaria parasite via increased or decreased blood feeding activity. Conflicts of interest between the malaria parasite and its vectors have been studied extensively, yet few of these studies account for the role of interactions between life history traits in the mosquito (Schwartz and Koella 2001). Data presented here confirm that *An. gambiae* females are capable of shifting allocation of resources between life history traits in response to limited nutritional resources. If infection with *Plasmodium* spp. is physiologically stressful for the mosquito, the ability for *An. gambiae* females to alter the proportional allocation of resources between reproduction and somatic functions will certainly impact the vector-parasite relationship. The potential impact of this on the epidemiology of malaria is likely to be complex and warrants further study.

A secondary goal of this study was to determine the power of the experimental design to detect the effects of nutritional limitation on *An. gambiae*. The experimental design was successful in detecting differences in the fecundity and starvation resistance of *An. gambiae* subjected to different diet regimes. Induction of metabolic stress *in An. gambiae* females due to limitation of sugar and blood was inferred by the trend towards reduced longevity and fecundity in mosquitoes reared on a lower concentration of sucrose and in mosquitoes provided with only 1 blood meal in their 1st gonotrophic cycle. Therefore, a

similar experimental design was employed to determine if infection with *P. berghei* induces stress in an anopheline vector (Chapter 4).

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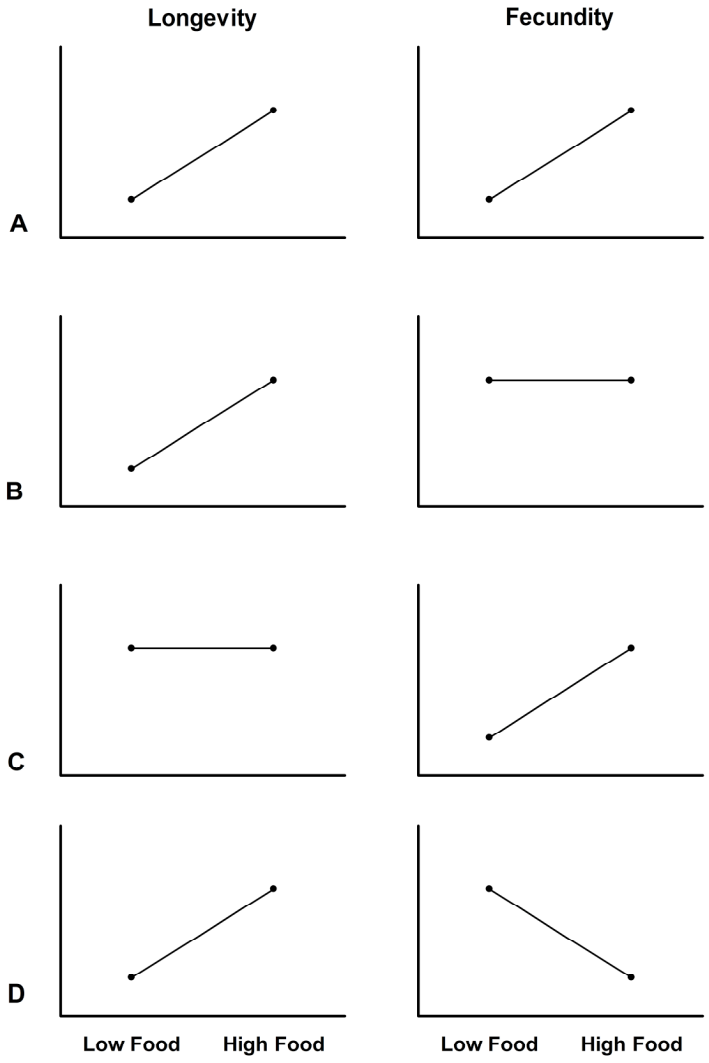

Figure 2-1 **Potential evidence of reallocation of resources between somatic and reproductive functions in an omnivore, *Anopheles gambiae*. A: Results predicted if *An. gambiae* females are not capable of reallocation or if diet is not limiting. Females on a restricted diet (Low Food) have reduced longevity and fecundity compared to females provided with greater nutritional resources (High Food). B-D: Results predicted if *An. gambiae* are capable of reallocating resources between somatic and reproductive functions and diet is limiting. B: Females on a restricted diet (Low Food) moderately increase the proportional allocation of resources into reproduction to maintain fecundity at the expense of longevity. C: Females on a restricted diet (Low Food) moderately increase the proportional allocation of resources into somatic functions to maintain longevity at the expense of fecundity. D: Females on a restricted diet (Low Food) greatly increase the proportional allocation of resources into reproduction to increase fecundity at the expense of longevity.**

Table 2-1 **Mean longevity of *An. gambiae* females subjected to four different diet treatments, with corresponding internal control groups. Longevity is measured in days, following removal from food. Means with different letters are significantly different (Tukey's HSD, $\alpha = 0.05$).**

Diet	n	Mean days survived ± SE
Treatment group		
2.5% sucrose, 1 blood meal	30	4.40 ± 0.19 (A)
2.5% sucrose, 2 blood meals	29	4.62 ± 0.20 (A)
10% sucrose, 1 blood meal	29	5.83 ± 0.15 (B)
10% sucrose, 2 blood meals	30	6.00 ± 0.17 (B)
Internal control		
2.5% sucrose, 1 blood meal	23	14.22 ± 0.40 (C)
2.5% sucrose, 2 blood meals	21	16.48 ± 0.49 (D)
10% sucrose, 1 blood meal	25	17.00 ± 0.33 (D, E)
10% sucrose, 2 blood meals	23	17.78 ± 0.24 (E)

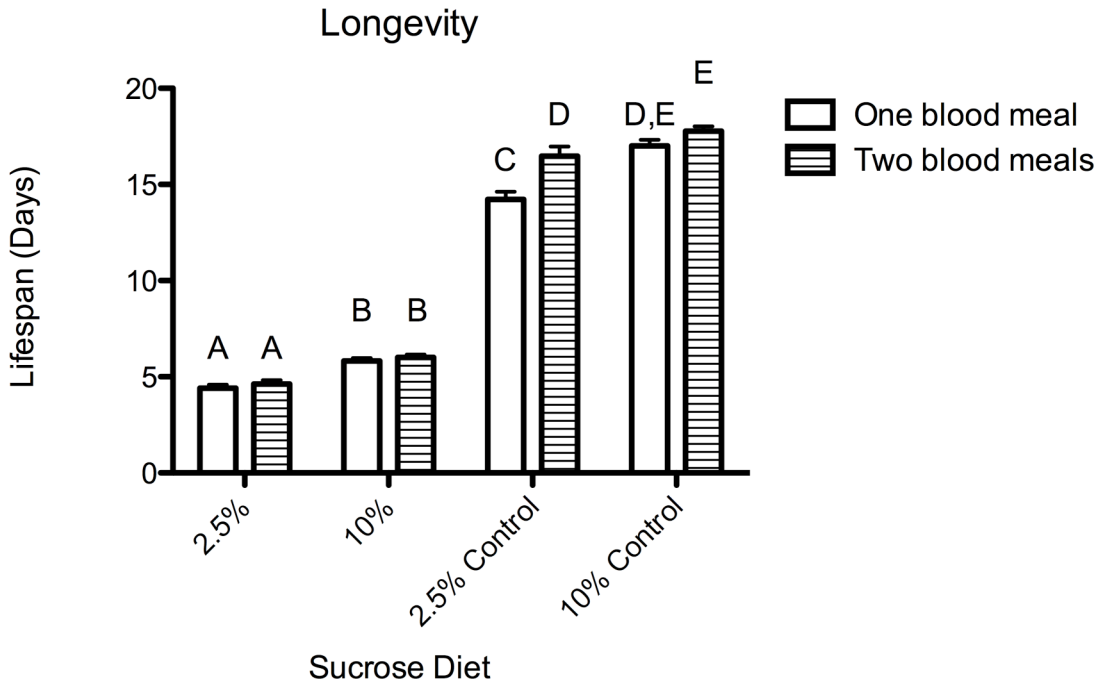

Figure 2-2 Longevity *of An. gambiae* females subjected to four different diet treatments, with corresponding internal control groups. Longevity is measured in days, following removal from food. Columns with different letters are significantly different (Tukey's HSD, $\alpha = 0.05$).

Table 2-2 **Mean fecundity over 2 gonotrophic cycles of *An. gambiae* females subjected to four different diet treatments, with corresponding internal control groups. Means with different letters are significantly different (Tukey's HSD, $\alpha = 0.05$).**

Diet	n	Mean fecundity ± SE
Treatment group		
2.5% sucrose, 1 blood meal	30	81.60 ± 2.47 (A,B,C)
2.5% sucrose, 2 blood meals	29	72.83 ± 1.12 (C)
10% sucrose, 1 blood meal	29	63.10 ± 1.84 (D)
10% sucrose, 2 blood meals	30	83.80 ± 1.78 (A,B)
Internal control		
2.5% sucrose, 1 blood meal	23	87.09 ± 2.74 (A)
2.5% sucrose, 2 blood meals	21	75.29 ± 2.12 (B,C)
10% sucrose, 1 blood meal	25	57.64 ± 3.25 (D)
10% sucrose, 2 blood meals	23	85.04 ± 1.46 (A.B)

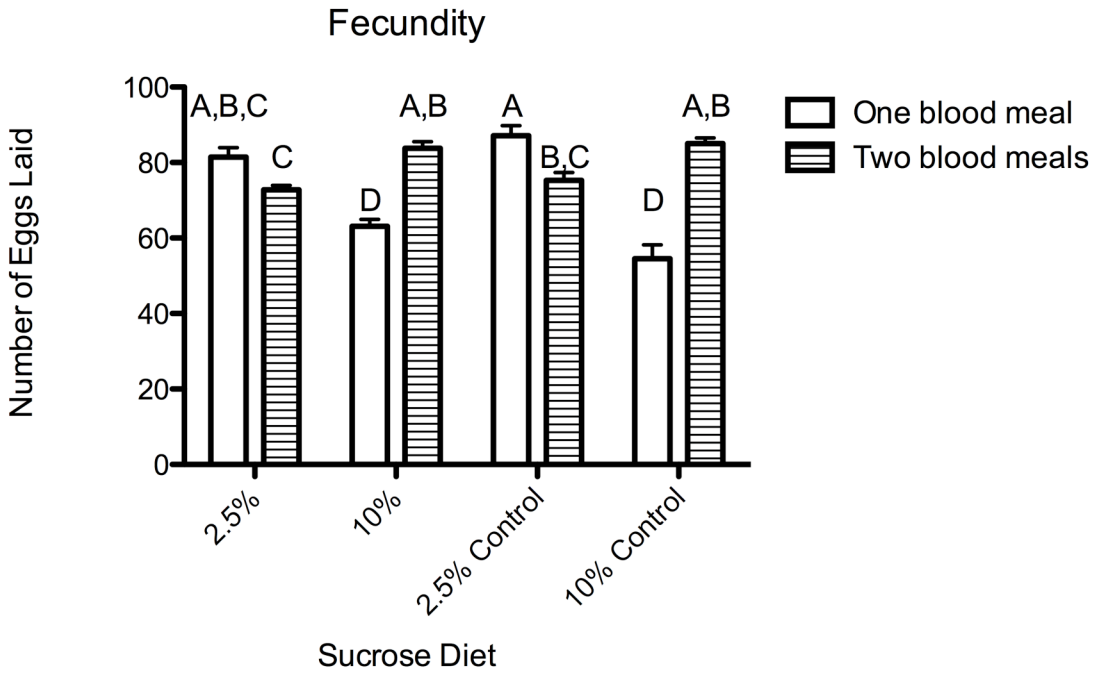

Figure 2-3 Fecundity over 2 gonotrophic cycles of *An. gambiae* females subjected to four different diet treatments, with corresponding internal control groups. Columns with different letters are significantly different (Tukey's HSD, α = 0.05).

Table 2-3 Effect of sucrose diet type, number of blood meals in 1st gonotrophic cycle, and interactions on the longevity of *An. gambiae* females. * indicates result is statistically significant (ANOVA, $\alpha = 0.05$).

Model effect	Sum of squares	F ratio	p value
Sucrose Diet	132.8265	54.35	<0.0001*
Number of Blood Meals	28.1281	11.5108	0.0003*
Continued Access to Sucrose	6315.0639	2584.285	<0.0001*
Sucrose Diet*Number of Blood Meals	13.0287	5.3317	0.002*
Sucrose Diet*Continued Access to Sucrose	2.4303	0.9945	0.3198
Continued Access to Sucrose*Number of Blood Meals	6.4301	2.114	0.1699
Sucrose Diet*Continued Access to Sucrose*Number of Blood Meals	3.5639	1.0021	0.2575

Table 2-4 Effect of sucrose diet type, number of blood meals in 1st gonotrophic cycle, and interactions on the fecundity of *An. gambiae* females over 2 gonotrophic cycles. * indicates result is statistically significant (ANOVA, $\alpha = 0.05$).

Model effect	Sum of squares	F ratio	p value
Sucrose Diet	2652.374	7.1386	0.0001*
Number of Blood Meals	2466.281	19.9133	<0.0001*
Sucrose Diet*Number of Blood Meals	15238.361	41.0126	<0.0001*

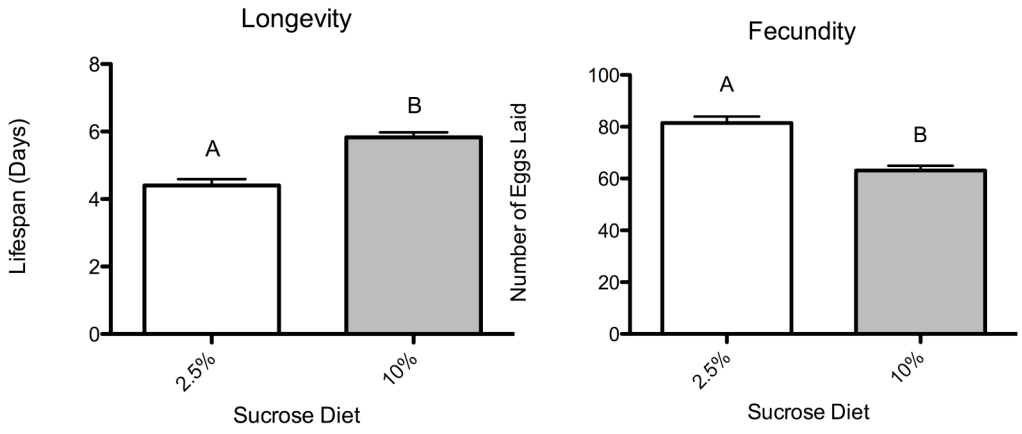

Figure 2-4 When provided with only 1 blood meal in the 1st gonotrophic cycle females reared on a low sucrose diet increase fecundity at the expense of longevity. This pattern indicates the ability of *An. gambiae* females to greatly alter the proportional allocation of resources between somatic and reproductive functions in response to a restricted diet. Columns with different letters are significantly different (Tukey's HSD, $\alpha = 0.05$).

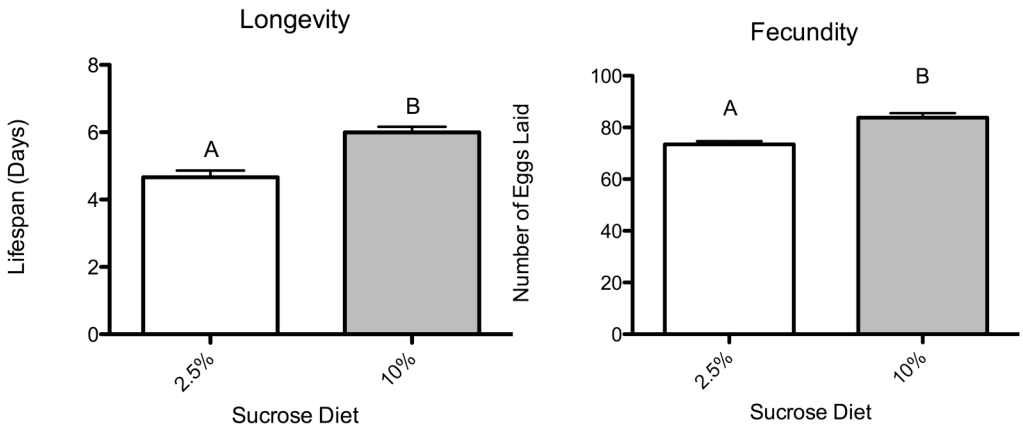

Figure 2-5 When provided with 2 blood meals in the 1st gonotrophic cycle, females reared on a low sucrose diet have reduced longevity and fecundity compared to those reared on a high sucrose diet. This pattern indicates that this diet was not limiting for *An. gambiae* females. Columns with different letters are significantly different (Tukey's HSD, $\alpha = 0.05$).

CHAPTER 3: A MOSQUITO CALLED LWANDA

3.1 Introduction

There remains much about the nutritional ecology of *An. gambiae* adults that is unknown, although there is increasing evidence to suggest that nutrition plays a critical role in the vectorial capacity of this insect (Chapter 2, Ma and Roitberg, unpublished manuscript). Females of this species feed on both blood and plant sugars. The interactions between blood and sugar feeding, somatic and reproductive energy reserves, and fitness are complex and have only been well studied in the laboratory. Nutrients acquired through blood feeding can be directed towards either somatic or reproductive functions, the proportional allocation between these 2 functions varies with body size (Briegel 1990) and existing nutrient reserves (Chapter 2). Small females allocate a greater proportion of the caloric content of a blood meal towards somatic lipid reserves in the fat body and require larger blood meals to initiate oogenesis compared to large females (Briegel 1990). Smaller females are also more likely to require more than one blood meal in their initial gonotrophic cycle to produce eggs (Fernandes and Briegel 2005). It is hypothesized that small females have low levels of somatic energy reserves and are therefore required to allocate nutrients towards somatic tissue to prevent starvation (Briegel 2003, Fernandes and Briegel 2005). Nutrients acquired through sugar feeding also can contribute to both somatic and reproductive metabolic reserves. Provision of sugar in the female diet increases the lipid content of somatic reserves (Briegel 1990) and

increases the probability that small females will produce eggs from their 1st blood meal (Fernandes and Briegel 2005). Availability of sugar meals also influences blood feeding behaviour. When sugar and blood are both available, females exhibit a preference towards taking sugar meals in the period immediately following emergence (Foster and Takken 2004). Sugar-fed females also take smaller, but more frequent blood meals than females without access to sugar (Gary and Foster 2006). In addition, sugar-fed females are able to allocate their increased somatic lipid reserves towards reproduction and use somatic lipids to produce yolk lipids (Briegel 1990, Fernandes and Briegel 2005). There is also some evidence that moderate amounts of sugar increase the lifespan *of An. gambiae* females (Straif and Beier 1996, Okech *et al*. 2003). Additionally, evidence is provided in Chapter 2 that longevity and fecundity are influenced by nutrition. When amounts of sugar and blood were restricted in the 1st gonotrophic cycle, females increased their reproductive output resulting in decreased longevity.

Together, the results of these studies indicate that nutrition acquired through blood and sugar meals impacts the somatic energy reserve status and reproductive physiology of *An. gambiae* females, which consequently affects the blood feeding and reproductive behaviours of females. Therefore, the nutritional ecology of females has implications for the vectorial capacity of *An. gambiae* via its effects on blood feeding and reproductive behaviours. Any factor that increases the frequency of blood feeding and/or longevity of an individual has the

potential to increase the probability of that individual acquiring and transmitting *Plasmodium* spp. (Beier 1996).

In contrast to females, males feed exclusively on plant sugars. Males obtain these sugars primarily from floral or extra-floral nectaries (Foster 1995), but can also feed on honeydew, damaged fruits, sap, or leaves (Clements 1992). Extra-floral nectaries also may provide males with a source of proteins and lipids as they contain these nutrients in addition to sugars (Wäckers 2005). There is evidence that males are highly attracted to plant odours soon after emergence (Foster and Takken 2004) and that feeding on plant nectars increases male survival (Gary and Foster 2004, Impoinvil *et al.* 2004). In the absence of sugar, male *An. gambiae* only survive 48-72 hours (Takken *et al.* 1998). Sugar also appears to be necessary for mating activity in males of most mosquito species studied (Gary and Foster 2004). Anophelines mate in swarms, a behaviour that requires males to sustain flight for an extended period of time (Yuval *et al.* 1994, Yuval 2006). Males rely on stored carbohydrates for flight, and use up to 50% of their available carbohydrate reserves during flight in a mating swarm (Yuval *et al.* 1994). The ability of males to initiate swarming behaviour also increases as carbohydrate reserves increase (Yuval *et al.* 1994). Therefore, it can be expected that availability of plant sugars, known to increase carbohydrate reserves in females (Fernandes and Briegel 2005), will increase the probability of mating success of males. Males without access to sugar or with limited carbohydrate reserves would not likely meet the high energy demands required for swarming and mating (Gary and Foster 2004).

Much of our knowledge of the nutritional ecology and energy reserve status of *An. gambiae* is derived from laboratory studies, due to the inherent difficulties of monitoring such small, mobile animals in the field. However, there are differences in the energy reserves of field populations and laboratory colonies of *An. gambiae* (Huho *et al*. 2007). This variation likely arises from the differences in laboratory and natural habitats. Laboratory conditions such as temperature, humidity, and availability of food are tightly regulated (Huho *et al*. 2007). In contrast, conditions in natural habitats vary across temporal and spatial scales. For example, rainfall in Western Kenya varies throughout the year, with the vast majority of rain occurring during the rainy seasons (March to May and October to November) (Manda *et al*. 2007). Increased rainfall increases the number and permanence of larval habitats, leading to a rapid increase in *An. gambiae* population size (Koenraadt *et al.* 2003). The impact of this variation in larval habitats on adult fitness parameters (such as energy reserves) has not yet been studied (Koenraadt *et al.* 2003). Variation in other environmental conditions such as availability of nectar, have been shown to increase longevity of laboratory-reared mosquitoes (Gary and Foster 2004). Such factors are also likely to affect energy reserves and fitness in field-populations of *An. gambiae*. The nutritional ecology and energy reserve status of *An. gambiae* has not been well studied in the field. However, because of the potential impact of nutrition on fitness via reproductive success (Roitberg and Foster, personal communication) and longevity, there is a great need for further investigation in this field.

Research questions

In this study, I conducted field and laboratory experiments to examine key aspects of the nutritional ecology of *An. gambiae* and to answer 3 research questions.

Firstly, what is the somatic energy reserve status of *An. gambiae* males and females at emergence? This is valuable information because we know that reserve status affects physiological processes and behavioural choices (Briegel 1990, Fernandes and Briegel 2005, Chapter 2). Investigations of female physiology indicate that the period immediately following emergence and during the 1st gonotrophic cycle is of particular importance (Fernandes and Briegel 2005, Chapter 2). This period is likely of critical importance for males as well since most males complete mating within 76-100 hours of emergence (Chambers and Klowden 2001). As mentioned above, reserve status and nutritional ecology have not been frequently investigated for males of this species. However, a recent study indicates that there is likely sexual dimorphism in the energy budget of *An. gambiae* (Huho *et al*. 2007). Including both males and females in this study allows for further investigation of the possibility of such sex-specific differences.

Secondly, can males and females accumulate somatic energy reserves through post-emergence feeding? The reserves available at emergence are carried over from the larval state and reflect food availability and reserve accumulation during the larval period (Briegel 1990). Studies of larval nutrition suggest that increased food availability produces larger adults (Lyimo and

Takken 1993) with more nutritional reserves (Timmermann and Briegel 1999). Compared with *Aedes aegypti*, *An. gambiae* emerges with lower energy reserves (Briegel 1990). This may be a result of different larval feeding habits. *Aedes aegypti* are capable of feeding at various depths throughout the water column while *An. gambiae* feed almost exclusively at the surface, which tends to contain a lower concentration of suspended organic matter (Briegel 2003). Data from lab studies indicate that sugar feeding and blood feeding can increase somatic and reproductive energy reserves (Briegel 1990, Fernandes and Briegel 2005). However, it is not yet clear whether adult *An. gambiae* in field populations increase their somatic energy reserves through sugar or blood feeding. This information is important because of the apparent undernourished condition of *An. gambiae* at emergence compared to other mosquito species and the effect of somatic reserve accumulation on fitness.

Lastly, how does the somatic energy reserve status of a field population compare to that of a laboratory population? Studies of *An. gambiae* and other species show that lab and field populations can differ in key fitness components (Huho *et al*. 2007). For example, males from a field population of *An. gambiae* were larger and contained more lipids than males in a laboratory colony (Huho *et al*. 2007). We need to know how laboratory and field populations vary in fitness parameters such as quantity of somatic energy reserves because this variation potentially impacts proposed vector control programs based on genetic modification. The fitness of genetically modified mosquitoes compared to their wild counterparts will determine, in part, the success of any such program (Huho

et al. 2007, Marrelli *et al*. 2007). Understanding the differences between field and laboratory populations will also help to determine the limits of inference possible when extrapolating the results of laboratory studies to field populations and vice versa.

3.2 Materials and methods

Field study site

Initial laboratory work, dissections and preparation of specimens were conducted at the Mbita Point Training and Research Centre of the International Centre of Insect Physiology and Ecology (ICIPE). This facility is located in Mbita, a village on the shores of Lake Victoria in Suba District, Nyanza Province, western Kenya. Field collections were conducted in the village of Lwanda, located approximately 12 km to the east of Mbita. Houses extend from the shoreline of Lake Victoria up into a highland area. Suba District experiences two rainy seasons; the “long rains” between March and May, and the “short rains” between October and November, with a mean annual rainfall of 700-1200mm (Manda *et al*. 2007). Larval and adult mosquitoes can be found in the area in the rainy and dry seasons (Manda *et al*. 2007). Adult mosquitoes are most commonly found inside the mud-and-thatched homes (Okech *et al.* 2003). Females blood feed during the night and are most active between 1 and 6 am (Githeko *et al*. 1996). Larvae can be found in a variety of aquatic habitats ranging from small puddles to the fringes of the lake (Munga *et al.* 2007). Due to the year-round presence of anophelines, malaria is endemic in the area (Mutero *et al*. 1998); with *An. gambiae* being the principle vector of *P. falciparum*,

although *Anopheles arabiensis* Patton and *Anopheles funestus* Giles also contribute to transmission of *P. falciparum* (Shililu *et al*. 2003).

Field collections

Field collections were conducted in the village of Lwanda over a period of 4 weeks during the rainy season (April-May) of 2006. Three types of samples were collected from the anopheline population in this village: Teneral, Minimum Irreducible Amounts, and Adult.

Teneral samples consisted of mosquitoes that were killed and frozen within one hour of eclosion. Mosquitoes in this category were used to determine the quantity of somatic energy reserves carried over from the larval stage and available after eclosion (Briegel 2003). Minimum Irreducible Amount (MIA) samples consisted of mosquitoes that were starved to death following eclosion. Mosquitoes in this category were used to determine the quantity of somatic reserves at emergence that are unavailable for metabolism to prevent starvation. The amount of somatic energy reserves in a mosquito that has been starved to death after eclosion represents the amount that the mosquito cannot access to maintain somatic functions and may include structural components and reproductive tissues (Briegel 1990, Van Handel 1984). Teneral and MIA samples were obtained by collecting anopheline pupae from larval habitats in the lowland and lakeside areas of the village. Pupae were taken to the laboratory at ICIPE and placed in small wax-lined cardboard bowls filled with water from the larval habitat. The bowls were placed inside metal-framed cages measuring approximately 20 x 20 x 20 cm with mesh side panels and a cotton sleeve for

access. Cages were inspected hourly for mosquitoes that had eclosed. Newly eclosed mosquitoes were visually identified as *Anopheles funestus* or *An. gambiae sensu lato*. *An. funestus* were discarded. *Anopheles gambiae* were alternately assigned to Teneral or MIA categories. Mosquitoes in the Teneral category were knocked down using chloroform and placed in a freezer at -20°C. Mosquitoes in the MIA category were transferred to a new cage and starved to death. Access to water was provided, ensuring that death was a result of starvation and not dehydration. MIA cages were checked hourly and any dead mosquitoes were placed in a freezer. Mosquitoes were removed from the freezer each day for further processing. One wing was removed from each mosquito and winglength was measured from the alular notch to the distal tip. The ovaries were removed from female mosquitoes. All mosquitoes were then placed in a drying oven at 100°C for 1 hour to remove all water from the specimens and then stored in sealed microcentrifuge tubes at room temperature until nutrient extractions were performed. This method of preservation and storage is recommended by Van Handel (1985a) to prevent enzymatic and bacterial degradation of samples, and eliminates the need for a method of freezing during transport. Teneral and MIA samples were also collected during the dry season (December 2006 - March 2007) and processed in the same manner. Results refer to samples collected during the rainy season unless otherwise stated.

Adult samples were collected at dawn and dusk from indoor and outdoor habitats in the village of Lwanda. Indoor habitats consisted of houses constructed from wood and mud walls with thatched or corrugated iron roofs.

This type of construction is typical of the region. Adults were collected by visually locating mosquitoes resting on surfaces of the home, aided by a flashlight covered with red Plexiglas™ (to avoid detection of the flashlight by mosquitoes). Mosquitoes were collected using an aspirator constructed from a 15 ml plastic centrifuge tube separated from plastic tubing by cotton mesh, and immediately knocked down using chloroform and placed on ice to reduce metabolism. Mosquitoes were found most commonly in darker areas of the houses, on dark surfaces such as walls, wooden furniture, and in the thatched roofs. Outdoor habitats consisted of dark clay pots with a wide mouth and a small hole near the bottom, placed in shaded vegetation. Mosquitoes were collected from these outdoor habitats by placing the cotton sleeve of a mesh cage over the mouth of the pot and blowing into the hole in the pot. Mosquitoes were aspirated from the cage, knocked down using chloroform, and placed on ice.

Three types of sites within the village of Lwanda were sampled for adult mosquitoes: Lakeside, Lowland, and Highland. Indoor and outdoor habitats were sampled in all 3 categories of sites. Lakeside sites were marshy or gravel-covered areas within 300 metres of Lake Victoria. The human population of the village was densest in this area. Vegetation in these sites was sparser than in the Lowland and Highland sites and typically consisted of short grasses and flowering bushes. There were also fewer non-human animals found around Lakeside sites compared to the other site types, although chickens, goats and dogs were found around one Lakeside house. Lowland sites were located farther away from the lake, with more space between houses compared to the

Lakeside area. All collection sites in the Lowland area contained crops, typically corn and tomatoes. Most sites also contained grasses, flowering bushes, and trees. Animals such as cows, goats and dogs were found in the Lowland area. Highland sites were farthest away from the lake, in an elevated region of the village. Vegetation consisted of crops (usually corn), grasses, flowering bushes, and trees and was typically denser than in the Lakeside and Lowland sites. Animals found in the Highland area included chickens, cows, dogs, and cats.

All adult samples were stored in a -20°C laboratory freezer at ICIPE for 1 to 7 days. Samples then were removed from the freezer and identified as *An. gambiae s. l.* or *An. funestus*. *An. funestus* samples were discarded. The abdomen of *An. gambiae s. l*. females was examined for the presence of a blood meal and eggs. Only 'empty' females were included in the study. Females that contained a blood meal at any stage of digestion or were in the process of developing eggs were excluded. Excluding these specimens ensures that the nutrients extracted are not part of a blood meal, are primarily somatic in nature, and are not being actively used for reproduction. Next, 1 wing was removed from each mosquito. Winglength was measured from the alular notch to the distal tip, excluding fringe scales. The ovaries were removed from female mosquitoes and classed as nulliparous or parous based on the presence or absence of tracheolar skeins (Detinova 1962). Nulliparous females have not yet produced any eggs, and the tracheoles surrounding the ovaries are tightly coiled at the terminal ends. The coils are referred to as skeins (Detinova 1962). Parous females have produced eggs and oviposited at least once, a process that stretches out and

irreversibly unwinds the skeins (Detinova 1962). Determination of reproductive status using this method is frequently used to estimate the age of female mosquitoes (Hayes and Wall 1999, Penilla *et al*. 2002). Nulliparous females are assumed to be, on average, younger than parous females (Detinova 1962, Hayes and Wall 1999, Penilla *et al.* 2002). Following wing measurement and classification of reproductive status, all mosquitoes were then placed in a drying oven at 100°C for 1 hour and then stored in sealed microcentrifuge tubes at room temperature until nutrient extractions were performed.

Laboratory colony

Anopheles gambiae sensu stricto (Ifakara strain) were reared in a growth chamber at 28°C (± 2°C) and 75% relative humidity (± 10%). This colony originated from Njag, Tanzania and has been in laboratory culture for approximately eight years.

Larvae were reared under high density or low density conditions, intended to generate adults that differ in body size as described by Lyimo *et al.* (1992). For the low density treatment, 100 1st instar larvae were placed into a 32 x 46 x 6 cm plastic tray containing 3 litres of distilled water. For the high density treatment, 1000 1st instar larvae were placed into each plastic tray, also containing 3 litres of distilled water. Three low density and three high density trays were prepared. Each tray was fed Nutrafin® Basix Staple Tropical Fish Food *ad libitum* daily. Food that had accumulated on tray bottoms was siphoned off periodically and distilled water was added as needed to maintain a 3-litre volume in each tray. Disposable plastic pipettes were used to transfer pupae

daily to glass bowls that were then placed into 30 x 30 x 30 cm Plexiglas™ cages with mesh sides and a cotton sleeve for access. Mosquitoes from the low and high density treatments were placed in separate cages. Furthermore, each pupa was randomly assigned during collection to one of two categories, Teneral, or Minimal Irreducible Amounts (MIA). The resulting four categories of cages are as follows: low density-teneral, high density-teneral, low density-MIA, and high density-MIA. These will henceforth be referred to as large-bodied teneral, small-bodied teneral, large-bodied MIA, and small-bodied MIA, respectively.

Mosquitoes in the teneral and MIA groups were handled in the same manner as the field-collected mosquitoes. Teneral samples were knocked down using chloroform within one hour of eclosion and then placed in a freezer. This allows for determination of the somatic energy reserves of small and large-bodied laboratory mosquitoes at emergence. MIA mosquitoes were allowed access to distilled water only and were thereby starved to death. MIA cages were examined every hour and any dead mosquitoes were removed and stored at -20°C. MIA samples allow for the determination of the somatic energy reserves that cannot be mobilized to prevent starvation (Briegel 1990, Fernandes and Briegel 2005).

Mosquitoes were removed from the freezer within two days for further processing. One wing was removed from each mosquito and winglength was measured from the alular notch to the distal tip. The ovaries were removed from female mosquitoes. All mosquitoes were then placed in a drying oven at 100°C

for 1 hour and then stored in sealed microcentrifuge tubes at room temperature until nutrient extractions were performed.

Nutrient extraction and biochemical analyses

Protein extraction and quantification using the Bradford assay

Each mosquito was homogenized in a microcentrifuge tube containing 100 μl of phosphate-buffered saline with a glass rod. The glass rod was then rinsed with 100 μl of phosphate-buffered saline into the microcentrifuge tube to give a total volume of 200 μl in the tube. Samples were centrifuged at 8.6 x g for one minute and 30 μl of the supernatant was transferred to a 16 x 125 mm glass test tube for protein quantification using the Bradford assay. The remaining supernatant and pellet were stored in a -80°C for carbohydrate and lipid extractions.

A Quick-Start™ Bradford Protein Assay Kit from Bio-Rad Laboratories was used to determine the amount of protein in each sample. Briefly, a standard curve was prepared from bovine gamma globulin, with concentrations of 0, 1.5, 2.5, 5, 10, 15, 20, 30, and 40 μg/ml. One ml of Bradford reagent was added to each standard and each sample. Tubes were then vortexed briefly and incubated at room temperature for 5 minutes.

Absorbance of standards and samples were read on a Beckman Du 640 spectrophotometer at a wavelength of 595 nm. A standard curve was generated from the standards by plotting μg of protein vs. absorbance. The amount of protein in μg contained in each 30 μl sample was then calculated from the

standard curve. This value was then multiplied by 6.667 to calculate the amount of protein in μg contained in the whole mosquito. This correction was required because only 30 μl of the original 200 μl was used for the Bradford assay. The value of protein in μg was converted to calories using a conversion factor of 0.004 calories/μg (Takken *et al*. 1998).

Carbohydrate and lipid extraction

The procedures described by Van Handel (1985a, 1985b) and Van Handel and Day (1988) were used to extract and quantify the amount of carbohydrates (glycogen and sugars) and lipids in each mosquito. The remaining 170 μl of each sample was thawed at room temperature, vortexed to resuspend the pellet, and transferred to a 15 ml centrifuge tube. Samples were vortexed again following the addition of 200 μl of a 2% sodium sulfate solution. Mixing with the sodium sulfate solution dissolves all the carbohydrates in the sample (Van Handel and Day 1988). A 1:1 solution of chloroform-methanol was then added to each sample to give a total volume of 3 ml and samples were vortexed. Addition of the chloroform-methanol mixture dissolves lipids and precipitates glycogen, leaving other carbohydrates (sugars) in solution (Van Handel and Day 1988). Samples were then centrifuged at 6000 rpm for 10 minutes. The supernatant, containing lipids and sugars, was transferred to a new centrifuge tube. The pellet, containing glycogen, was set aside for analysis using the hot anthrone assay. Distilled water was used to increase the volume of the supernatant to a total of 5 ml. When the aqueous portion of the supernatant is increased, two layers form. The bottom layer contains lipids dissolved in chloroform and the

upper layer contains sugars dissolved in methanol and water (Van Handel and Day 1988). Following addition of distilled water, each sample was vortexed and then centrifuged at 3000 rpm for 10 minutes. The upper layer (sugars) was transferred to a 16 x 125 mm glass test tube. These test tubes were placed in a hot water bath at 100°C and samples were evaporated to an approximate volume of 200 μl. The rate of evaporation was increased through the use of an evaporator that passed air over each test tube. Once evaporation to 200 μl was complete the tubes were removed from the water bath and set aside for analysis using the hot anthrone assay. The bottom fraction (lipids) was transferred to a different 16 x 125 mm glass test tube. These tubes were placed in a hot water bath at 100°C and evaporated to dryness. Once evaporation was complete the samples were removed from the hot water bath and set aside for analysis using the vanillin assay.

Glycogen and sugar quantification using the hot anthrone assay

Anthrone reagent was prepared as described by Van Handel (1985a). This reagent changes colour from yellow to green when added to samples containing carbohydrates and can be used in conjunction with a standard curve to quantify the amount of sugar and glycogen in a mosquito (Van Handel 1985a). A standard curve for glucose was prepared by placing 10, 25, 50, 100, 150, and 200 μl of 1 mg/ml glucose (in 25% ethanol) into 16 x 125 mm glass test tubes. A blank was also prepared by pipetting 200 μl of 25% ethanol into a test tube. 5 ml of anthrone reagent was added to each sugar and glycogen sample and to each standard (including the blank). Glycogen samples were first transferred to 16 x

125 mm glass test tubes. Transfer of the glycogen pellet into the test tube was facilitated by first adding 1 ml of anthrone to the pellet and vortexing. Following the addition of 5 ml of anthrone, all samples and standards were vortexed and incubated in a water bath at 100°C for 17 minutes. Samples and standards were placed on ice immediately following incubation and allowed to cool to room temperature.

Absorbance of the blank, standards, and samples were read on a Beckman Du 640 spectrophotometer at a wavelength of 625 nm. A standard curve was generated from the blank and standards by plotting μg of glucose vs. absorbance. The amount of sugars or glycogen in μg contained in each sample was then calculated from the standard curve. This value was then multiplied by 1.176 to calculate the amount of sugars or glycogen contained in the whole mosquito. This correction was required because only 170 μl of the original 200 μl was used for the hot anthrone assay. The value of sugar and glycogen in μg was converted to calories using a conversion factor of 0.004 calories/μg (Takken *et al*. 1998).

Lipid quantification using the vanillin assay

Vanillin reagent was prepared as described by Van Handel (1985b). This reagent changes from colourless to pink when added to samples containing lipids and can be used in conjunction with a standard curve to quantify the amount of lipid in a mosquito (Van Handel 1985b). A standard curve for lipids was prepared by placing 10, 25, 50, 100, 150, and 200 μl of 1 mg/ml lipid (vegetable oil in

chloroform) into 16 x 125 mm glass test tubes. A blank was also prepared by pipetting 200 μl of chloroform into a test tube. The blank and standards were then evaporated to dryness in a hot water bath at 100°C. Next, 200 μl of concentrated sulphuric acid was added to each standard, blank, and sample. All tubes were then mixed by vortexing, and incubated at 100°C in a hot water bath for 10 minutes. All tubes were then cooled to room temperature, and 5 ml of vanillin reagent was added to each sample and standard (including the blank). Each tube was vortexed and incubated at room temperature for 5 minutes.

Absorbance of the blank, standards, and samples were read on a Beckman Du 640 spectrophotometer at a wavelength of 525 nm. A standard curve was generated from the blank and standards by plotting μg of lipid vs. absorbance. The amount of lipids in μg contained in each sample was then calculated from the standard curve. This value was then multiplied by 1.176 to calculate the amount of lipids contained in the whole mosquito. This correction was necessary because only 170 μl of the original 200 μl was used for the vanillin assay. The value of lipid in μg was converted to calories using a conversion factor of 0.009 calories/μg (Takken *et al*. 1998).

Statistical analyses

Winglength^3 was used as an estimate of volumetric body size as it is correlated with dry body mass (Koella and Lyimo 1996), fecundity (Hogg *et al*. 1996, Lyimo and Takken 1993) and survival (Lehmann *et al.* 2006). The caloric value of somatic energetic reserves was divided by winglength^3 to generate a

size-specific caloric content (SSCC) for comparisons between males and females, Teneral, MIA, and Adult samples, and laboratory and field samples. Adjusting caloric values for body size allows for comparison between samples while eliminating size as a confounding factor. For the comparisons listed above, the SSCC of Teneral and Adult samples was then adjusted by subtracting the average SSCC of MIA samples. A sex-specific average was calculated and used for males and females.

An initial analysis of the total caloric content, lipid, protein, sugar, and glycogen content showed that these data were not normally distributed (D'Agostino and Pearson normality test, $p<0.05$). Therefore, non-parametric tests (Mann-Whitney U test, Kruskal-Wallis modified analysis of variance, and Dunn's multiple comparison test) were used to test for differences in somatic energy reserves. Data for proportional lipid, protein, sugar, and glycogen content were transformed (arcsine transformation) prior to analysis by multiple analysis of variance (MANOVA). Analysis of winglength showed that these data were normally distributed (D'Agostino and Pearson normality test, $p>0.05$). Therefore, parametric tests (Student's t test, analysis of variance, and Tukey's honestly significant difference test) were used to test for differences in body size. The relationships between body size and somatic energy reserves were analyzed using Spearman's correlation coefficient. Statistical analyses were conducted using JMP 6.0 (SAS Institute Inc. 2005) and GraphPad Prism 4.00 (GraphPad Software Inc. 2005). Graphs were generated using GraphPad Prism 4.00.

Using Spearman's correlation coefficient, it was determined that the relationship between body size and somatic energy reserves is not isometric. Therefore, the ratio between these two variables is not constant throughout the distribution of *An. gambiae* body size. Another method of statistically accounting for body size is to use the cubic value of winglength as a covariate in analysis of variance models. This type of analysis was also conducted, however the addition of body size as a covariate did not result in a change in the significance of any factors. Therefore, body size was accounted for by dividing the caloric value of each reserve by winglength^3. This method allowed for the use of non-parametric tests and is used by other authors in the field of mosquito nutritional ecology (Briegel 2003, Fernandes and Briegel 2005, Koella and Lyimo 1996).

3.3 Results

Somatic energy reserves of a field population at emergence

Forty-nine teneral samples were collected, 24 of which were female. Fifty-four MIA samples were collected, 28 of which were female. Winglength of teneral females (mean = 3.38 mm) was significantly greater than winglength of teneral males (mean = 3.16 mm) ($t = 7.423$, $p < 0.0001$). Female winglength ranged from 3.18 to 3.60 mm, male winglength ranged from 2.94 to 3.30 mm.

When adjusted for winglength and minimum irreducible amounts to allow for comparisons independent of body size and structural components, teneral males contained significantly more somatic energy reserves in total caloric content, lipid, and protein than teneral females (Table 3-1, Figure 3-1). The

sugar and glycogen content of teneral males was also greater than females, although the difference was not statistically significant (Table 3-1, Figure 3-1).

The proportional allocation between different classes of somatic energy reserves (lipid, protein, etc.) was similar in females and males. Multiple analysis of variance showed that the distribution of calories between the 4 classes of reserves did not vary significantly between females and males ($F = 0.9803$, $p = 0.4278$). Lipids accounted for the majority of calories in females and males (64% and 67%, respectively), followed by protein (24% in females and 21% in males) (Figure 3-2). Sugar accounted for the smallest proportion of total caloric content in field-collected mosquitoes at emergence (5% in females and males), while glycogen accounted for 7% and 6% of total caloric content in females and males, respectively (Figure 3-2).

Starvation with access to water allowed for a determination of the minimum irreducible amounts, or the amount of somatic energy reserves metabolized to prevent starvation. The % mobilization of each type of somatic energy reserves was calculated by dividing the average size-specific caloric content of MIA samples by the average size-specific caloric content of teneral samples, subtracting this value from 1, then multiplying by 100. Males mobilized a greater percentage (34%) of their total caloric content than females (12%). Males mobilized 30% of teneral lipid, 48% of teneral protein, 28% of teneral sugar, and 31% of teneral glycogen. Females mobilized 7% of teneral lipid, 16% of teneral protein, 35% of teneral sugar, and 25% of teneral glycogen. Most

mosquitoes died within 2 days of emergence, and the longest survival time was 3 days.

Somatic energy reserves of adults in a field population

Five hundred and sixty-seven samples were collected, 198 of which were female (35%). Of these, 156 males and 133 females were analyzed for somatic energy reserves (lipids, protein, sugars, and glycogen). Winglength of adult females (mean = 3.24 mm) was significantly greater than winglength of adult males (mean = 3.10 mm) ($t = 6.238$, $p < 0.0001$). Female winglength ranged from 2.70 to 3.72 mm, male winglength ranged from 2.58 to 3.60 mm.

The lipid, protein, sugar, glycogen, and total caloric content of male and female adults were positively correlated with body size (Table 3-2, Figure 3-3). The correlation between each type of somatic reserve and body size was hypoallometric (slope less than 1 and greater than 0), but was only statistically significant for lipid, protein, and total caloric content in females (Table 3-2).

A preliminary analysis showed that the date of collection and the time of collection (am or pm) did not have a significant impact on the quantity of somatic energy reserves; therefore these factors were excluded from the statistical model. Sex (female or male), collection method (indoor or outdoor), and site type (lakeside, lowland, or highland) each had a significant effect on the quantity of somatic energy reserves (Tables 3-3, 3-5, 3-6, and 3-7), therefore these effects and their interaction terms were included in statistical models for further investigation.

When adjusted for winglength and minimum irreducible amounts to allow for comparisons independent of body size and structural components, the total caloric content of mosquitoes varied between those collected indoors and outdoors (Table 3-3). The total caloric content of mosquitoes collected from indoor habitats was significantly greater than mosquitoes collected from outdoor habitats. The mean size-specific total caloric content of mosquitoes collected from indoor habitats was 0.1372 (SEM = 0.00789). The mean size-specific total caloric content of mosquitoes collected from indoor habitats was 0.1020 (SEM = 0.009329). This difference is statistically significant (Mann-Whitney U = 6841, p = 0.0217). Total caloric content of field-collected adults did not vary significantly between males and females or between collection sites, although the total caloric content of females was slightly greater than that of males (Tables 3-3, 3-4, Figure 3-4A).

When adjusted for winglength and minimum irreducible amounts to allow for comparisons independent of body size and structural components, the lipid content of mosquitoes varied between females and males, and between indoor and outdoor habitats (Table 3-5). Female mosquitoes contained significantly more lipid than males (Table 3-4, Figure 3-4B). The lipid content of mosquitoes collected from indoor habitats was significantly greater than mosquitoes collected from outdoor habitats. The mean size-specific lipid content of mosquitoes collected from indoor and outdoor habitats were 0.1247 (SEM = 0.0075) and 0.0922 (SEM = 0.0085), respectively. This difference is statistically significant (Mann-Whitney U = 6957, p = 0.0346).

When adjusted for winglength and minimum irreducible amounts to allow for comparisons independent of body size and structural components, sex and collection site type had significant effects on the amount of protein in adult mosquitoes (Table 3-6). There was a significant interaction between sex and collection site type (Table 3-6) that required a separate ANOVA to determine the effects of each factor separately (Zar 1999). Site type had a significant effect on protein content in both females ($F = 0.306$, $p = 0.0481$) and males ($F = 9.2151$, $p = 0.0002$). Females collected from lowland sites contained more protein than males ($F = 8.3557$, $p = 0.0043$), as did females collected from highland sites ($F = 3.1201$, $p = 0.0337$). Females collected from lakeside sites contained less protein than males although this result was not statistically significant ($F = 1.9231$, $p = 0.1705$). The effects of sex and collection site type are graphically represented in Figure 3-5. Protein content was greatest in mosquitoes collected from highland sites and was least in mosquitoes collected from lowland sites (Figure 3-5). The mean SSCC protein content of mosquitoes collected from lakeside sites were 0.00446 (n=31) and 0.008673 (n=35) for females and males, respectively. The mean SSCC protein content of mosquitoes collected from lowland sites were 0.006894 (n=79) and 0.002199 (n=105) for females and males, respectively. The mean SSCC protein content of mosquitoes collected from highland sites were 0.01866 (n=23) and 0.006224 (n=16) for females and males, respectively. Overall, female mosquitoes contained significantly more protein than males (Table 3-4, Figure 3-4C).

When adjusted for winglength and minimum irreducible amounts to allow for comparisons independent of body size and structural components, sex had a significant effect on the amount of sugar in adult mosquitoes (Table 3-7). Male mosquitoes contained significantly more sugar than females (Table 3-4, Figure 3-4D). Glycogen content was not significantly affected by sex, collection method, or site type (Table 3-10). Males contained slightly more glycogen than females, although the difference was not statistically significant (Table 3-4, Figure 3-4E).

The proportional allocation between different classes of somatic energy reserves (lipid, protein, etc.) varied between adult females and males (MANOVA, $F = 16$, $p < 0.0001$). Subsequently, ANOVA tests were conducted to determine if each class of reserve varied significantly between the sexes (Zar 1999). The majority of calories in both sexes were derived from lipids (79% in females and 78% in males, $F = 0.8122$, $p = 0.3682$) (Figure 3-6). However, protein accounted for a greater proportion of total calories in females (13%) than in males (8%) ($F = 24.97$, $p < 0.0001$), and sugar accounted for a greater proportion of total calories in males (10%) than in females (5%) ($F = 39.34$, $p < 0.0001$) (Figure 3-6). Glycogen accounted for the smallest proportion of total caloric content in both females (4%) and males (5%) ($F = 2.97$, $p = 0.0857$) (Figure 3-6).

Accumulation of somatic energy reserves of a field population post-emergence

A comparison of the size-specific caloric content (adjusted for minimum irreducible amounts) of teneral and adult mosquitoes shows that both males and females accumulate somatic energy reserves post-emergence (Figure 3-7).

Males emerge with significantly more somatic energy reserves (mean size-specific total caloric content = 0.0643) than females (mean size-specific total caloric content = 0.0233). As described above, there is not a statistically significant difference in the total caloric content of adult males and females (Table 3-4, Figures 3-4A, 3-7).

The use of reproductive status (nulliparous or parous) as proxy for age produces 3 age categories for females: teneral, nulliparous, and parous. A comparison of the size-specific caloric content (adjusted for minimum irreducible amounts) of somatic energy reserves in these 3 categories confirms that females accumulated reserves post-emergence. There was a significant increase in the total caloric content of females post-emergence, with no difference between nulliparous and parous mosquitoes (Table 3-9, Figure 3-8A). This increase in caloric content was due primarily to an increase in lipid. The size-specific lipid content of females increased significantly post-emergence, with no difference in the lipid content of nulliparous and parous females (Table 3-9, Figure 3-8B). There was no significant change in the protein, sugar, or glycogen content post-emergence, although there was a trend (not statistically significant) for an increase in sugar content with age (Table 3-9, Figure 3-8C, D, E).

Dry season samples and comparison with rainy season samples

17 teneral samples were collected during the dry season, 13 of which were female. 11 MIA samples were collected, 7 of which were female. There was no significant difference between the winglength of teneral females and males collected in the dry season ($t = 0.9098$, $p = 0.3773$). Female winglength

ranged from 2.56 to 3.21 mm, with a mean of 2.97 mm. Male winglength ranged from 2.63 to 3.01 mm, with a mean of 2.88 mm. Mosquitoes collected in the dry season were significantly smaller than those collected in the rainy season (Figure 3-9).

When adjusted for winglength and minimum irreducible amounts to allow for comparisons independent of body size and structural components, males collected in the dry season emerge with significantly more protein compared with females (Table 3-10). Males also tended to emerge with more somatic energy reserves in total caloric content and glycogen compared to females, although this was not statistically significant (Table 3-10). Females tended to emerge with more lipid and sugar than males however, this was not statistically significant (Table 3-10).

Females collected during the dry season emerged with significantly more somatic energy reserves in total caloric content, lipid, protein, and sugar than females collected during the rainy season (Figure 3-10A to 3-10D). There was no difference in the glycogen content of females collected during the dry and rainy seasons (Figure 3-10E). Males collected during the dry season also emerged with more somatic energy reserves than those collected during the rainy season. The total caloric content and protein content of dry season males was significantly greater than rainy season males (Figures 3-11A, 3-11C). Males collected in the dry season tended to emerge with more lipid and glycogen than males collected in the rainy season, although this was not statistically significant

(Figures 3-11B, 3-11E). There was no difference in the sugar content of males collected in the dry and rainy seasons (Figure 3-11D).

The proportional allocation between different classes of somatic energy reserves (lipid, protein, etc.) was very similar in teneral females and males (Figure 3-12). Multiple analysis of variance showed that the distribution of calories between the 4 classes of reserves did not vary significantly between females and males ($F = 1.2$, $p = 0.3544$). The majority of calories in both males and females were derived from lipids (85% and 83%, respectively), while glycogen accounted for the smallest proportion of total caloric content (1% in females and 3% in males) (Figure 3-12). Protein accounted for 11% and 12% of the total caloric content of females and males, respectively, while sugar accounted for 3% and 2% of the total caloric content of females and males.

The proportional allocation between different classes of somatic energy reserves (lipid, protein, etc.) varied between mosquitoes collected in the dry and rainy seasons (MANOVA, $F = 22.1$, $p < 0.0001$). Subsequently, ANOVA tests were conducted to determine if each class of reserve varied significantly between the seasons (Zar 1999). Mosquitoes collected in the dry season contained a greater proportion of lipids than those collected in the rainy season (85% and 66% respectively, $F = 80.39$, $p < 0.0001$). Dry season samples contained a smaller proportion of protein than those collected in the rainy season (11% and 23% respectively, $F = 44.27$, $p < 0.0001$). Mosquitoes collected in the dry season also contained a smaller proportion of sugar (3% and 5% respectively, F

= 4.34, p = 0.0411) and glycogen (2% and 7% respectively, F = 11.15, p = 0.0014) than those collected in the rainy season.

Starvation with access to water allowed for determination of the minimum irreducible amounts, as described above for dry season samples. Males and females mobilized approximately the same amount of their total caloric content (24% and 22%, respectively). Males mobilized 14% of teneral lipid, 81% of teneral protein, 40% of teneral sugar, and 70% of teneral glycogen. Females mobilized 20% of teneral lipid, 32% of teneral protein, 38% of teneral sugar, and 56% of teneral glycogen.

Somatic energy reserves of a laboratory colony at emergence

Winglength of teneral mosquitoes was affected by sex and larval density (Table 3-11). Winglength of females (mean = 3.09 mm) was greater than winglength of males (mean = 2.80 mm), and winglength of mosquitoes reared at low larval densities (mean = 3.06 mm) was greater than winglength of mosquitoes reared at high larval densities (mean = 2.83 mm) (Figure 3-13).

When adjusted for winglength and minimum irreducible amounts to allow for comparisons independent of body size and structural components, there were no statistically significant differences in the somatic energy reserves of laboratory-reared females and males (Table 3-12). The total caloric content and lipid content of laboratory-reared females at emergence was greater than laboratory-reared males, although these differences were not statistically significant (Table 3-12). This pattern is the opposite of that seen for field-

collected mosquitoes (Figure 3-14). The sugar content of laboratory-reared males was greater than females, although not significantly different (Figure 3-14). The protein and glycogen content of teneral laboratory-reared males and females was similar (Figure 3-14).

The proportional allocation between different classes of somatic energy reserves (lipid, protein, etc.) was similar in teneral laboratory-reared females and males (Figure 3-15). Multiple analysis of variance showed that the distribution of calories between the 4 classes of reserves did not vary significantly between females and males ($F = 1.63$, $p = 0.1804$). The majority of calories in both sexes were derived from lipids (71% in females and 72% in males), while glycogen accounted for the smallest proportion of total caloric content (8% in females and 7% in males) (Figure 3-15). Thirteen percent of the total caloric content of females was derived from protein, 11% of total caloric content of males was derived from protein. Sugar accounted for 8% and 10% of the total caloric content of females and males, respectively (Figure 3-15).

Starvation with access to water allowed for determination of the minimum irreducible amounts, as described about for field-collected samples. Laboratory-reared males and females mobilized approximately the same amount of their total caloric content (21% and 22%, respectively). Males mobilized 9% of teneral lipid, 44% of teneral protein, 76% of teneral sugar, and 55% of teneral glycogen. Females mobilized 16% of teneral lipid, 16% of teneral protein, 65% of teneral sugar, and 58% of teneral glycogen. Most mosquitoes died within 3 days and the longest survival time was 4 days.

Comparison of laboratory-reared and field-collected mosquitoes

Laboratory-reared mosquitoes emerged with more somatic energy reserves than field-collected mosquitoes (Figure 3-14). Laboratory-reared females emerged with significantly more somatic energy reserves in total caloric content, lipid, sugar, and glycogen than field-collected females (Figure 3-14). Laboratory-reared females also emerged with more protein than field-collected mosquitoes although the difference was not statistically significant (Figure 3-14). Laboratory-reared males tended to emerge with more somatic energy reserves in total caloric content, lipid, sugar, and glycogen than field-collected males, although this trend was not statistically significant (Figure 3-14).

The proportional allocation between different classes of somatic energy reserves (lipid, protein, etc.) varied between laboratory-reared and field-collected mosquitoes (MANOVA, $F = 28$, $p < 0.0001$). Subsequently, ANOVA tests were conducted to determine if each class of reserve varied significantly lab and field collections (Zar 1999). Laboratory-reared mosquitoes emerged with proportionally more lipid than field-collected mosquitoes (72% and 66% respectively, $F = 6.43$, $p = 0.0127$). Laboratory-reared mosquitoes emerged with proportionally more sugar than field-collected mosquitoes (9% and 5% respectively, $F = 22.68$, $p < 0.0001$). In contrast, laboratory-reared mosquitoes emerged with proportionally less protein than field-collected mosquitoes (12% and 23% respectively, $F = 74.57$, $p < 0.0001$). There was no significant difference in proportional allocation of glycogen between laboratory-reared and field-collected mosquitoes (7% and 7%, $F = 0.4716$, $p = 0.4938$).

3.4 Discussion

Physiological status in the period immediately following emergence is critically important for both male and female *An. gambiae* (Fernandes and Briegel 2005, Chambers and Klowden 2001). Teneral reserves provide the energy required for initial flights and can impact adult survival and female feeding behaviour (Takken *et al*. 1998). The results of this study are consistent with works by other researchers (Briegel 1990, Briegel 2003, Lyimo and Takken 1993) in showing that compared to other mosquito species, *An. gambiae* emerge in physiologically poor condition with few somatic energy reserves carried over from the larval stage. Compared to *Ae. aegypti* anophelines emerge with relatively fewer somatic energy reserves (Briegel 1990, Briegel 2003). This tendency to emerge in poor condition may explain the propensity of anophelines to blood feed very soon after emergence and to take multiple blood meals in their 1st gonotrophic cycle (Briegel 2003).

To my knowledge, this is the first study that directly compares the somatic energy reserves of males and females in a field population of *An. gambiae*. Results of this investigation show that field-collected males emerge in better relative condition than females, in both the rainy and dry seasons (Figure 3-1, Table 3-10). This may reflect differences in larval diet, larval growth strategies, or conservation of reserves during pupation and metamorphosis. Males may be more efficient feeders as larvae, or conserve a greater proportion of their larval reserves throughout metamorphosis. To my knowledge, these hypotheses have not been tested. Because mating occurs in swarms and requires substantial

carbohydrate reserves (Yuval *et al.* 2004, Yuval 2006), males that emerge with greater energy reserves may enjoy a higher mating success. This relationship between energy reserves and overall mating success has not been measured, although it has been shown that the ability of males to initiate swarming behaviour increases as carbohydrate reserves increase (Yuval *et al.* 1994). It is also possible that there is a greater need for males to acquire lipid and protein in the larval stage, since these nutrients are not as readily available in the diet of adult males. Females acquire protein directly from blood meals, and are able to convert blood meals into somatic protein and lipid reserves (Briegel 1990). Males feed only on plant juices, primarily sugars found in nectar (Foster 1995). To accumulate lipid and protein post-emergence, males would be required to convert carbohydrates acquired through sugar feeding into lipids or protein via costly metabolic pathways. Males also may acquire lipid and protein directly through feeding on extra-floral nectaries, which typically have high lipid and protein content (Wäckers 2005). Both sexes will feed on floral and extra-floral nectaries in a laboratory setting, resulting in an increase in longevity (Gary and Foster 2004). However, neither the preference for floral vs. extra-floral nectaries, nor the efficiency of conversion between reserve types (lipid, sugar, protein, and glycogen) have been assessed in *An. gambiae*.

This pattern of sexual dimorphism in physiological condition is seen in many other species, especially in those with polygynous mating systems or where female mate choice plays an important role (Krebs and Davies 1993). For example, large-bodied male seaweed flies enjoy greater mating success than

small males due to female preference for large males, where body size was used as a proxy for physiological condition (Crean *et al.* 2000). Sexual dimorphism is also common among Aves, where male birds tend to mature in better condition than females (Dunn *et al*. 2001). Sexual dimorphism in body size is especially pronounced in avian species that are polygynous or mate in leks (Dunn *et al.* 2001, Oakes 1992). In these types of mating systems, large males may gain a fitness advantage through female preference for large males or through a competitive advantage over smaller competitors (Oakes 1992). Since relatively little is known about the mating behaviour of *An. gambiae*, it is not certain what the role of female choice or polygyny plays in the sexual dimorphism seen in this study. There is some evidence that male anophelines compete for mates, a small proportion of males mate multiply and are responsible for the majority of the matings while some males never mate (Yuval 2006). However, there is also evidence that some females mate multiply (Tripet *et al.* 2003). Regardless, nutritional status and somatic energy reserve levels are likely to be key components of the reproductive success of male *An. gambiae*. Since swarming is a metabolically costly activity, a male must increase its somatic energy reserves in order to increase the time spent swarming and thereby increasing its probability of mating success (Yuval *et al*. 1994).

As well as varying with gender, the physiological status of mosquitoes at emergence varied between the rainy and dry seasons. Mosquitoes emerging in the dry season tended to be smaller, but contained greater amounts of reserves per unit of body size than those collected in the rainy season (Figures 3-9, 3-10,

3-11). *Anopheles gambiae* may emerge smaller during the dry season due to a reduction in the number and size of larval habitats. A reduction in the number and size of larval habitats will increase the density of larvae in each habitat. Increased density has been shown to decrease body size at emergence (Figure 3-13, Lyimo *et al*. 1992), decrease larval survival, and increase intra-specific predation (Koenraadt *et al*. 2004). The combination of these factors may explain the seemingly better condition of teneral *An. gambiae* in the dry season. Relatively few larval habitats exist in the dry season and while average body size and survival may be reduced due to high larval densities, increased competition and cannibalism may increase the amount of nutrients available to surviving larvae.

The results of this study also show that *An. gambiae* males and females are able to utilize a proportion of their teneral reserves to prevent starvation when food is unavailable. Mobilization of teneral reserves allowed field-collected mosquitoes to survive 1-2 days without food, and laboratory reared mosquitoes survived slightly longer, approximately 2-3 days. The longer survival time in laboratory-reared mosquitoes likely reflects their greater somatic energy reserve content per unit of body size. The results of this study are consistent with the results of previous work showing that *An. gambiae* mobilizes teneral reserves under starvation conditions (Briegel 1990). The ability of *An. gambiae* to mobilize a proportion of teneral reserves may be an adaptation to poor physiological condition at emergence. According to the results presented here, *An. gambiae* have a window of approximately 2 days following emergence to acquire food

before starving to death. Field-collected males mobilized a greater proportion of their teneral reserves compared to females. Additionally, field-collected males utilized lipid, protein, sugar, and glycogen approximately equally, while females utilized substantially more sugar and glycogen than lipid and protein. This likely reflects the existence of critical values for lipid and protein content for egg production (Briegel 2003). Females may be unwilling to utilize this reproductively critical portion of their lipid and protein reserves to prevent starvation, while males do not have this restriction.

The somatic energy reserves of field-collected *An. gambiae* adults increase hypo-allometrically with body size (Table 3-2, Figure 3-3). The correlation between body size and amount reserves was statistically significant for total calories, lipid, and protein in females, and for total calories and lipid in males (Table 3-2). A hypo-allometric relationship between body size and reserves is consistent with the relative-efficiency hypothesis described by Blanckenhorn *et al*. (2007), and is common in insect species (Glazier 2005). This hypothesis suggests that larger individuals have a more efficient metabolism than smaller individuals because size-specific metabolic rate decreases with an increase in body size (Blanckenhorn *et al.* 2007). In other words, the hypo-allometric relationship between body size and energy reserves is a reflection of a hypo-allometric relationship between body size and metabolic rate. If larger mosquitoes have a more efficient metabolism compared to smaller conspecifics, large mosquitoes may have a metabolic advantage. For example, a large mosquito would be able to survive longer on each unit of size-specific energy

reserves. This possibility could be confirmed experimentally by checking for a correlation between body size and starvation resistance.

As for teneral samples, there was sexual dimorphism in the somatic energy reserves of field-collected adult *An. gambiae*. Although there was no difference in the total caloric content of males and females, females contained significantly more lipid and protein than males while males contained significantly more sugar (Table 3-4, Figure 3-4). Lipid also constituted a greater proportion of total calories in females compared to males, and sugar constituted a greater proportion of total calories in males. This may reflect differences in the adult diet of males and females and suggests that females favour lipogenesis over gluconeogenesis, while the reverse is true for adult males. Lipogenesis is the conversion of ingested carbohydrates and some amino acids into lipids via conversion into acetyl-CoA; gluconeogenesis is the conversion of ingested amino acids into carbohydrates via conversion to pyruvate (Clements 1995). Females acquire protein and lipid directly from blood meals (Briegel 1990), and synthesize somatic lipid and protein stores prior to oocyte development (Briegel 1990, Fernandes and Briegel 2005). This tendency for *An. gambiae* females to synthesize somatic lipid reserves may compensate for small blood meals and the relatively low efficiency at which they convert blood meals into eggs (Fernandes and Briegel 2005). It may be more efficient for *An. gambiae* to convert somatic reserves into yolk lipids and proteins rather than drawing them directly from a blood meal, although to my knowledge this has not been tested. Human blood (the preferred host of *An. gambiae*) also has a relatively low isoleucine content

compared to other mammals, and this may limit the efficiency of using blood meal protein directly towards egg production (Briegel 1990).

Compared with females, relatively little is known about the feeding habits of adult males (Gary and Foster 2006). It is thought that male *An. gambiae* feed exclusively on plant nectar as adults (Clements 1995, Foster 1995, Gary and Foster 2006). Although males are highly attracted to plant odours (Gary and Foster 2004) and will feed on floral and extra-floral nectaries in semi-field conditions (Gary and Foster 2004, Manda *et al*. 2007) the feeding preferences of males in a natural environment are unknown (Gary and Foster 2006). Males likely acquire sugar directly from plant nectar, accounting for their greater sugar content compared to females. It is also possible that males can acquire some lipid and protein directly from extra-floral nectaries (Wäckers 2005), as well as through conversion of sugar via catabolic pathways. Males are also likely to favour gluconeogenic pathways over lipogenic pathways due to the importance of flight for male mating success. As described above, mating in swarms requires sustained flight. Swarming males have been shown to use a large proportion of their sugar and glycogen reserves, while lipid reserves were not significantly affected (Yuval *et al*. 1994).

The results of this study indicate that the somatic energy reserves of *An. gambiae* also vary with environmental factors such as habitat, in addition to varying with physical parameters such as body size and sex (Table 3-13). Mosquitoes collected from indoor habitats contained significantly greater amounts of somatic energy reserves in total caloric content and lipid content. For

female mosquitoes, this likely reflects the increased proximity to and availability of human hosts in indoor habitats. The link between increased lipid content and indoor habitats is less clear for male mosquitoes. It is possible that vegetation on which males feed in the immediate vicinity of the huts has greater lipid content than the vegetation in the vicinity of the outdoor collection sites. This possibility could be investigated by determining which species of plants males feed on, characterizing their nutritional content, and testing for variation in feeding preferences or plant species composition with proximity to human habitations. It is also important to keep in mind that the outdoor habitats used in this study were introduced to the environment for the purposes of this investigation, and that only one type of outdoor habitat was sampled. Therefore, one cannot eliminate the possibility that there was a bias in the samples collected from these outdoor habitats towards mosquitoes that have a preference for this particular type of habitat.

There was an interesting interaction between gender and collection site on the protein content of field-collected mosquitoes. Overall, mosquitoes collected from highland sites had the highest protein content, followed by lakeside then lowland sites (Figure 3-5). Females contained more protein than males, except at lakeside sites where the protein content of males was greater (Figure 3-5). Similar to the effect of indoor and outdoor habitats on lipid content, this pattern likely reflects variation in the types and amounts of food sources at different collection sites. Interestingly, the availability of plants was lowest at lakeside sites, where male protein content exceeded that of females. It is possible that

there is a plant species at lakeside sites not identified in this study that has high nectary protein content. An increased dependence of males on plant feeding compared to females may then explain the higher protein content of males at lakeside sites. However, a detailed survey of the number and diversity of plant species would be required to definitively determine if there is a correlation between vegetation and protein content at the 3 different collection sites included in this investigation.

A comparison of teneral and adult samples shows that both males and females increase their somatic energy reserves post-emergence (Figure 3-7). This result implies that both males and females feed following emergence and that the nutrients acquired are used to synthesize somatic energy reserves. Post-emergence feeding negates the difference in total caloric content between males and females (males emerge with a greater total caloric content than females), although adult females contain more lipid and protein than adult males (Table 3-4, Figure 3-4B, C).

The determination of female reproductive status produced 3 age categories for female mosquitoes in this study, resulting in a finer scale for the examination of accumulation of somatic energy reserves with age. A comparison of teneral, nulliparous, and parous females shows that females increase their total caloric content and lipid content significantly post-emergence, with a trend towards an increase in sugar content (Table 3-9, Figure 3-8). As described above, an increase in somatic energy reserves in females could result from the conversion of nutrients found in a blood meal into somatic reserves, primarily

lipid reserves (Briegel 1990, Fernandes and Briegel 2005). The increase in sugar content of adult females may also be an indication of plant feeding in this species. Female *An. gambiae* have rarely been observed feeding on plant materials in the field (Gary and Foster 2004), however females will feed readily on sugars in the laboratory and will feed on floral and extra-floral nectaries under laboratory and semi-field conditions (Gary and Foster 2004, Manda *et al*. 2007). Sugar feeding can increase female survival (Chapter 2, Straif and Beier 1996, Okech *et al*. 2003), physiological condition (Briegel 1990), and can increase fecundity (Chapter 2, Fernandes and Briegel 2005). The study design used in this investigation does not allow for a determination of the origin of any reserves acquired post-emergence. Therefore, it is not possible to determine if the increase in somatic reserves seen in field-collected females is due to sugar feeding or blood feeding. Given the ability of females to feed on either material, it is likely a combination of sugar and blood that results in an increase in somatic energy reserves. *Anopheles gambiae* females are opportunistic feeders and take advantage of any food source available (Fernandes and Briegel 2005), especially given their poor nutritional status at emergence.

Adult females contain more somatic energy reserves than teneral females, but there is no difference in the reserves of nulliparous and parous females (Figure 3-8). These data suggest that female *An. gambiae* likely take a meal (sugar or blood) very soon after emergence. Since females that have not yet produced any eggs but have increased their somatic energy reserves, we can infer that *An. gambiae* females synthesize somatic reserves, primarily lipids, prior

to devoting nutrients towards reproduction. This is consistent with the results of many other studies that indicate *An. gambiae* often requires multiple blood meals before producing eggs, and that initial meals are used to increase somatic reserves (Briegel 1990, Fernandes and Briegel 2005). The propensity to take multiple blood meals and to start feeding soon after emergence increases the vectorial capacity of this species (Chapter 2, Briegel 2003). It should be noted that there might be a bias in the adult samples towards mosquitoes in relatively good nutritional condition. Mosquitoes emerging in very poor condition may not survive long enough to reach one of the collection sites included in this study. Therefore, the adult samples likely include only those mosquitoes who emerged in good condition, or who found a food source rapidly.

The third goal of this study was to compare the somatic energy reserve status of a laboratory colony at emergence to the field-collected samples. There is growing interest in such comparisons between laboratory-reared and field-collected *An. gambiae*, as part of assessing the feasibility of genetic modification as part of vector control strategies. Laboratory colonies are generally maintained under optimal conditions, with little variation in conditions over time and where food and mates are virtually unlimited. Under such conditions, it would generally be expected that laboratory-reared mosquitoes would enjoy greater nutritional health than wild populations. The results of this investigation support this hypothesis as laboratory-reared mosquitoes emerged in better condition than field-collected mosquitoes (Figure 3-14). This result has important implications for the extrapolation of laboratory experiments to wild populations. In this case

the nutritional health, a key indicator of fitness, of *An. gambiae* was significantly greater in laboratory-reared mosquitoes. If laboratory colonies are to be used to approximate wild populations, this may result in an underestimation of the effects of a particular stress on *An. gambiae* fitness, since laboratory-reared mosquitoes have more somatic energy reserves to buffer the effects of stress. The greater starvation resistance in laboratory-reared mosquitoes also supports this possibility.

A recent study by Huho *et al*. (2007) examined the differences between laboratory-reared and field-collected male *An. gambiae*. In contrast to the results presented here, the Huho study concluded that field-collected males contained more somatic energy reserves than laboratory-reared males. Direct comparisons between these 2 studies are difficult due to differences in data analyses. Protein was not assayed in the Huho study, although in this investigation it accounted for approximately 12% and 21% of the caloric content in lab-reared and field-collected males, respectively. Additionally, somatic energy reserves in my study are adjusted for winglength to produce size-specific values. This treatment is valid due to the positive correlation between winglength and reserves, and allows for the comparison of reserve status between groups independent of size (Briegel 2003). This adjustment based on body size was not done in the Huho study, although field-collected males were significantly larger than laboratory-reared males (Huho *et al*. 2007).

Including both sexes in this study allows for detection of sexual dimorphism in somatic energy reserves. Interestingly, the pattern of sexual

dimorphism in somatic energy reserves at emergence was different from the pattern seen in field-collected samples. Laboratory-reared females emerge with more reserves than males, although this was only a trend and not statistically significant (Figure 3-14). Briegel's 1990 study similarly found evidence of sexual dimorphism in teneral laboratory-reared *An. gambiae*, with females containing slightly more reserves than males. In comparison, field-collected males emerge with more reserves than females (Figures 3-1, 3-14). This may be a reflection of the high quality diet provided in the lab, or the release from some of the selective pressures experienced by males in wild populations. Sugar is readily available to adults, and swarming may be less energetically costly for laboratory-reared males due to the proximity of females and relatively small size of cages.

Somatic energy reserve status had been shown to impact physiological processes such as oogenesis (Briegel 1990) as well as behaviours such as feeding preferences in females (Foster and Takken 2004) and swarming in males (Yuval *et al*. 1994). The results of this study indicate that the somatic energy reserves of *An. gambiae* vary with physical and physiological parameters such as body size, sex, age, and reproductive status, as well as with environmental factors such as habitat and season (Table 3-13). Based on this, it is likely that the nutritional status of *An. gambiae* also varies within its habitat range, which includes a large proportion of sub-Saharan Africa (della Torre *et al*. 2005). This investigation highlights the need for more study of the nutritional ecology of *An. gambiae*. Further details on how diet influences energy reserve levels may help elucidate the relative importance of larval vs. adult diet, and sugar feeding vs.

blood feeding in females. The results presented here also confirm that difficulties may arise when using laboratory-reared mosquitoes to draw inferences about field populations.

3.5 Literature cited

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Table 3-1 **Mean size-specific caloric content (SSCC) of somatic energy reserves in teneral field-collected *An. gambiae*. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). * indicates a statistically significant difference between somatic energy reserves of females and males (Mann-Whitney test, $\alpha = 0.05$).**

Reserve type	n	Mean SSCC ± SE	Mann-Whitney U	p-value
Total calories				
Female	24	0.0233 ± 0.0058	121	0.0004*
Male	25	0.0643 ± 0.0096		
Lipid				
Female	24	0.0133 ± 0.0044	140	0.0012*
Male	25	0.0396 ± 0.0074		
Protein				
Female	24	0.0073 ± 0.0015	117	0.0003*
Male	25	0.0189 ± 0.0027		
Sugar				
Female	24	0.00377 ± 0.0008	294	0.9020
Male	25	0.0049 ± 0.0012		
Glycogen				
Female	24	0.0057 ± 0.0016	298	0.9749
Male	25	0.0070 ± 0.0019		

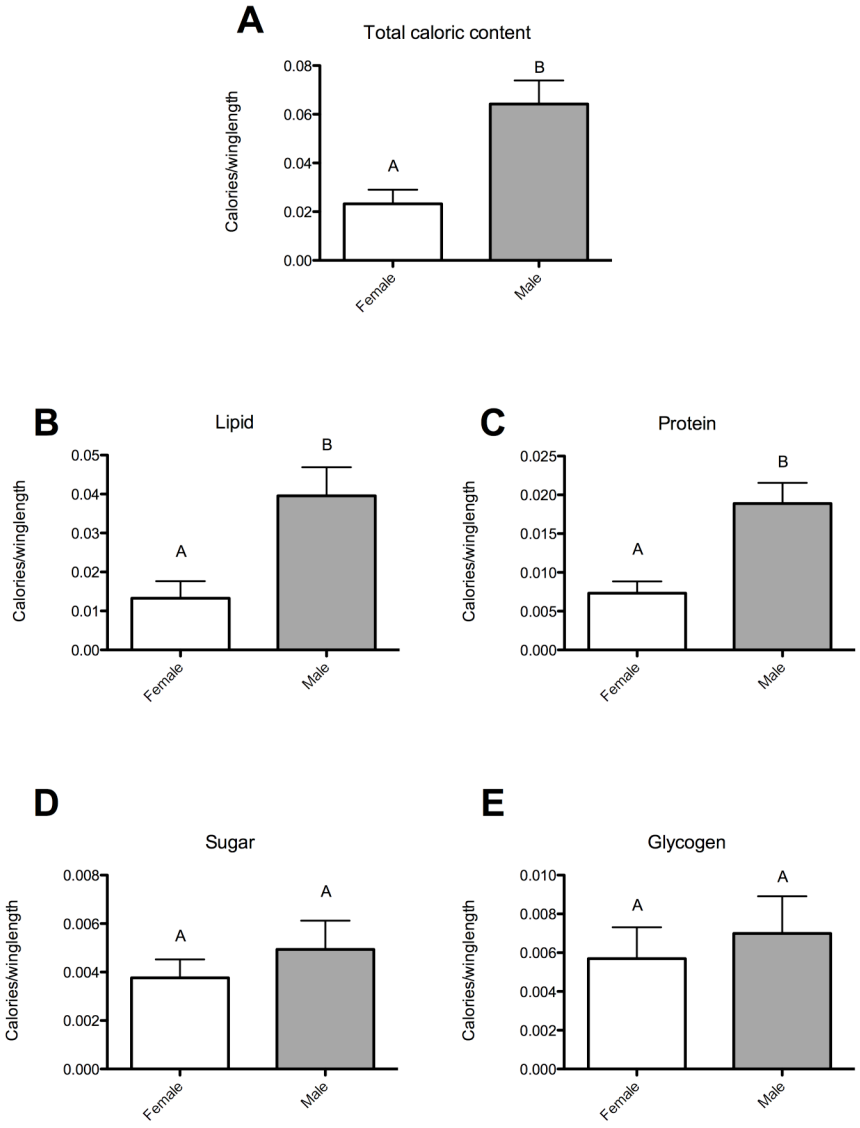

Figure 3-1 Size-specific caloric content (SSCC) of somatic energy reserves in teneral field-collected *An. gambiae*. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). Columns with different letters are significantly different (Mann-Whitney test, $\alpha = 0.05$).

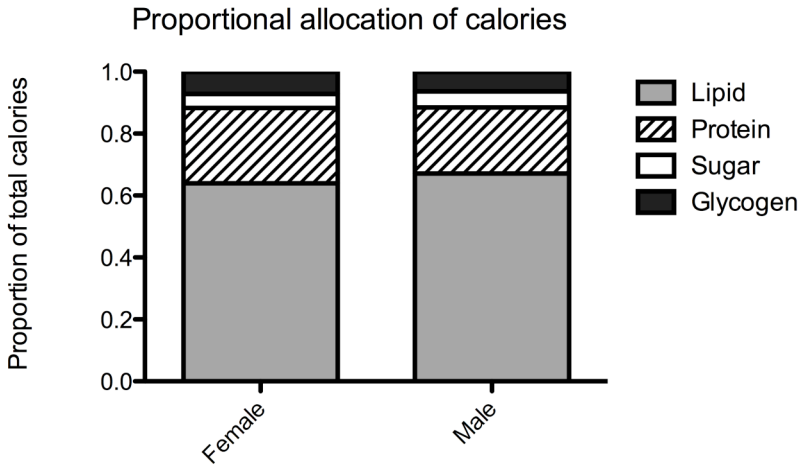

Figure 3-2 Proportional allocation of different classes of somatic energy reserves in teneral field-collected *An. gambiae*. * indicates a statistically significant effect of gender on proportional allocation of reserves (ANOVA, $\alpha = 0.05$).

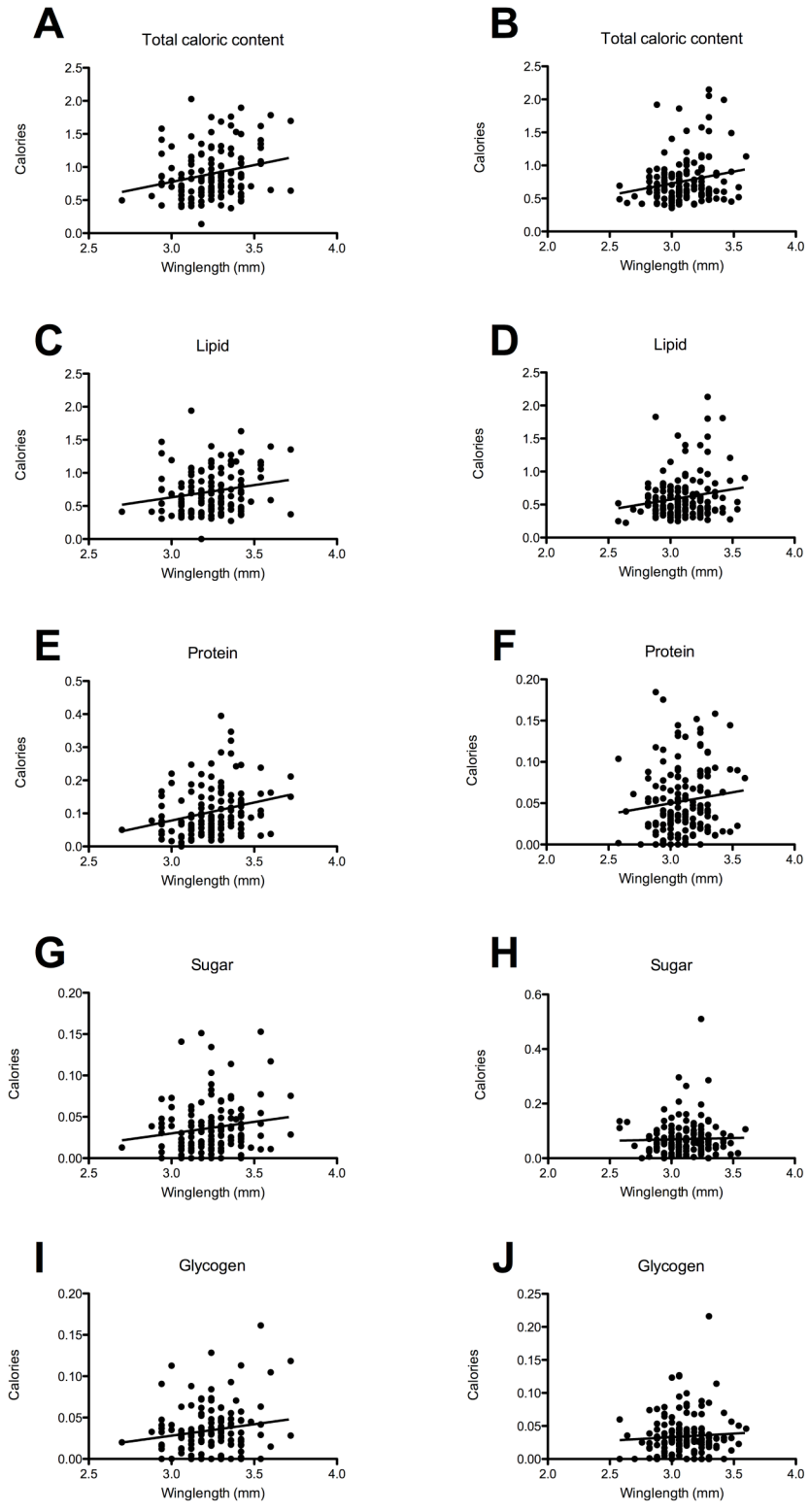

Figure 3-3 Relationship between body size and somatic energy reserves in field-collected *An. gambiae* females (A, C, E, G, I) and males (B, D, F, H, J).

Table 3-2 Correlation between adult mosquito body size and somatic energy reserves in a field population. * indicates significant correlation ($\alpha = 0.05$).

Reserve type	Slope	r^2	Spearman correlation coefficient (r)	p-value
Female				
Total calories	0.5100	0.0590	0.2178	0.0118*
Lipid	0.3704	0.0400	0.2001	0.0209*
Protein	0.1088	0.0676	0.3047	0.0004*
Sugar	0.0280	0.0256	0.1604	0.0661
Glycogen	0.0277	0.0281	0.1051	0.2287
Male				
Total calories	0.3588	0.0393	0.1343	0.0947
Lipid	0.3113	0.0312	0.1128	0.1608
Protein	0.0265	0.0153	0.1427	0.0756
Sugar	0.0103	0.0009	0.0273	0.7335
Glycogen	0.0107	0.0044	0.0398	0.6221

Table 3-3 Effect of sex, collection method (indoor or outdoor), and site type (lakeside, lowland, or highland) on the total size-specific caloric content (SSCC) of field-collected *An. gambiae* adults. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). * indicates a statistically significant effect (Kruskal-Wallis ANOVA, $\alpha = 0.05$).

Total caloric content (SSCC)			
Model effect	Sum of squares	F ratio	p-value
Sex	0.0296	2.6446	0.1050
Collection method	0.0639	5.7059	0.0176*
Site type	0.0079	0.3518	0.7037
Sex*collection method	0.00003	0.0031	0.9556
Sex*site type	0.0454	2.0296	0.1333
Collection method*site type	0.0225	1.0045	0.3676
Sex*collection method*site type	0.0139	0.6194	0.5390

Table 3-4 **Mean size-specific caloric content (SSCC) of somatic energy reserves in field-collected *An. gambiae* adults. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). * indicates a statistically significant difference between somatic energy reserves of females and males (Mann-Whitney test, $\alpha = 0.05$).**

Reserve type	n	Mean SSCC ± SE	Mann-Whitney U	p-value
Total calories				
Female	133	0.1340 ± 0.00940	9788	0.4079
Male	156	0.1222 ± 0.008504		
Lipid				
Female	133	0.1254 ± 0.008542	8965	0.0466*
Male	156	0.1077 ± 0.008234		
Protein				
Female	133	0.008397 ± 0.001383	8976	0.0478*
Male	156	0.004064 ± 0.0006499		
Sugar				
Female	133	0.007874 ± 0.001149	7084	<0.0001*
Male	156	0.01581 ± 0.001552		
Glycogen				
Female	133	0.003941 ± 0.0005960	9857	0.4437
Male	156	0.004543 ± 0.0006523		

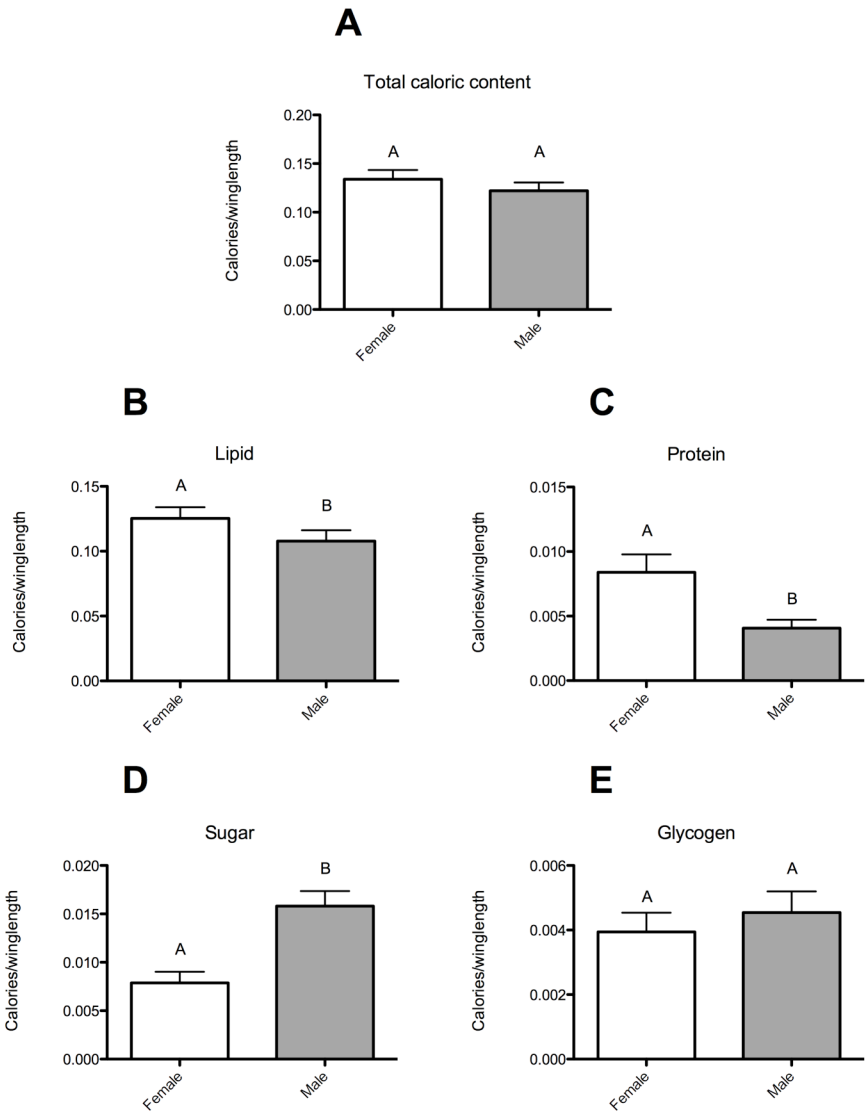

Figure 3-4 Size-specific caloric content (SSCC) of somatic energy reserves in field-collected *An. gambiae* adults. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). Columns with different letters are significantly different (Mann-Whitney, $\alpha = 0.05$).

Table 3-5 **Effect of sex, collection method (indoor or outdoor), and site type (lakeside, lowland, or highland) on the size-specific caloric content (SSCC) of lipid in field-collected *An. gambiae* adults. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). * indicates a statistically significant effect (Kruskal-Wallis ANOVA, $\alpha = 0.05$).**

Lipid (SSCC)			
Model effect	Sum of squares	F ratio	p-value
Sex	0.0420	3.8805	0.0499*
Collection method	0.0639	5.7059	0.0176*
Site type	0.0079	0.3518	0.7037
Sex*collection method	0.00003	0.0031	0.9556
Sex*site type	0.0454	2.0296	0.1333
Collection method*site type	0.0225	1.0045	0.3368
Sex*collection method*site type	0.0139	0.6194	0.5390

Table 3-6 **Effect of sex, collection method (indoor or outdoor), and site type (lakeside, lowland, or highland) on the size-specific caloric content (SSCC) of protein in field-collected *An. gambiae* adults. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSSC). * indicates a statistically significant effect (Kruskal-Wallis ANOVA, $\alpha = 0.05$).**

Protein (SSCC)			
Model effect	Sum of squares	F ratio	p-value
Sex	0.0009	6.5182	0.0112*
Collection method	0.00001	0.0972	0.7554
Site type	0.0009	3.2489	0.0402*
Sex*collection method	0.00008	0.5590	0.4553
Sex*site type	0.0011	4.1321	0.0170*
Collection method*site type	0.0004	1.5653	0.2109
Sex*collection method*site type	0.0005	1.8674	0.1565

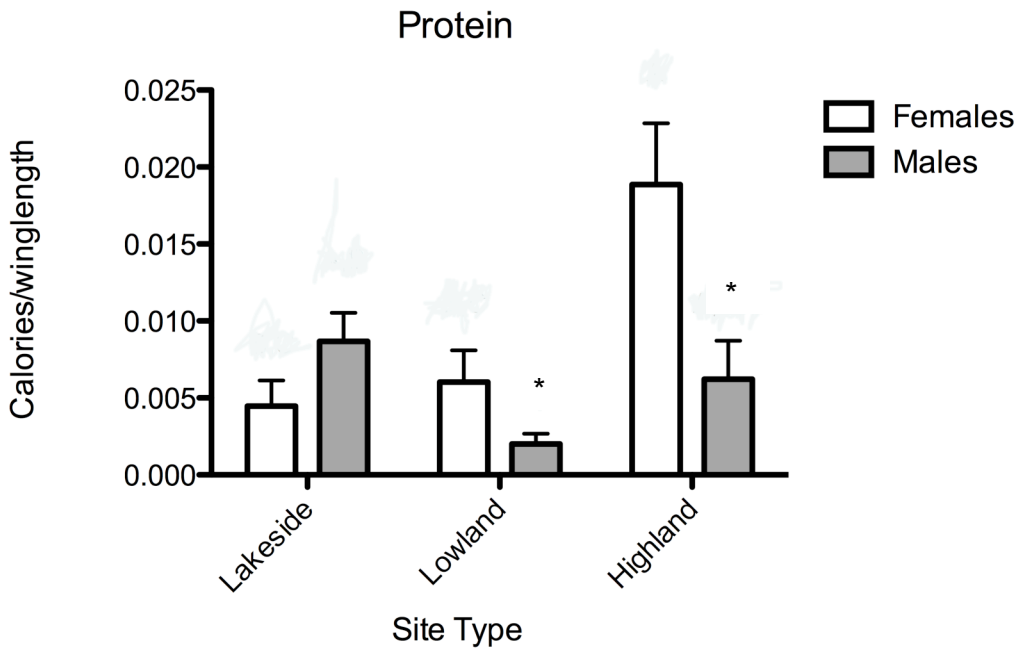

Figure 3-5 **Protein content (SSCC) in *An. gambiae* adults collected from lakeside, lowland, and highland sites. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). * indicates a significant difference between males and females for a particular collection site type (Kruskal-Wallis ANOVA, $\alpha = 0.05$).**

Table 3-7 **Effect of sex, collection method (indoor or outdoor), and site type (lakeside, lowland, or highland) on the size-specific caloric content (SSCC) of sugar in field-collected *An. gambiae* adults. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). * indicates a statistically significant effect (Kruskal-Wallis ANOVA, $\alpha = 0.05$).**

Sugar (SSCC)			
Model effect	Sum of squares	F ratio	p-value
Sex	0.0018	6.2757	0.0128*
Collection method	0.000002	0.0058	0.9392
Site type	0.0003	0.4548	0.6350
Sex*collection method	0.00003	0.1229	0.7262
Sex*site type	0.00009	0.1663	0.8468
Collection method*site type	0.0016	2.9131	0.0560
Sex*collection method*site type	0.0002	0.3750	0.6876

Table 3-8 Effect of sex, collection method (indoor or outdoor), and site type (lakeside, lowland, or highland) on the size-specific caloric content (SSCC) of glycogen in field-collected *An. gambiae* adults. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). * indicates a statistically significant effect (Kruskal-Wallis ANOVA, $\alpha = 0.05$).

Glycogen (SSCC)			
Model effect	Sum of squares	F ratio	p-value
Sex	0.00003	0.5567	0.4562
Collection method	0.00003	0.6878	0.4076
Site type	0.00002	0.1793	0.8360
Sex*collection method	0.00003	0.6122	0.4346
Sex*site type	0.0001	0.9095	0.4039
Collection method*site type	0.0002	0.3120	0.0889
Sex*collection method*site type	0.0001	0.9489	0.3884

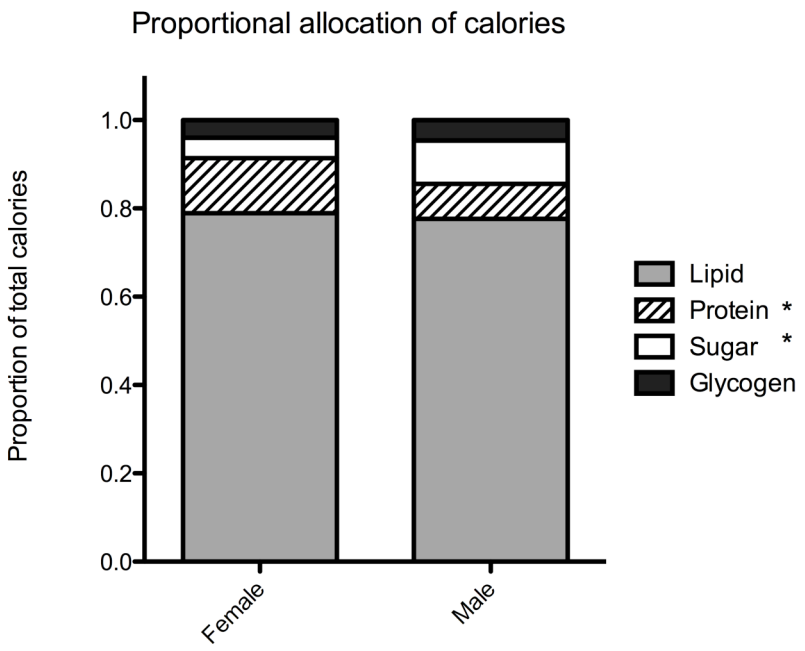

Figure 3-6 Proportional allocation of different classes of somatic energy reserves in field-collected *An. gambiae* adults. * indicates a statistically significant effect of gender on proportional allocation of reserves (ANOVA test, $\alpha = 0.05$).

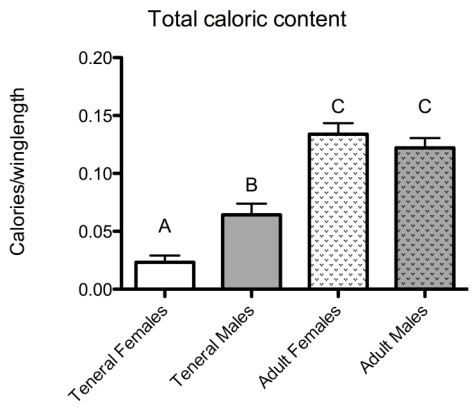

Figure 3-7 Accumulation of somatic energy reserves post-emergence in field-collected *An. gambiae*. Means with different letters are significantly different (Dunn's multiple comparison, $\alpha = 0.05$).

Table 3-9 **Mean size-specific caloric content (SSCC) of different types of somatic energy reserves in 3 different age classes of field-collected *An. gambiae* females. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). Means with different letters are significantly different (Dunn's multiple comparison, $\alpha = 0.05$).**

Reserve type and age class	n	Mean (SSCC) ± SE
Total calories		
Teneral	24	0.0233 ± 0.0058 (A)
Nulliparous	46	0.1210 ± 0.0169 (B)
Parous	87	0.1408 ± 0.0114 (B)
Lipid		
Teneral	24	0.0643 ± 0.0096 (A)
Nulliparous	46	0.1169 ± 0.0168 (B)
Parous	87	0.1298 ± 0.0096 (B)
Protein		
Teneral	24	0.0073 ± 0.0015 (A)
Nulliparous	46	0.006 ± 0.0022 (A)
Parous	87	0.0095 ± 0.0018 (A)
Sugar		
Teneral	24	0.0038 ± 0.0008 (A)
Nulliparous	46	0.0060 ± 0.0013 (A)
Parous	87	0.0088 ± 0.0016 (A)
Glycogen		
Teneral	24	0.0057 ± 0.0016 (A)
Nulliparous	46	0.0035 ± 0.0009 (A)
Parous	87	0.0042 ± 0.0008 (A)

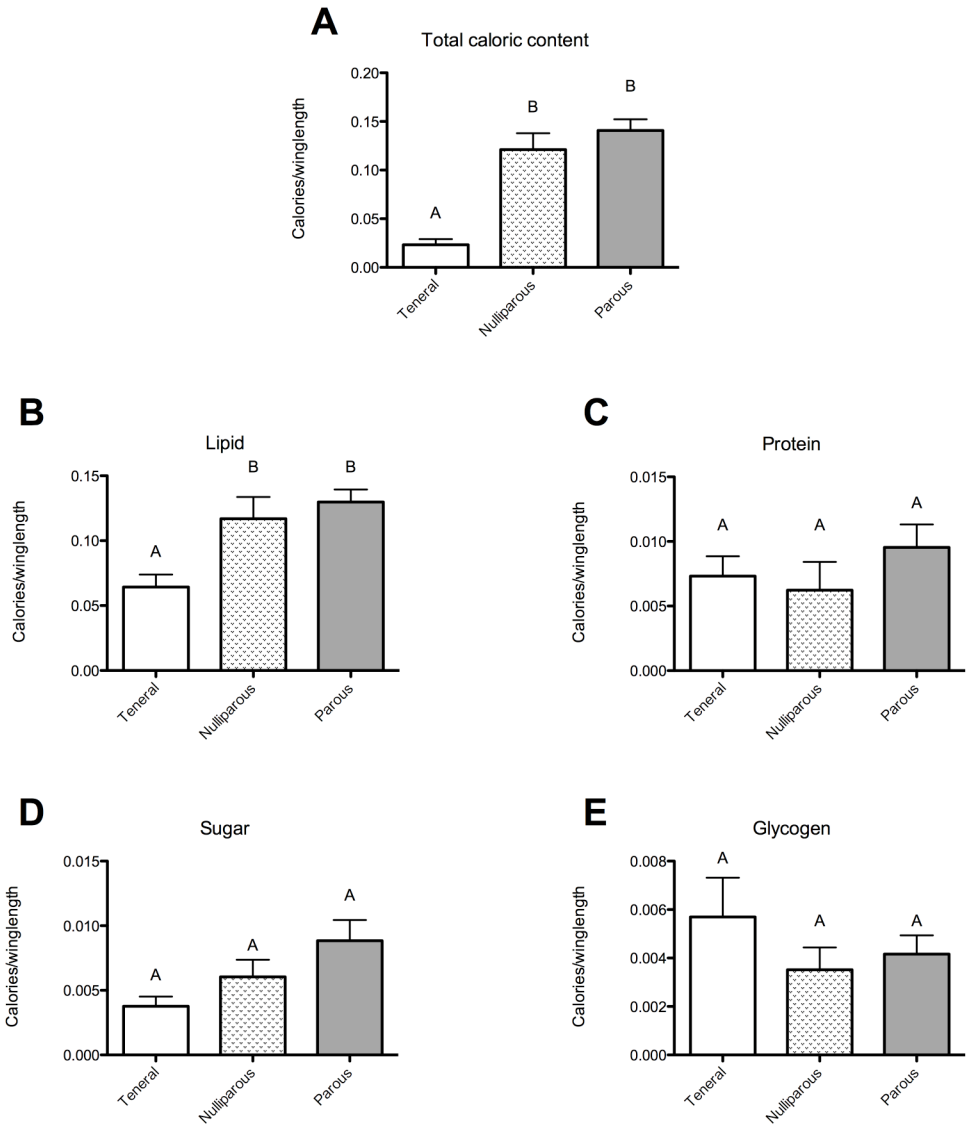

Figure 3-8 Accumulation of somatic energy reserves post-emergence in field-collected *An. gambiae* females. Columns with different letters are significantly different (Dunn's multiple comparison, $\alpha = 0.05$).

Figure 3-9 Body size in field-collected *An. gambiae* collected during the dry and rainy seasons. Columns with different letters are significantly different (Tukey's HSD, $\alpha = 0.05$).

Table 3-10 Mean size-specific caloric content (SSCC) of somatic energy reserves in *An. gambiae* collected during the dry season. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). * indicates a statistically significant difference between the somatic energy reserves of females and males (Mann-Whitney test, $\alpha = 0.05$).

Reserve type	n	Mean SSCC ± SE	Mann-Whitney U	p-value
Total calories				
Female	13	0.1332 ± 0.0301	22	0.6917
Male	4	0.1558 ± 0.0500		
Lipid				
Female	13	0.1117 ± 0.0285	23	0.7744
Male	4	0.0849 ± 0.0368		
Protein				
Female	13	0.0229 ± 0.0054	2	0.0074*
Male	4	0.0624 ± 0.0021		
Sugar				
Female	13	0.0071 ± 0.0040	21	0.6102
Male	4	0.0057 ± 0.0026		
Glycogen				
Female	13	0.0047 ± 0.0012	21	0.6102
Male	4	0.0132 ± 0.0090		

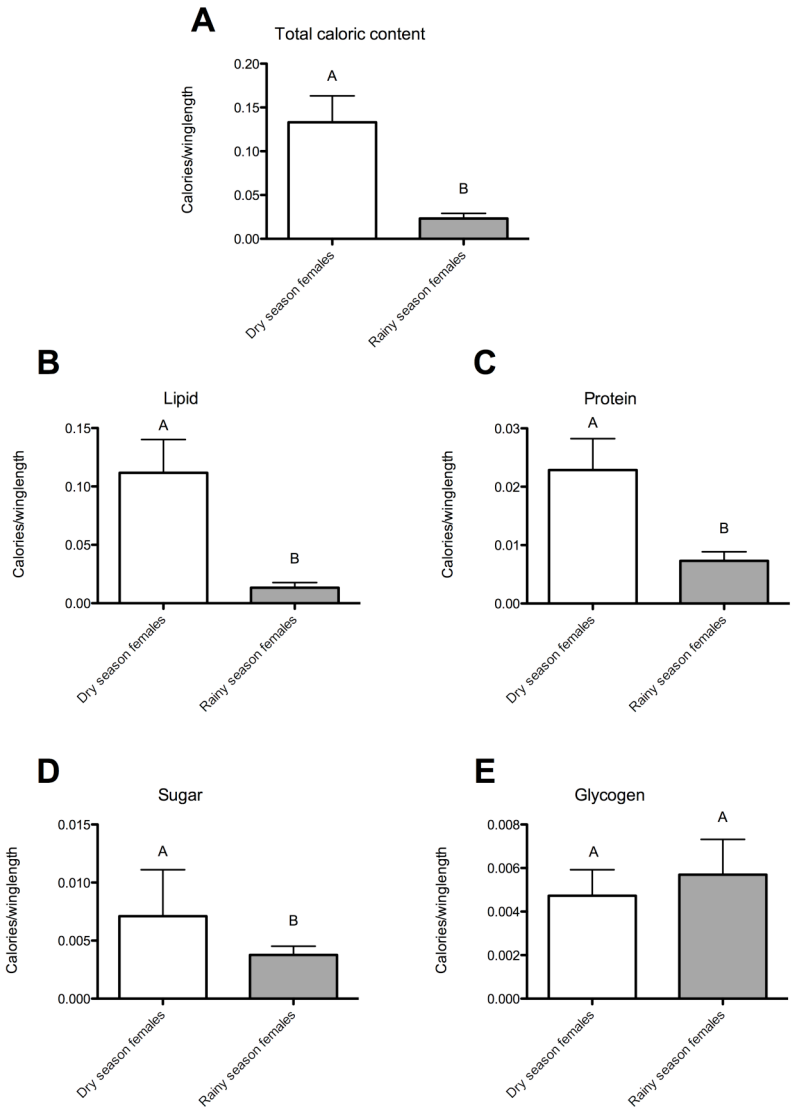

Figure 3-10 Variation in somatic energy reserves of field-collected *An. gambiae* females between dry and rainy seasons. All data have been adjusted by subtracting the average minimum irreducible amounts (MIA in SSCC). Columns with different letters are significantly different (Dunn's multiple comparison, α = 0.05).

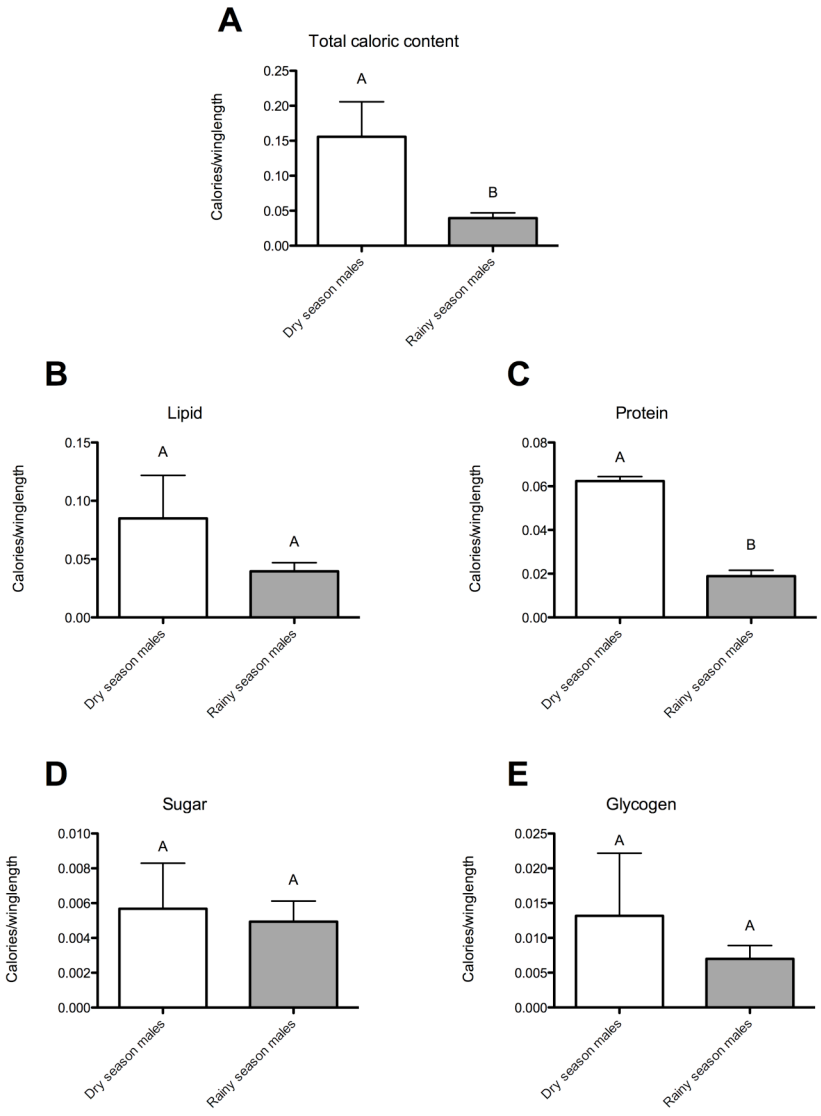

Figure 3-11 Variation in somatic energy reserves of field-collected *An. gambiae* males between dry and rainy seasons. All data have been adjusted by subtracting the average minimum irreducible amounts (MIA in SSCC). Columns with different letters are significantly different (Dunn's multiple comparison, α = 0.05).

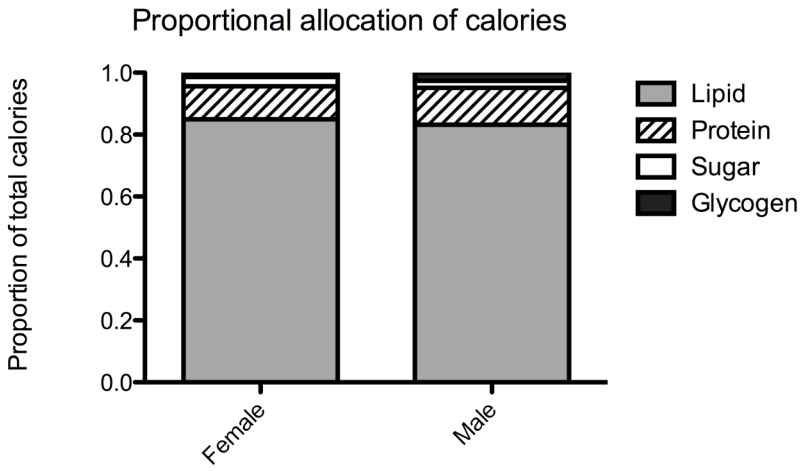

Figure 3-12 Proportional allocation of different classes of somatic energy reserves in teneral *An. gambiae* collected during the dry season. * indicates a statistically significant effect of gender on proportional allocation of reserves (ANOVA test, $\alpha = 0.05$).

Table 3-11 Effect of sex and larval density on the body size (winglength in mm) of laboratory-reared *An. gambiae*. * indicates a statistically significant effect (ANOVA, $\alpha = 0.05$).

Model effect	Sum of squares	F ratio	p-value
Sex	1.840	0.5359	< 0.0001*
Larval density	1.030	70.27	< 0.0001*
Sex*larval density	0.014	39.31	0.4659

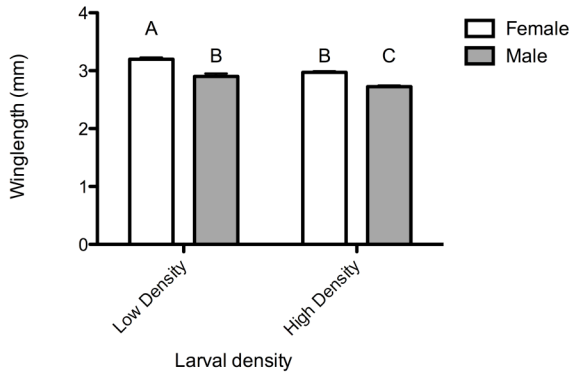

Figure 3-13 Effect of sex and larval density on the body size (winglength in mm) of laboratory-reared *An. gambiae*. Columns with different letters are significantly different (Tukey's multiple comparison test, $\alpha = 0.05$).

Table 3-12 Mean size-specific content (SSCC) of somatic metabolic reserves in teneral laboratory-reared *An. gambiae*. All data have been adjusted by subtracting average minimum irreducible amounts (MIA in SSCC). Means with different letters are significantly different (Mann-Whitney test, $\alpha = 0.05$).

Reserve type and sex	n	Mean (SSCC) ± SE
Total calories		
Female	27	0.1167 ± 0.0248 (A)
Male	30	0.0909 ± 0.0175 (A)
Lipid		
Female	27	0.0930 ± 0.0223 (A)
Male	30	0.0611 ± 0.0152 (A)
Protein		
Female	27	0.0148 ± 0.0034 (A)
Male	30	0.0151 ± 0.0019 (A)
Sugar		
Female	27	0.0160 ± 0.0012 (A)
Male	30	0.0241 ± 0.0028 (A)
Glycogen		
Female	27	0.0131 ± 0.0014 (A)
Male	30	0.0070 ± 0.0019 (A)

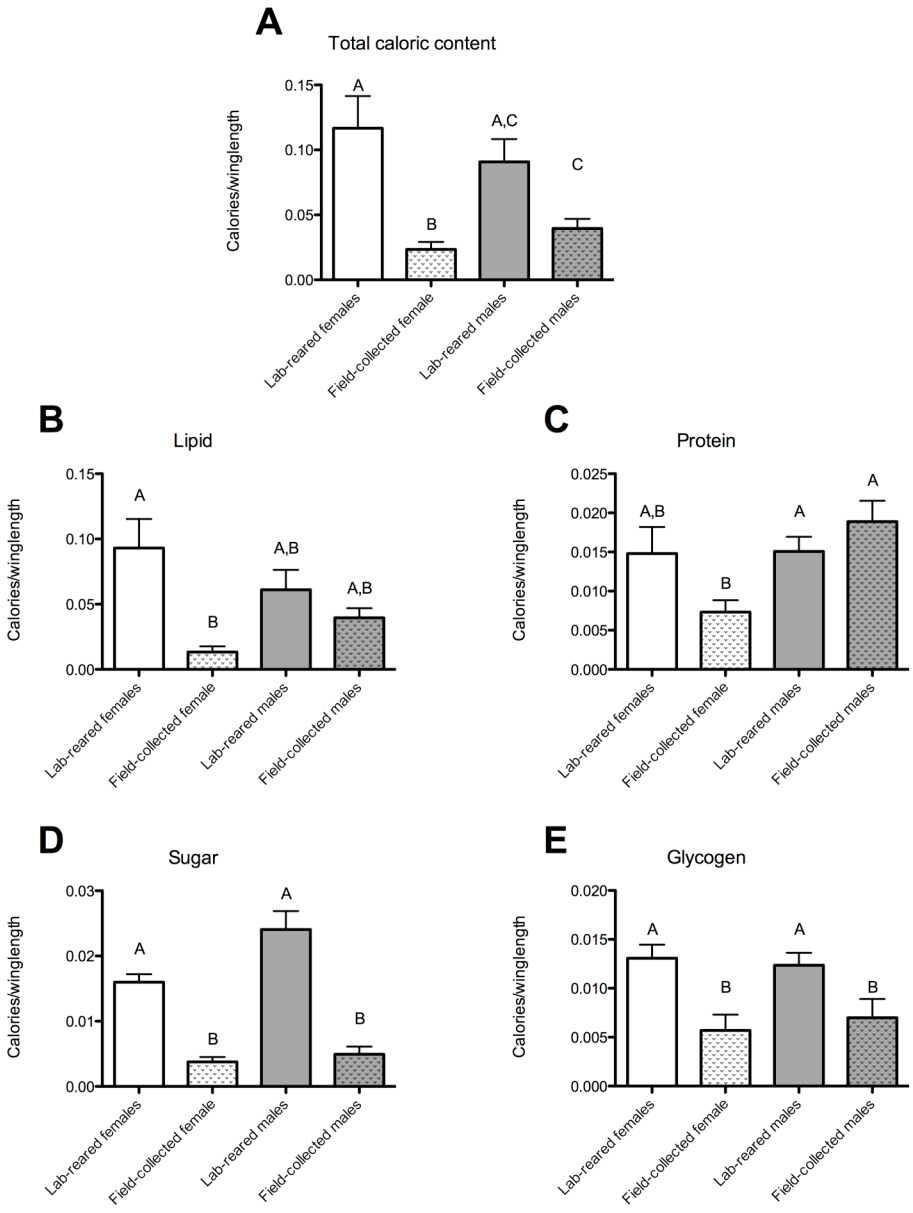

Figure 3-14 A comparison of the somatic energy reserves of laboratory-reared and field-collected *An. gambiae*. All data have been adjusted by subtracting average minimum amounts (MIA in SSCC). Columns with different letters are significantly different (Dunn's multiple comparison test, $\alpha = 0.05$).

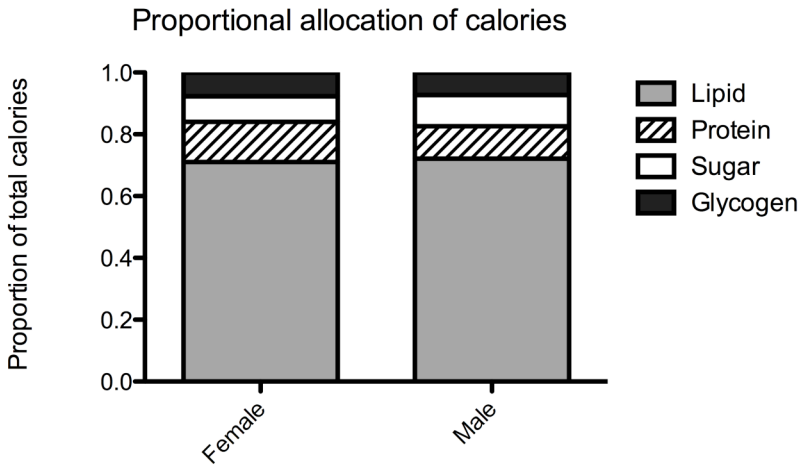

Figure 3-15 Proportional allocation of different classes of somatic energy reserves in teneral laboratory-reared *An. gambiae*. * indicates a statistically significant effect of gender on proportional allocation of reserves (ANOVA test, $\alpha = 0.05$).

Table 3-13 Factors that affect somatic energy reserves in *An. gambiae*, as measured by size-specific caloric content. "√" indicates that the factor has a significant effect.

Factor	Effect on somatic energy reserves
Sex	√
Age	√
Habitat (indoor vs. outdoor)	√
Season (dry vs. rainy)	√
Field-collected vs. lab-reared	√

Table 3-14 Factors that effect the distribution of somatic energy reserves between lipid, protein, sugar, and glycogen in *An. gambiae*. "√" indicates that the factor has a significant effect on the proportional allocation of calories between lipid, protein, sugar, and glycogen.

Factor	Effect on proportional distribution of somatic energy reserves
Sex	√
Season (dry vs. rainy)	√
Field-collected vs. lab-reared	√

CHAPTER 4: EFFECT OF *PLASMODIUM BERGHEI* ON FITNESS COMPONENTS IN *ANOPHELES STEPHENSI*

4.1 Introduction

Many studies have attempted to elucidate the effects of *Plasmodium* spp. on the fitness of mosquito vectors, yet this issue is still unresolved. Although most of the results reported in the literature indicate that malaria parasites decrease survival and fecundity of anophelines, other studies report no effect on anopheline fitness (Ferguson and Read 2002). Resolution of this issue is important because any factor that significantly alters longevity or reproductive output in vector species has the potential to affect malaria transmission (Beier 1996, Gu *et al*. 2006).

Much of the controversy about the effect of *Plasmodium* spp. on mosquito vectors may be attributed to the many factors that can affect vector-parasite relationships (Thomas *et al*. 2005). For example, mean intensity of infection, vector-parasite species pairings, and environmental conditions all have been shown to impact the effects of *Plasmodium* spp. on mosquitoes (Ferguson and Read 2002). Despite the problems inherent in extrapolating the results of laboratory studies to field populations (Chapter 3), there is still a need for laboratory studies to increase our basic understanding of the parasite-vector relationship. In this chapter I describe an investigation that asked a general

question about the vector-parasite relationship: does infection with a malaria parasite reduce the fitness of a mosquito vector?

The experimental design of this study was based on the design described in Chapter 2 of this thesis. This design was successful in detecting differences in fecundity and longevity of *An. gambiae* resulting from limitation of nutritional content of adult diet and was therefore determined to be suitable for detecting differences in fecundity and longevity in response to other potential stressors such as infection with *Plasmodium* spp. As in Chapter 2, the nutritional quality of the mosquito diet was altered by varying the concentration of sucrose solution provided and by varying the number of blood meals provided in the first gonotrophic cycle. Infection status was added as a 3rd factor in this study. The life history traits measured in this experiment were mosquito longevity and fecundity.

It is hypothesized that a reduction in the fitness of parasitized individuals is driven by a reduction in resources, or a reallocation of resources between fitness components in response to infection (Rivero *et al*. 2007). Reduction of resources in infected mosquitoes may be due to a high rate of metabolism and energetic resource use by the parasite (Schiefer *et al*. 1977). There also may be increased use of energy reserves by the mosquito to compensate for energetic costs associated with infection such as initiation of an immune response and repair of damaged midgut epithelium (Rivero *et al*. 2007). If a reduction in the value of a fitness component were due to reallocation of resources, one would predict a corresponding effect on another fitness component such as an increase in

reproductive output at the expense of longevity. Chapter 2 of this thesis demonstrates that *Anopheles gambiae* is capable of reallocating resources in this manner when the nutritional content of diet is limited.

Plasmodium berghei and Anopheles stephensi

Due to the inherent dangers of working with *Plasmodium falciparum*, a human malaria parasite, rodent and avian malaria parasites are commonly used as laboratory models. *Plasmodium berghei*, a rodent malaria was chosen for this study due to similarities with *P. falciparum* in basic biology, use of anopheline mosquitoes as vectors, susceptibility of laboratory mice, and for ease of comparison with other studies. *Plasmodium berghei* is found across central Africa, and its natural vectors and mammalian hosts include *Anopheles dureni* and *Thamnomys surdaster* (tree rat) (Sinden and Gilles 2002). *Anopheles stephensi* was chosen as the vector species for this investigation due to its susceptibility to *P. berghei*. *Anopheles stephensi* is a vector of *Plasmodium vivax* in Asia and the Middle East (Sinden 1997).

4.2 Materials and methods

Mosquito rearing conditions

Larvae were reared under the same conditions described in Chapter 2. Following emergence of adults into Plexiglas™ cages, the adult mosquitoes were provided with either a 2.5% or 10% w/v sucrose solution and transferred to a growth chamber in the Animal Care Facility. Conditions inside the growth chamber were maintained at 21°C (± 1°C) and 75% RH (± 10%). This lower

temperature (compared to Chapter 2) was necessary because *P. berghei* will not develop efficiently in *An. stephensi* outside the temperature range of 19-21°C (Sinden 1997).

Maintenance of *Plasmodium berghei* and infection of *Anopheles stephensi*

Plasmodium berghei, strain NK65A was obtained from American Type Culture Collection (ATCC, catalogue number 50175) and *An. stephensi* was donated by Dr. Rob Anderson (University of Winnipeg). Laboratory mice, *Mus musculus* (CD-1 strain) were used as the rodent host for *P. berghei*.

I followed the procedures described by Sinden (1997) for infection of mice and mosquitoes with *P. berghei*. For initial infection of mice, the *P. berghei* purchased from ATCC was warmed to 37°C and inoculated by the intraperitoneal route into a recipient mouse. Beginning on the 2nd day post-inoculation, a blood sample was taken from the saphenous vein to check for the presence of blood-stages of *P. berghei*. A thin blood smear was fixed in methanol and then stained with Giemsa stain (Sigma-Aldrich Inc., St. Louis, USA) diluted in phosphate-buffered saline (1:4 v/v). The prepared blood samples then were viewed at 1000 x magnification under oil immersion. The presence of blood-stages of *P. berghei* was determined by visual identification of trophozoites and schizonts using images in Landau and Boulard (1978) as a reference. If *P. berghei* was detected, then blood samples were taken approximately every 8 hours to monitor the course of infection as measured by parasitemia (the percentage of red blood infected with *P. berghei* in 3 fields of view) and the presence of gametocytes. The gametocyte is the only stage of *Plasmodium* spp. that is infective to the

mosquito (Sinden 1997). The presence of gametocytes can be detected visually in the stained blood smears, however this method does not allow for assessment of gametocyte infectivity (indicated by exflagellation of microgametocytes) (Sinden 1997). Therefore, the presence of potentially infective gametocytes was detected by exflagellation of microgametocytes. A small puff of air was breathed onto a drop of blood (the CO_2 in the breath and drop in temperature initiates exflagellation of microgametocytes) and a coverslip was placed over the blood and sealed with a thin layer of Vaseline® around the edges of the coverslip. The blood sample was checked every 1-2 minutes at 40 x magnification for exflagellation. Exflagellation is visible as small areas of whirling motion (Sinden 1997). If parasitemia was between 5 and 10% and exflagellation was observed in at least 3 fields of view (optimal conditions for transmission to mosquitoes, Sinden 1997), the mouse was prepared for blood feeding.

Prior to blood feeding, mice were anesthetized using a mixture of Ketamine (50 mg/kg) and Xylazine (5 mg/kg) administered using the intraperitoneal route as approved by the Simon Fraser University Animal Care Committee (permit number 741B-05). Using this treatment, mice were anesthetized for approximately 20 minutes, allowing mosquitoes to feed freely. The bellies of the mice were shaved to reduce the interference with blood feeding, and mice were then placed on the top of a cage of mosquitoes. Mosquitoes fed readily under these conditions. Following blood feeding but prior to recovery from anaesthesia, infected mice were euthanized using CO_2 and blood was collected via cardiac puncture for cryopreservation of *P. berghei*. The

blood was collected in a syringe containing 1 mg/mL heparin, diluted 1:1 (v:v) in a solution of 30% glycerol in phosphate-buffered saline, and 0.5 mL aliquots were placed in cryovials on dry ice. The samples were stored at -80 °C for 24 hours prior to transfer to liquid nitrogen. This method of cryopreservation ensures long-term viability of *P. berghei* and these samples can be thawed and used to infect subsequent mice by intraperitoneal injection (Sinden 1997).

If a mosquito acquires gametocytes in a blood meal, gametogenesis is initiated by a drop in temperature, a rise in pH (Sinden 1997), and the presence of xanthurenic acid in the mosquito midgut (Billker *et al*. 1998). Following the escape of gametocytes from red blood cells, microgametes are released from the microgametocytes, and subsequently fertilize macrogametes. The resulting zygote develops into an ookinete, a motile stage that crosses the midgut epithelium and develops into an oocyst between the basal lamina and basement membrane of midgut epithelial cells (Sinden 1997). Infection in the mosquito was confirmed by visual examination of the midgut on day 9 post-infection for the presence of oocysts, visible from day 5 onwards. To examine midgut tissue for the presence of oocysts, the entire midgut was dissected and stained with 0.1% mercurochrome (Sigma-Aldrich Inc., St. Louis, USA) in phosphate-buffered saline. Oocysts are visible as dark pink or red spheres.

Mosquitoes are able to transmit *Plasmodium* spp. to a mammalian host during blood feeding once the sporozoite stage of the parasite has invaded the salivary gland of the mosquito. Sporozoites are released from the oocyst and invade the salivary glands approximately 2 weeks after gametocytes are ingested

in a blood meal (Sinden 1997). Mosquitoes that were fed on *P. berghei*-infected mice were dissected 14-20 days after the infectious blood meal and their salivary glands examined for the presence of sporozoites. The presence of sporozoites was determined visually by pressing the salivary glands under a coverslip and examining at them 40 x magnification (Sinden 1997). Sporozoite-infected mosquitoes were used to infect mice through blood feeding. Following infection of an initial mouse and cohort of mosquitoes, a cycle of transmission between mice and mosquitoes was maintained through blood feeding. Mice were prepared for blood feeding as described above. Blood feeding by 6-10 sporozoite-positive mosquitoes was sufficient to infect mice with *P. berghei*.

Experimental design

As described in the introduction, I modified the experimental design used in Chapter 2, resulting in a 2 x 2 x 2 factorial design with 8 treatment groups. The 3 factors included were: concentration of sucrose diet (2.5% or 10%), number of blood meals in the 1st gonotrophic cycle (1 or 2), and infection status (fed on *P. berghei*-infected blood or on uninfected blood).

Experimental procedures were also very similar to those described in Chapter 2, with the following exceptions. All blood meals were provided by a laboratory mouse instead of a human host and on day 4 post-emergence. For mosquitoes in the "infected" treatment groups, the initial blood meal was provided by a mouse infected with *P. berghei* (parasitemia of 5-10% and presence of gametocytes confirmed by exflagellation). All subsequent blood meals were from an uninfected mouse. Following provision of the 1st blood meal, mosquitoes

were transferred to individual housing and provided with sucrose and an oviposition site as described in Chapter 2. Fecundity was determined by counting the number of eggs oviposited 96 hours after provision of the initial blood meal. A subsample of 5 mosquitoes from each treatment group was dissected 9 days following exposure to *P. berghei*-infected blood and assessed for the presence of oocysts. The prevalence of infection (the percentage of examined individuals containing oocysts) and mean intensity of infection (number of oocysts per infected mosquito) was recorded. On day 10 post-emergence, females were provided with an uninfected blood meal to initiate another gonotrophic cycle after which, (except for the subsamples used to estimate prevalence and mean intensity of infection) sucrose was removed and the mosquitoes were starved to death with access to water. The number of eggs oviposited by each individual was counted upon death of the individual, or 96 hours after the blood meal, whichever came first. The experiment was replicated 3 times.

Statistical analyses

The mean longevity and fecundity (± standard error) are reported for all treatment groups. Analysis of variance (ANOVA) was used to test for the effects of sucrose diet, number of blood meals in the 1st gonotrophic cycle, and infection status on longevity and fecundity. The sum of squares, F ratios, and p values are reported. If interaction terms were statistically significant, a separate ANOVA was conducted to determine the effects of each factor separately (Zar 1999).

Statistical analyses were conducted using JMP 6.0 (SAS Institute Inc. 2005) and graphs were generated using GraphPad Prism 4.00 (GraphPad Software Inc. 2005).

4.3 Results

Infection of mosquitoes with *Plasmodium berghei*

A cycle of transmission between *An. stephensi* and *P. berghei* was successfully maintained during 3 replicates of the experiment. The estimated prevalence and mean intensity of infection in mosquitoes is reported in Table 4-1. In all 3 replicates, 80-100% of the mosquitoes contained oocysts 9 days after feeding on an infected mouse (Table 4-1). The mean intensity ranged from 8 to 41 oocysts per mosquito (Table 4-1).

Longevity

Mean longevity of all treatment groups (following removal from food) is reported in Table 4-2. There was a significant interaction between the type of sucrose diet and infection status (Table 4-3) that required a separate ANOVA to determine the effects of each factor separately (Zar 1999). When mosquitoes fed on uninfected blood, the type of sucrose diet had a significant effect on longevity ($F = 7.3149$, $p = 0.0083$); uninfected mosquitoes reared on 2.5% sucrose survived longer (6 days) than those reared on 10% sucrose (4 days). When mosquitoes fed on infected blood, the type of sucrose diet did not have a significant effect on longevity ($F = 1.385$, $p = 0.2416$). When mosquitoes were reared on 2.5% sucrose, infection status had a significant negative effect on

longevity (F = 11.0367, p = 0.0012). Uninfected mosquitoes reared on 2.5% sucrose survived longer (7 days) than infected mosquitoes reared on 2.5% sucrose (5 days). In contrast, when mosquitoes were reared on 10% sucrose, infection status did not have a significant effect on longevity (F = 0.7806, p = 0.3792).

Fecundity

Mean fecundity of all treatment groups is reported in Table 4-4. There was a significant interaction between the type of sucrose diet and infection status (Table 4-5) that required a separate ANOVA to determine the effects of each factor separately (Zar 1999). When mosquitoes fed on uninfected blood, the type of sucrose diet did not have a significant effect on fecundity (F = 3.84, p = 0.0588). When mosquitoes fed on infected blood, the type of sucrose diet had a significant effect on fecundity (F = 4.314, p = 0.0400). Infected mosquitoes reared on 10% sucrose laid more eggs (39) than infected mosquitoes reared on 2.5% sucrose (28). When mosquitoes were reared on 2.5% sucrose, infection status had a significant effect on fecundity (F = 17.766, p < 0.0001). Uninfected mosquitoes reared on 2.5% sucrose laid more eggs (48) than infected mosquitoes reared on 2.5% sucrose (28). When mosquitoes were reared on 10% sucrose, infection status did not have a significant effect on fecundity (F = 0, p = 0.9979).

Infection significantly decreased mean fecundity in all diet treatments (Tables 4-4, 4-5). Infected mosquitoes laid an average of 33 eggs, compared to an average of 43 eggs in uninfected mosquitoes.

4.4 Discussion

The development of better methods in malaria control can benefit from a more thorough understanding of the effects of *Plasmodium* spp. on vector fitness and behaviour. Such knowledge may lead to the identification of conditions that limit vector survival and decrease transmission of *Plasmodium* spp. (Ferguson and Read 2002). Furthermore, understanding how variation in factors such as diet interact with *Plasmodium* spp. to impact vector fitness will increase the relevance of laboratory experiments to naturally acquired infections. In this study, I used a rodent malaria model to investigate the effects of a malaria parasite and variation in diet on mosquito fitness.

I was able to establish a cycle of transmission of *P. berghei* between a rodent host (*M. musculus*) and a vector *(An. stephensi*). Overall, infection with *P. berghei* reduced the fitness of *An. stephensi* as measured by fecundity over 2 gonotrophic cycles, but did not affect longevity following removal from food (Tables 4-2, 4-3, 4-4, 4-5). There was an interesting interaction between infection status and sucrose diet that affected both starvation resistance and fecundity (Tables 4-2, 4-4). When mosquitoes were reared on 2.5% sucrose, blood meals from mice infected with *P. berghei* significantly reduced starvation resistance and fecundity compared to blood meals from uninfected mice (Figure 4-1A, B). This result suggests that when sucrose is limited, infection with *P. berghei* can negatively affect fitness components of mosquitoes. Infection-induced reductions in starvation resistance and fecundity were not seen when mosquitoes were reared on 10% sucrose (Figure 4-1 C, D), suggesting that an

increase in sucrose concentration can compensate for the effect of *P. berghei* on mosquito fecundity.

Analysis of the interaction between infection status and sucrose diet also shows that mosquitoes fed on blood from *P. berghei*-infected mice and reared on 2.5% sucrose have reduced fecundity compared to those reared on 10% sucrose (Figure 4-2D). Mosquitoes fed on infected blood and reared on 2.5% sucrose also exhibit reduced starvation resistance compared to those reared on 10% sucrose, although the difference was not statistically significant (Figure 4-2C). These results are expected based on our current understanding of nutritional physiology that predicts an increase in food availability will increase the nutritional resources available for physiological processes such as egg production. The opposite pattern was seen in mosquitoes fed on uninfected mice. In these mosquitoes, individuals reared on 2.5% sucrose had increased starvation resistance (Figure 4-2A) and increased fecundity (not statistically significant) (Figure 4-2B). This result was unexpected. Unpredicted relationships between diet and life history components were also seen in the results presented in Chapter 2. Due to the use of different species in Chapter 2 and Chapter 4 studies and the introduction of *P. berghei* as a factor in Chapter 4, it is not easy to make direct comparisons between the 2 studies. However, it is clear that the mechanisms regulating the allocation of resources between somatic and reproductive functions in *Anopheles* are complex and are affected by diet.

The results of this study are generally consistent with work by other authors that indicate infection with *Plasmodium* spp. reduces mosquito fecundity (Ahmed *et a*l. 1999, Hogg and Hurd 1995, 1997). In *An. stephensi*, the reduction in fecundity results from apoptosis in follicular cells approximately half way through the gonotrophic cycle (Hopwood *et al*. 2001). An observation that infection with malaria parasites increases glucose use in midgut epithelium (Schiefer *et al*. 1977) prompted investigation into the possibility that metabolic resources mediate this reduction in fecundity. In a 2003 study by Rivero and Ferguson, *An. stephensi* were infected with *Plasmodium chabaudi*, and the lipid, protein, sugar, and glycogen content of mosquitoes were measured when the parasite had developed to the oocyst and sporozoite stages. Midguts and salivary glands were removed to exclude parasites from the analysis. Infection did not reduce the total amount of metabolic reserves in mosquitoes, and resulted in an increase in sugar content (Rivero and Ferguson 2003). The authors hypothesized that the results likely reflect increased sugar feeding in infected mosquitoes (Rivero and Ferguson 2003). Similarly, a 2006 study by Gray and Bradley found that infection with *Plasmodium gallinaceum* does not change the metabolic rate of *Aedes aegypti*. Together these studies suggest that sugar feeding plays an important role in modulating the effects of *Plasmodium* spp. on vector fecundity, although the mechanism behind this interaction remains unresolved. Increased sugar feeding may benefit both the parasite and the vector. Increased sugar feeding can reduce blood feeding (Straif and Beier 1996) and thereby limit blood-feeding related mortality (Rivero and Ferguson

2003). Reduction in vector mortality may increase both vector and parasite fitness. Sugar feeding also may benefit the mosquito by increasing the resources available to repair any parasite-induced damage to midgut tissue (Rivero and Ferguson 2003).

Many other studies have tested the effects of malaria parasites on mosquito survival, with conflicting results. Some studies indicate that *Plasmodium* spp. reduce mosquito survival while others indicate no effect (Ferguson and Read 2002). There are many factors that may account for these conflicting reports, including the vector and parasite species studied, mean intensity of infection, diet, and environmental conditions such as temperature and humidity (Ferguson and Read 2002). The results presented in this chapter support the hypothesis that diet has a significant effect on the impact of *P. berghei* on mosquito survival.

In contrast to the results presented in Chapter 2, there was no evidence of a reallocation of resources between fecundity and longevity in response to limitation of sugar or blood, or in response to infection with *P. berghei*. When mosquitoes were reared on a diet limited in carbohydrates, infection with *P. berghei* decreased both longevity and fecundity (Figure 4-1). This result suggests that the reduction in measures of fitness incurred in *An. stephensi* infected with *P. berghei* are due to a limitation or reduction in nutritional resources and not due to a reallocation of resources (see Chapter 2). The second major contrast between the results of Chapters 2 and 4 is that the number of blood meals in the 1st gonotrophic cycle did not affect longevity or

fecundity in the Chapter 4 study. In the Chapter 2 study, provision of a second blood meal increased longevity and fecundity, except in mosquitoes reared on 2.5% sugar (Tables 2-1, 2-2, Figures 2-1, 2-2). This result highlighted the importance of the initial blood meal to *An. gambiae*, likely to compensate for limited energy reserves at emergence. One hypothesis for the different results in Chapter 4 is that *An. gambiae* may be more flexible in resource allocation between somatic and reproductive functions and/or more constrained in teneral reserves than *An. stephensi*. This hypothesis could be tested by a repetition of the Chapter 2 study including species (*An. stephensi* or *An. gambiae*) as a 3rd factor in addition to sucrose and blood. It is also possible that an increase in blood intake did not affect fecundity in this study due to the different nutritional quality of mouse blood compared to human blood. Human blood is lower in isoleucine content then rodent blood, and isoleucine is a limiting factor in egg production (Clements 1992). Fertility and fecundity are greater when mosquitoes are fed on rodent blood, and the addition of isoleucine to human blood increases egg production (Chang and Judson 1977).

Although there are inherent difficulties in extrapolating the results from a non-normal laboratory model of vector-parasite interactions to naturally occurring vector-parasite pairings, non-normal laboratory models still provide valuable information because it is generally easier to detect parasite effects in these systems due to the tendency for increased intensity of infection. The greater intensity of infection in non-normal vector-parasite associations tends to result in greater reductions in vector survival than in natural vector-parasite pairings

(Ferguson and Read 2002). In naturally occurring vector-parasite relationships, mosquitoes have evolved cellular and molecular defences that decrease parasite survival and intensity (Cohuet *et al*. 2006, Ferguson and Read 2002). There is also evidence that some mechanisms governing vector-parasite interactions are different in laboratory models using non-normal associations and naturally acquired infections. For example, it has been shown that expression of genes regulating mosquito humoral immune response can affect the development of *P. berghei* in *An. gambiae* (Osta *et al*. 2004). Leucine-rich repeat immune protein 1 decreases parasite survival from the ookinete to oocyst stage while C-type lectin 4 and C-type lectin mannose binding 2 increases parasite survival during the same period (Osta *et al*. 2004). However, a 2006 study by Cohuet *et al*. found that these same genes did not affect the development of *P. falciparum* in *An. gambiae*. Additionally, relationships between physiological factors can vary significantly between laboratory colonies and field populations (Chapter 3).

Despite these differences between non-normal laboratory models and natural vector-parasite associations, laboratory studies still provide valuable information about vector-parasite interactions, especially parasite effects on vector fitness. Greater oocyst burdens in non-natural pairings exacerbate fitness reductions in infected mosquitoes, making parasite effects on vector fitness easier to detect than in natural pairings. Laboratory studies also can indicate the conditions under which a parasite may affect vector fitness.

The relationship between malaria parasites and anophelines is complex, and varies with diet of the vector. The majority of studies examining the effect of

Plasmodium spp. on anophelines indicate that the parasite reduces mosquito fitness components (longevity and fecundity) (Ferguson and Read 2002). The results of the investigation presented here suggest that availability of sugar in the mosquito diet can interact with the effects of *P. berghei* infection to affect mosquito fitness. The interaction between mosquito diet and *Plasmodium* spp. on mosquito fitness, especially in *An. gambiae* infected with *P. falciparum* is a subject worthy of further investigation. Increasing our understanding of vector-parasite interactions, particularly the mechanisms that govern these relationships may eventually provide a basis for preventing the development of *Plasmodium* spp. in its mosquito vector and breaking the cycle of malaria transmission.

4.5 Literature cited

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Table 4-1 **Prevalence and mean intensity of *P. berghei* infections in *An. stephensi* for 3 replicates of the experiment. Means are reported for each combination of sugar and blood diets and were estimated from a subsample of 5 mosquitoes from each group.**

Treatment	Replicate	Prevalence	Mean intensity
2.5% sucrose, 1 blood meal	1	80%	23
2.5% sucrose, 1 blood meal	2	80%	20
2.5% sucrose, 1 blood meal	3	100%	8
2.5% sucrose, 2 blood meals	1	80%	40
2.5% sucrose, 2 blood meals	2	100%	33
2.5% sucrose, 2 blood meals	3	80%	21
10% sucrose, 1 blood meal	1	100%	14
10% sucrose, 1 blood meal	2	100%	20
10% sucrose, 1 blood meal	3	80%	29
10% sucrose, 2 blood meals	1	100%	22
10% sucrose, 2 blood meals	2	100%	41
10% sucrose, 2 blood meals	3	100%	32

Table 4-2 **Mean longevity of *An. stephensi* females subjected to different diet treatments. Mosquitoes in the infected treatment groups were fed on mice infected with *P. berghei*. Mosquitoes in the uninfected treatment groups were fed on uninfected mice.**

Treatment	n	Mean ± SE
Infected		
2.5% sucrose, 1 blood meal	38	3.63 ± 0.17
2.5% sucrose, 2 blood meals	28	5.71 ± 0.50
10% sucrose, 1 blood meal	35	4.20 ± 0.21
10% sucrose, 2 blood meals	18	6.44 ± 0.53
Uninfected		
2.5% sucrose, 1 blood meal	24	5.63 ± 0.62
2.5% sucrose, 2 blood meals	21	7.38 ± 1.02
10% sucrose, 1 blood meal	25	3.84 ± 0.19
10% sucrose, 2 blood meals	17	5.71 ± 0.67

Table 4-3 **Effect of sucrose diet type, infection status, and number of blood meals in the 1st gonotrophic cycle on the longevity of *An. stephensi* females. * indicates a statistically significant effect (ANOVA, $\alpha = 0.05$).**

Model effect	Sum of squares	F ratio	p value
Sucrose diet	13.977	2.3856	0.1241
Infection status	19.6293	3.3503	0.0687
Number of blood meals	8.0626	2.949	0.0875
Sucrose diet*infection status	67.74463	11.5626	0.0008*
Sucrose diet*number of blood meals	0.22079	0.0377	0.8463
Infection status*number of blood meals	1.48825	0.254	0.6148
Sucrose diet*infection status*number of blood meals	0.00803	0.0014	0.9705

Table 4-4 **Mean fecundity of *An. stephensi* females subjected to different diet treatments. Mosquitoes in the infected treatment groups were fed on mice infected with *P. berghei*. Mosquitoes in the uninfected treatment groups were fed on uninfected mice.**

Treatment	n	Mean ± SE
Infected		
2.5% sucrose, 1 blood meal	38	25.76 ± 2.98
2.5% sucrose, 2 blood meals	28	31.75 ± 3.68
10% sucrose, 1 blood meal	35	41.97 ± 6.14
10% sucrose, 2 blood meals	18	31.78 ± 6.69
Uninfected		
2.5% sucrose, 1 blood meal	24	52.75 ± 7.98
2.5% sucrose, 2 blood meals	21	42.14 ± 2.53
10% sucrose, 1 blood meal	25	39.80 ± 0.90
10% sucrose, 2 blood meals	17	36.65 ± 2.50

Table 4-5 Effect of sucrose diet type, infection status, and number of blood meals in the 1st gonotrophic cycle on the fecundity of *An. stephensi* females. * indicates a statistically significant effect (ANOVA, $\alpha = 0.05$).

Model effect	Sum of squares	F ratio	p value
Sucrose diet	14.6081	0.0239	0.8774
Infection status	4805.1812	7.8467	0.0056*
Number of blood meals	965.725	1.5770	0.2107
Sucrose diet*infection status	3598.4198	5.8761	0.0162*
Sucrose diet*number of blood meals	227.8069	0.3720	0.5426
Infection status*number of blood meals	273.031	0.4459	0.5051
Sucrose diet*infection status*number of blood meals	1671.1191	2.7289	0.1001

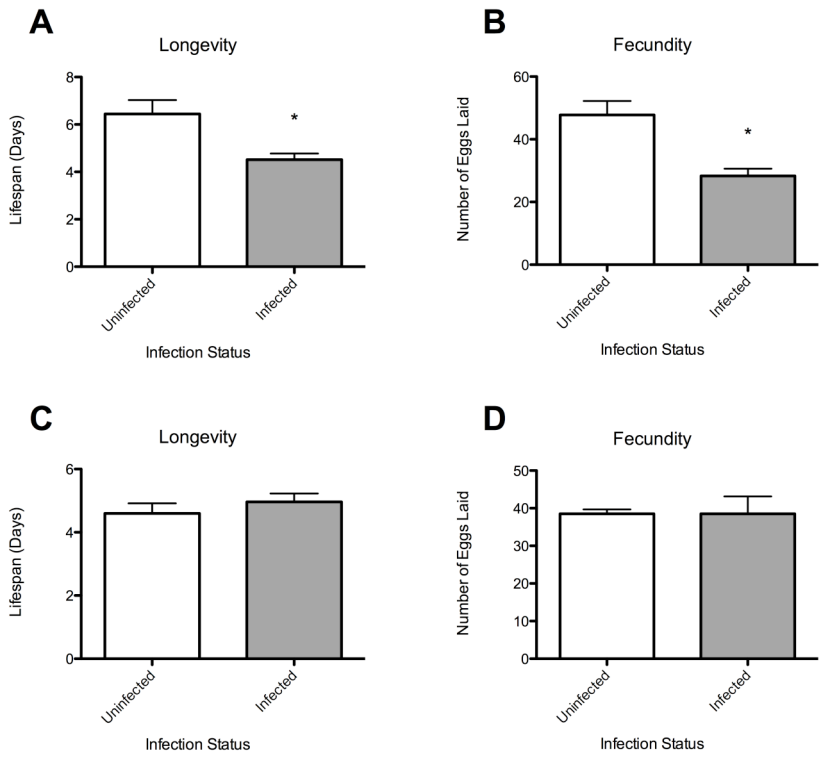

Figure 4-1 The effect of infection with *P. berghei* on starvation resistance and fecundity of *An. stephensi* reared on different concentrations of sucrose. A and B: reared on 2.5% sucrose. C and D: reared on 10% sucrose. * indicates a significant difference between infected and uninfected mosquitoes (ANOVA, α = 0.05).

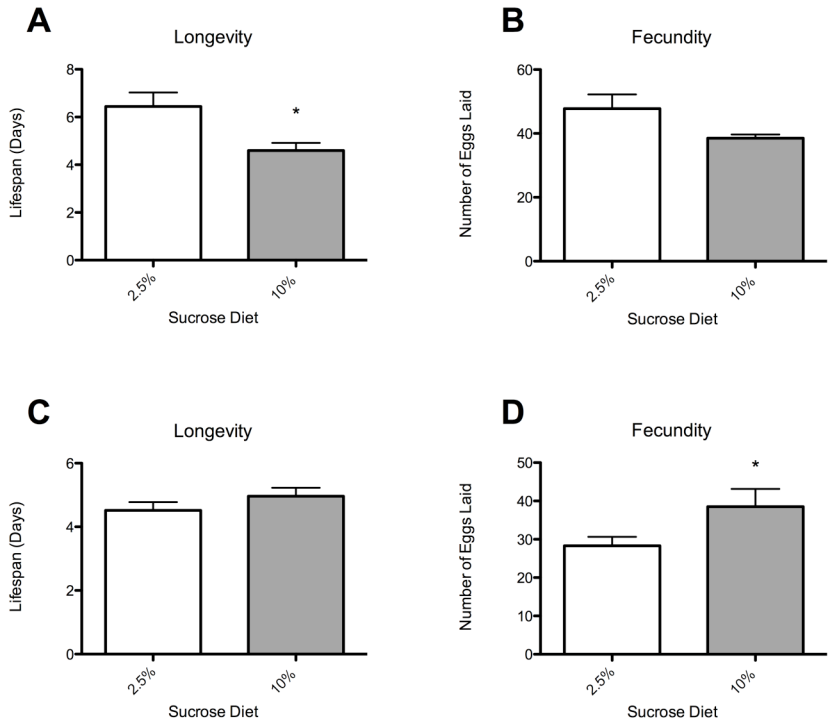

Figure 4-2 The effect of sucrose diet on starvation resistance and fecundity of *An. stephensi*. A and B: blood fed from uninfected mice. C and D: blood fed from mice infected with *P. berghei*. * indicates a significant difference between mosquitoes reared on 2.5% and 10% sucrose (ANOVA, $\alpha = 0.05$).

CHAPTER 5: CONCLUSIONS

The primary goal of this thesis was to examine the role of nutrition in determining mosquito fitness in the context of vectorial capacity. To accomplish this goal I asked 3 research questions. Firstly, can *Anopheles gambiae* females alter the proportional allocation of nutritional resources between somatic and reproductive functions? Secondly, what is the somatic metabolic reserve status of a field population of *An. gambiae*; how does reserve status vary within a population, and does it differ from that of a laboratory colony? Lastly, do malaria parasites affect vector fitness and does the nutritional intake of the vector alter the impact of *Plasmodium* spp. on vector fitness?

The data presented in this thesis resulted in 4 major findings. Firstly, the use of a constant value for survivorship in the classic model of vectorial capacity is problematic, as survivorship and longevity of *An. gambiae* females is dynamic. Longevity is greatly influenced by nutritional intake, and indirectly influenced by the mosquitoes themselves, which have the ability to the way they allocate resources between somatic and reproductive functions in response to changes in their nutritional intake. Secondly, the physiological condition of *An. gambiae* (as estimated by somatic energy reserves) varies within a population based on sex, age, season, and habitat, and varies between wild populations and laboratory colonies. Third, nutritional intake can impact the effects of malaria parasites on vector fitness. Lastly, sugar and blood are both important components of the female diet in this medically important insect. In this chapter, I review the main

conclusions of the thesis, and suggest directions for future work in the field of vector nutritional ecology.

5.1 Summary of major findings

In Chapter 2, laboratory manipulations of blood and sugar availability were employed to determine if female *An. gambiae* are capable of changing the proportional allocation of resources between somatic and reproductive functions in response to changes in diet. The results indicated that females exhibit flexibility in the proportional allocation of resources between somatic and reproductive functions. Individuals reared on a diet restricted in sugar content and number of blood meals allocated an increased proportion of their nutrient intake towards current reproductive effort at the expense of longevity, allowing females to indirectly alter their own longevity. In general, increasing the concentration of sucrose and the number of blood meals both increased longevity and fecundity. However, provision of a 2nd blood meal did not completely compensate for the negative effects of a low sucrose diet. These results show that both blood and sugar are important to *An. gambiae*, especially during the 1st gonotrophic cycle, and that while females can alter the allocation of nutritional resources between soma and reproduction in response to nutritive stress, this flexibility is limited. Mosquitoes provided with the most restrictive diet exhibited an interesting and unexpected deviation from the pattern of increased fecundity with increased blood intake. When females were reared on a low sucrose diet, those provided with only 1 blood meal produced more eggs than those provided with 2 blood meals. This result highlights the complexity of the

relationship between the 2 components of the female diet and suggests that the utilization of blood towards eggs may be altered by the availability of sucrose.

Chapter 3 presented the results of an analysis of the somatic energy reserves of a field population and a laboratory colony of *An. gambiae*. This investigation showed that the amount and distribution of somatic energy reserves varies with physiological factors such as sex and age, and with environmental factors such as season and habitat type. Field-collected males emerged with greater quantities of somatic energy reserves compared to females, a pattern of sexual dimorphism that may reflect the high energetic demands of the swarming behaviour that is essential for male mating success. Both sexes accumulated reserves post-emergence, primarily lipid, implying that males and females feed after emergence. The data also suggest that females likely need to feed very soon after emergence and energy acquired through feeding is used to increase somatic energy reserves prior to reproduction. This data also implies that females who are unable to feed very soon after emergence are not likely to survive long, suggesting that limiting food availability might be an effective method of reducing population size and therefore vectorial capacity of *An. gambiae*. This is consistent with predictions developed by Ma and Roitberg (unpublished manuscript), which suggest limiting sugar sources near oviposition sites is an effective method to limit vector population size. Another major though not surprising finding of these experiments was that laboratory-reared mosquitoes emerge with more somatic energy reserves than those collected from a field population. Laboratory colonies are frequently used as proxies for

field populations, yet data presented in Chapter 3 suggests that laboratory-reared mosquitoes may differ in important physiological parameters from their wild counterparts. This could potentially reduce the accuracy of predictions based on laboratory colonies.

Based on the experimental designed in Chapter 2, I was able to establish a protocol to detect the effect of *Plasmodium berghei* on an anopheline vector and the impact of vector nutrition on the vector-parasite relationship. Infection with *P. berghei* reduced the longevity and fecundity of *Anopheles stephensi* when mosquitoes were reared on a low sucrose diet, and this effect was not seen when mosquitoes were reared on a high sucrose diet. This suggests that sucrose may compensate for the negative effects of infection with *P. berghei*. Increased sugar feeding and any resulting increase in longevity are likely to be beneficial to both the vector and the parasite. If this effect is also seen in *An. gambiae* infected with *Plasmodium falciparum*, it is possible that limiting the availability of sugar sources in wild populations may decrease vectorial capacity of this mosquito by reducing the survival of both the vector and the parasite.

Results presented in this thesis indicate that sugar feeding is an important component of the adult diet in *An. gambiae* females and can affect fitness, sometimes in unexpected ways. The sugar content of diet was shown to affect the allocation of energy between reproductive and somatic functions and an increase in sucrose concentration tends to increase longevity and fecundity in *An. gambiae*. Increased sucrose concentration also compensated for the negative effects of *P. berghei* on vector fitness. Additionally, data collected from

a field population suggest that sugar feeding may be a naturally occurring behaviour; the increase in sugar content of field-collected females provides indirect evidence of feeding on plant sugars by females.

When considered together, the major findings presented in this thesis indicate that nutrition is an important determinant of *An. gambiae* fitness and should be considered when discussing the vectorial capacity of *An. gambiae* populations. The results of this work show that the survival term in the vectorial capacity equation is dynamic because it is a function of blood and sugar availability. Blood and sugar availability can affect *An. gambiae* survival in non-linear and sometimes unexpected ways due to the ability of females to change the way they allocate resources between longevity and fecundity.

5.2 Suggestions for future studies

The data presented in this thesis provides evidence that nutrition can affect behaviour, physiological processes and the cost of infection with *Plasmodium* spp. in anophelines, and is a topic worthy of further study.

The complexity of the relationship between sugar and blood and their impacts on female fitness provides many opportunities for future research. For example, it could prove valuable to measure how each type of somatic energy reserve (lipid, protein, sugar, and glycogen) varies as sugar and blood availability are manipulated. One could also measure the efficiency of conversion between each type of somatic reserve in males and females. These data would produce a more precise energy budget, help establish the relative importance of sugar and

blood towards somatic and reproductive functions in females, and determine the limits of flexibility in resource allocation in this mosquito.

Future studies should also examine the impact of nutrition on the interactions between *An. gambiae* and *Plasmodium falciparum*, the deadliest human malaria parasite. As a starting point, an experimental design similar to that described in Chapter 4 could be utilized to achieve this goal. Ideally, naturally acquired infections should be used. This would require collection of wild *An. gambiae* for use in the experiment, and testing for *P. falciparum* infection by detecting expression of parasite-specific genes such as circumsporozoite protein following completion of the experiment. If a pattern of fitness reduction due to infection and compensation by increased sucrose availability is seen, there is an opportunity to exploit this phenomenon to reduce transmission of *P. falciparum*. Decreased food (blood and sugar) availability may reduce longevity of infected mosquitoes. This would, in turn, reduce parasite transmission by reducing the number of infected vectors, and by reducing the probability that an infected mosquito will survive long enough for *P. falciparum* to develop to the sporozoite stage. This is predicted by the current vectorial capacity model if a simple mortality increase is included but results from my thesis indicate that this effect would be highly non-linear due to the mosquito's allocation plasticity.

Nutrition affects vector fitness both directly and indirectly and may provide future opportunities for vector control. Therefore, the nutritional ecology of *An. gambiae* is a subject worthy of future research and should be considered during the development of vector control strategies.