EFFECT OF BLOOD MEAL SIZE ON MOSQUITO RESPONSE TO DISTURBANCE WHILE BLOOD FEEDING ON A SIMULATED HOST

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Effect of blood meal size on mosquito response to disturbance while blood feeding on a simulated host

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ABSTRACT

I tested the hypothesis that blood feeding mosquitoes are more likely to leave the host in response to disturbance when they are almost compared with partially full. I studied behavioural responses of female *Anopheles gambiae* and *Aedes aegypti* and assessed the influence of blood meal size and feeding duration on the likelihood that they would leave the membrane when disturbed. An artificial feeder was used as a simulated host. Both species showed an increase in blood meal weight and fecundity with feeding duration. For both species, a positive correlation was found between feeding duration and the tendency to leave when disturbed. A*edes aegypti* demonstrated a positive correlation between blood meal weight and the tendency to leave, however no such relationship was found in *An. gambiae.* These data support the hypothesis that the amount of blood in the mosquito mid-gut influences their decision to remain on defensive hosts.

DEDICATION

To Mom, Dad and Andrew.

ACKNOWLEDGEMENTS

I would like to thank Dr. Bernie Roitberg for allowing me to join the Roitberg lab, for giving me the opportunity to take on this project and for continuing to help me in many ways throughout the years. Thank you Dr. Carl Lowenberger for agreeing to be one of my supervisors, your help and advice with my thesis is greatly appreciated. Ian Gordon, I thank you for all of your ideas and help with Plexiglas® and pendulums. Bruce Leighton, without your ability to listen, advise and join me on Fridays – I don't know where I would be. Thank you Chris Beach for your AutoCAD work and thank you to all of my lab mates for making the Roitberg lab so enjoyable.

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1. INTRODUCTION

1.1. Mosquitoes and Disease

Mosquitoes have a greater influence on human health throughout the world than any other insect. The present global burden of mosquito-borne diseases is enormous, amounting to hundreds of millions of new infections and deaths annually. While this burden pre-eminently applies to the anophelines, owing to their role as vectors of human malaria parasites, it also pertains to the genus *Aedes*, which includes important vectors of dengue and yellow fever. Malaria, dengue fever, yellow fever, and filariasis are four of the most important diseases of the tropical and subtropical parts of the world today and they are examples of a long list of diseases caused by transmission of pathogens from infected mosquitoes to vertebrate hosts during blood feeding.

On a worldwide basis, malaria, in its various forms, is the most important vector-borne disease. There are four human malaria parasites of the genus *Plasmodium* (*Plasmodium falciparum, P. ovale, P. malariae* and *P. vivax*) and they are all transmitted by members of the genus *Anopheles. Plasmodium vivax* and *P. falciparum* are the most common and *P. falciparum* is the most deadly type of malaria infection. Malaria is endemic in parts of Asia, Africa, Central and South America, Oceania and certain Caribbean Islands (WHO 2004). Malaria parasites infect 300-500 million people and cause 1-3 million deaths annually. The economic toll of malaria is also great. In some countries with a heavy malaria burden, the disease may account for as much as 40% of public health expenditure, 30-50% of inpatient admissions, and up to 50% of outpatient visits (WHO 2004).

Since the early recognition by Patrick Manson in 1878 - that mosquitoes were responsible for transmitting pathogens to humans - tremendous efforts have been made towards defining epidemiological patterns of diseases and determining the most effective means of reducing mosquito populations. During the past sixty years, the use of insecticides and physical measures to control mosquitoes has had a significant impact on improving human health and well being throughout the world. The appearance of residual insecticides in the early 1940's significantly reduced or eliminated many vector-borne diseases in various parts of the world. However, with the advent of insecticide resistance, progress toward disease control has suffered considerable setbacks and it is now apparent that chemical biocides are not in themselves a sufficient means of reducing or eliminating mosquito-borne diseases.

Other approaches to malaria control have focused on the human host, such as protection by vaccines and prophylaxis by suppressive chemotherapy to minimize opportunities for infection (Kaslow 1990, Ramasamy *et al.* 1997). Interruption of transmission is technically difficult in many parts of the world because of limitations in approaches and tools for malaria control. As a result, attention has turned to vector management where careful consideration is given to aspects of vector ecology including vector-host interaction. As such, it is necessary to broaden the knowledge of vector biology to achieve a greater understanding of disease patterns and the relationships that can influence them.

A mosquito's status as a vector of disease depends on many facets of its ecology and behaviour such as life span, distribution, immunities, preference for feeding on humans, biting rate and tendency to enter houses (Burkot *et al.* 1988, Koella 1991, Levine *et al.* 2004). Because the epidemiology of mosquito-borne diseases is so intimately related to the blood feeding habits of the female, knowledge of behavioural

patterns expressed during blood feeding is vital to understanding host-vector relationships and their respective roles in disease transmission. Such insights might improve mosquito management decisions and current mathematical models of mosquito-borne diseases. There is no doubt that mathematical epidemiology is a useful component of an efficient disease control program where models are based on relevant, verified data rather than incorporating assumptions about mosquito behaviour and parasite interaction with its vector (Koella 1991).

Below I discuss current knowledge of the behaviour and ecology of two key mosquitoes, *Anopheles gambiae* and *Aedes aegypti,* the species used in this experimental study.

1.2. *Anopheles gambiae*

The *Anopheles gambiae* complex is the most efficient vector system for the deadliest malaria parasite in Africa, *P. falciparum.* The complex is primarily responsible for approximately 80% of morbidity and mortality in sub-Saharan Africa (Levine *et al.* 2004). The *An. gambiae* complex includes 7 genetically distinct sibling species that can be identified on a cytogenetic basis or using molecular genetics (Coluzzi *et al.* 2002). There are five inland and two coastal species. Inland species collectively called *An. gambiae sensu lato* (*s.l.)* include *An. gambiae sensu stricto* (*s.s.*) Giles, *An. arabiensis* Patton, *An. quadriannulatus* Theobald species A and B, and *An. bwambae* White. The two coastal members of the *An. gambiae* complex that favour brackish water for larval development include *An. melas* Theobald in West Africa and *An. merus* Donitz in East Africa (Hunt *et al.* 1998).

There is significant variation between anopheline species in their choice of breeding site, distribution, host preference, resting-site preference and vectorial status

(Burkot 1988, Clements 1992). Within the *An. gambiae* complex, *An. gambiae (s.s.)* is the most efficient vector of human malaria because of its highly anthropophilic character, its preference for feeding indoors (endophagy), resting indoors (endophily) and ability to support *Plasmodium* parasites (Alavi *et al.* 2003). *Anopheles gambiae* is a nocturnal feeder, with most of the blood feeding occurring after midnight (Lindsay *et al.* 2002). Females preferentially feed off the lower extremities of the body including the feet (De Jong and Knols 1995).

Anopheles gambiae (s.s.) has a continent-wide distribution in Africa, from Madagascar to Senegal (Craig *et al.* 1999). It breeds mainly in small, temporary pools of sunlit water, devoid of vegetation. Particularly important are breeding sites created by human activity such as pools created by cattle footprints, grooves tracked by vehicle tires or irrigated fields.

1.3. Aedes aegypti

Aedes aegypti is the primary vector for the viruses that cause dengue and yellow fever (Putnam and Scott 1995). This species is found throughout most tropical and subtropical world regions, including North America, however it rarely occurs beyond latitudes of 40 degrees N to 40 degrees S. The northern and southern limits of distribution appear to be related to temperature (Christophers 1960).

This species is particularly abundant in towns and cities and is closely associated with human dwellings. In contrast to many other house-frequenting species, *Ae. aegypti* is active during the day with two periods of biting activity, one in the morning for several hours after daybreak and the other in the afternoon for several hours before dark. Whether in nature or in the laboratory setting, it feeds readily and consistently under most circumstances when given the opportunity. *Aedes aegypti* is highly anthropophilic

with a tendency to forego feeding on plant carbohydrates (Harrington *et al.* 2001). Foraging indices from a Thai study determined that *Ae. aegypti* fed on humans more than any other hosts available, including dogs, bovines, cats, chickens, and swine (Day *et al.* 1994).

Eggs are laid singly on damp surfaces just above the water line. Artificial containers (flower pots, rain gutters, bird baths) are often used as oviposition sites however, in tropical climates, eggs may also be laid in natural water-retaining cavities in tree holes and herbaceous plants. Eggs can withstand long periods of desiccation (more than a year) but will hatch when flooded by deoxygenated water.

1.4. Selection of Mosquito Species for Study

Anopheles gambiae and *Ae. aegypti* are species of particular interest in this study for several reasons. First, due to their importance as vectors of numerous human diseases, a greater understanding of their blood feeding patterns and host contact is essential to evaluate disease transmission patterns. Secondly, *An. gambiae* and *Ae. aegypti* have several ecological and behavioural attributes in common which give rise to their vector potential including: (1) high local abundance, although often seasonal; (2) good dispersal and colonizing ability; (3) ability to exploit different man-made environments (e.g. buildings, rice fields, vehicles); (4) a strong preference for feeding on human blood and (5) ability to support specific parasites or viruses.

Thirdly, the physical and behavioural differences between *An. gambiae* and *Ae. aegypti* make them excellent species to compare blood feeding behaviour and response to host disturbance. *Aedes aegypti* is a robust mosquito whereas *An. gambiae* has a comparatively smaller body size. And although both species are anthropophilic, their blood feeding behaviour and activity patterns differ significantly. *Aedes aegypti* is a

diurnal feeder and does not exhibit prediuresis, whereas *An. gambiae* is a nocturnal feeder with a comparatively longer feeding duration primarily due to their concentration of erythrocytes during prediuresis (Briegel *et al.* 1978). Anopheline mosquitoes are known to extrude considerably large amounts of red rectal fluid while ingesting blood. This process has been termed prediuresis and involves the passage of blood-derived fluids through the distended midgut into the hemolymph. The fluids are then excreted via the Malpighian tubules (Briegel and Rezzonico 1985, Vaughan *et al.* 1991) (Fig. 1.1). Erythrocytes are too large to pass and are concentrated in the midgut lumen (Vaughan *et al.* 1991). The process of prediuresis represents an efficient mechanism of concentrating the blood protein to allow the female to acquire a large amount of protein in a midgut limited to a small volume (Briegel and Rezzonico 1985).

Below I focus on blood feeding, a key aspect of mosquito behaviour contributing to their importance as disease vectors.

1.5. Hematophagy

"Hematophagy" is defined as feeding on the blood of a host organism by another organism. Natural cycles of mosquito-borne diseases depend on transmission of pathogens to human hosts during the act of blood feeding (Titus and Ribeiro 1990, Ribeiro and Francischetti 2003). Most female mosquitoes require a blood meal to obtain amino acids from erythrocytes and plasma protein digestion to synthesize yolk proteins for egg production (Hurd 2003). Autogenous mosquitoes do not require blood for egg production and develop their ovaries in the absence of blood feeding. Blood feeding can result in ingestion of pathogens, which the mosquito can transmit to hosts during subsequent blood feeding.

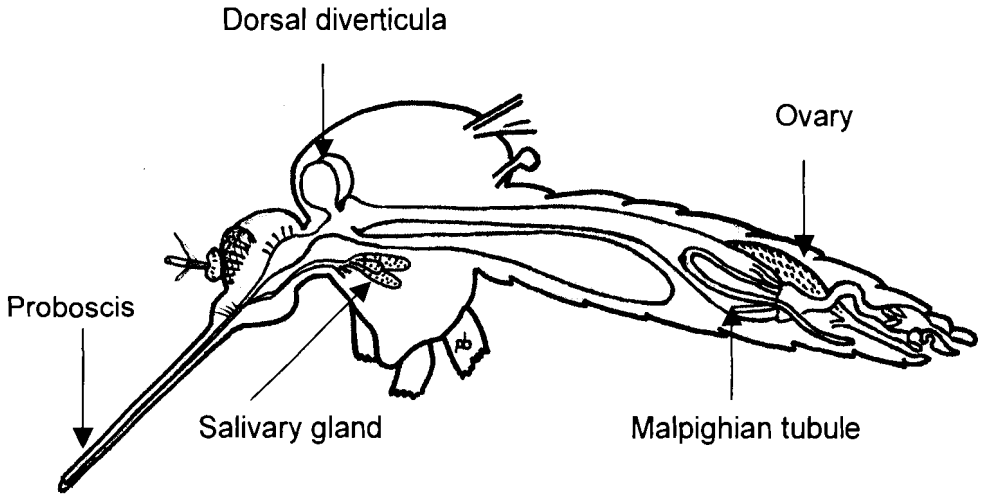

Figure 1.1 Diagram of the mosquito internal anatomy (diagram courtesy of Dr. P. Belton).

Blood feeding in mosquitoes can be divided into (1) blood searching and (2) blood feeding behaviours (Ribeiro *et al.* 1985, Ribeiro 1988).

1.5.1. Blood Searching Behaviour

Blood-searching behaviour is governed by two behavioural components, probing and giving up (Ribeiro *et al.* 1985). Once the mosquito lands on a host it searches for a suitable site to insert its mouthparts. The fascicle penetrates the host's skin and curves anteriorly, probing the tissue for blood in a series of rhythmic thrusts about 0.5 mm below the skin surface. During probing, the mosquito lacerates blood vessels and causes hemorrhages, increasing the relative volume of blood in the skin being probed. Laceration of the blood vessels triggers host hemostatic responses that consist of platelet aggregation, vasodilation and blood coagulation (Ribeiro 2000). Mosquitoes counteract hemostatic responses and facilitate blood feeding by injecting saliva that contains antihistamines, vasodilators and anticoagulants (Dhar and Kumar 2003, Ribeiro and Francischetti 2003). Saliva is injected during the probing phase and is continuous throughout the entire feeding process (Golenda *et al.* 1995).

A probe results either in successful blood location or re-probing. Re-probing involves complete withdrawal of the mouthparts and usually is followed by an attempt to probe at an adjacent site (Christophers 1960). If blood is located the mosquito senses purinergic phagostimulants, such as ATP and ADP, which signal contact between their feeding stylets and the blood (Ribeiro *et al.* 1984, Friend and Smith 1977). At this point, they shift from blood searching to blood feeding behaviour.

1.5.2. Blood Feeding Behaviour

Gordon and Lumsden (1939) described two types of blood feeding: (1) pool feeding, in which a capillary is ruptured by the tip of the fascicle and the blood is sucked from the small pool formed by the ensuing hemorrhage and (2) capillary feeding, in which the blood is taken up as a result of the fascicle penetrating into the lumen of a capillary.

A major difference between the blood feeding process of *An. gambiae* and *Ae. aegypti* is the ability of the former to concentrate erythrocytes while taking a blood meal through prediuresis (Briegel and Rezzonico 1985). In a study with *An. aquasalis, Ae. aegypti, Haemagogus janthinomys,* and *Culex quinquefasciatus,* Chadee and Beier (1996) found tremendous generic differences in parameters of the feeding process, including gut-filling, prediuresis and the total feeding time. The duration of prediuresis usually accounted for most of the differences in total feeding times.

The volume of blood ingested varies between and within species and is influenced by the gut dimension, duration of feeding and the rate of blood uptake (Ribiero 1987). Feeding duration and gut-filling times tend to be less variable among members of the same species (Ribeiro 1988, Vaughan *et al.* 1991). Differences in feeding duration have also been attributed to blood meal source, and genetic and environmental factors including larval habitats, available nutrients and climate (Chadee *et al.* 2002).

The rate of blood uptake is determined by the distribution of blood vessels in the host. Differences in skin vascularity influence the likelihood of penetrating a capillary while searching for blood. Studies with *Ae. aegypti* have shown that fast feeding (<2 min) may be associated with capillary feeding whereas mosquitoes taking blood from a hematoma may imbibe blood more slowly (Chadee *et al.* 2002).

Once the mosquito has fed to repletion, the fascicle is withdrawn and unless disturbed, engorged mosquitoes often remain on the host with the proboscis resting on the skin for several minutes (Christophers 1960).

1.6. The Blood Meal and Egg Production

Blood feeding triggers egg development in anautogenous mosquitoes. In most cases, a batch of eggs matures for each blood meal (gonotrophic concordance) although many species take several blood meals before eggs mature (gonotrophic discordance). The number of eggs produced in each batch varies greatly between species. For example, *An. maculipennis melanon* lays up to 500 eggs in the first gonotrophic cycle (Shannon and Hadjinicalao 1941), *Cx. pipiens* lays 250-400 (Christophers 1945), and *An. gambiae* and *Ae. aegypti* generally produce less than 100 eggs per gonotrophic cycle (Day *et al.* 1994,Takken *et al.* 1998, Woke *et al.* 1956). The number of ovarioles present in each ovary ultimately limits fecundity. The number of ovarioles can vary widely between and within species and it is positively correlated with body size (Briegel 1990).

The lower limit of fecundity is metabolic (Briegel 1985). Laboratory studies show that mosquito fecundity is affected by at least the following factors: body size and teneral reserves (Briegel 1990), source of the blood meal, i.e. host species, (Colless and Chellapah 1960, Taylor and Hurd 2001), size of the blood meal (Lea *et al.* 1978, Roitberg and Gordon 2005) and parasite infection (Hogg and Hurd 1995). Many of these factors are interlinked, however the size of the blood meal has been shown to play a major role in mosquito fecundity.

Most mosquitoes require blood to mature their eggs. From this it follows that the volume of blood ingested should influence fecundity such that the number of eggs laid in

a gonotrophic cycle is a positive function of the amount of blood ingested (Edman and Lynn 1975). Edman and Lynn (1975) found that in both mated and unmated female *Cx. nigripalpus,* larger blood meals resulted in corresponding increases in the number of females that developed eggs and in the number of eggs per female. In studies with *Ae. aegypti,* Colless and Chellapah (1960) discovered that the quantity of blood ingested during a single feed varied from trace amounts to nearly 5 mg, and mosquitoes that ingested less than 0.5 mg failed to initiate vitellogenesis and egg maturation. Blood meals above this size were positively correlated with the amount of blood ingested. The positive correlation between blood meal intake and fecundity is well established for many other species including *Ae. triseriatus* (Jalil 1974), *Ae. atropalpus* (Kalpage and Brust 1974), *Cx. tarsalis* (Downe and Archer 1975) and *An. elutus* (Yoeli and Mer 1938), though the quantitative relationships are still poorly understood (Roitberg and Gordon 2005).

1.7. Trade-offs Associated With Blood Feeding

Given that feeding longer will increase the size of the blood meal, why would a mosquito leave the host before ingesting a blood meal sufficient for maximum fecundity?

For mosquitoes, blood feeding has associated risks. First, important aspects of the feeding behaviour of any mosquito species include the defensive actions of the host in response to mosquito feeding. Feeding to repletion usually occurs in cases where there is no interference, such as in the laboratory where mosquitoes are offered an anaesthetised or restrained host. Under these conditions, mosquitoes will generally ingest a maximum blood volume physiologically determined by the limits upon the gut and body wall. Under natural conditions however, the act of feeding has a mortality risk since the pain sometimes caused by the feeding stylets and the hypersensitive response to salivary components induces nearly all hosts to display evasive or defensive

behaviour, resulting in ingestion of smaller blood meals, injury or death. Therefore, defensive behaviour in response to biting mosquitoes is an important factor in blood feeding success (Edman and Scott 1987). Second, a blood-fed mosquito has dramatically increased mass, resulting in slower flight and a greater risk of predation (Roitberg *et al.* 2003). Thus, remaining on a host to ingest a larger blood meal can increase reproductive output but may also shorten the lifespan. Since there is both a benefit and cost to remaining on a vertebrate host, mosquitoes face trade-offs in terms of blood feeding strategies.

Hematophagous insects are expected to balance the costs and benefits of blood feeding in an adaptive manner and to make decisions based on their physiological condition (Lima and Dill 1990). This may be true in mosquitoes, where feeding persistence will match the blood meal size required to mature a large batch of eggs and trade-off decisions will be made with regard to egg-batch size and the risk of mortality. Roitberg and Gordon (2005) suggested that females interrupted by hosts before they imbibe a blood meal that can be converted into the maximum number of eggs must 'decide' whether to try for more blood (and risk the accrued payoff) or to desist and lay fewer eggs. In their study with *An. gambiae,* they analyzed the fecundity payoff curve for blood feeding females and determined that females receive diminishing marginal returns with increasingly large amounts of blood (Fig.1.2). They hypothesized that mosquitoes should be more willing to leave the host when mostly versus partially full.

Very interesting in the above connection is the study by Woke *et al.* (1956). Their results showed a similar decelerating fecundity curve for blood fed *Ae. aegypti,* where fecundity was positively correlated with increased blood meal size up to a blood meal weight of 2mg, at which point there was no increase in egg production. In another study with *Ae. hexodontus,* Barlow (1955) found that the number of eggs laid was proportional

to the size of blood meal between 1 and 5 mg. Analysis of these results presents an important assumption: after ingesting a certain amount of blood, the mosquito gains little or nothing from a greater amount of blood and due to the danger incurred from the host, fitness should be greatest when the female minimizes the time spent on the host when fecundity (in terms of blood meal size) has reached a maximum. Based on this evidence, the amount of blood in the midgut is expected to influence mosquitoes' decisions to remain on defensive hosts (Roitberg and Gordon 2005).

My research is an elaboration of the hypothesis presented by Roitberg and Gordon (2005) wherein I compare the response of *An. gambiae* and *Ae. aegypti* to host disturbance during blood feeding.

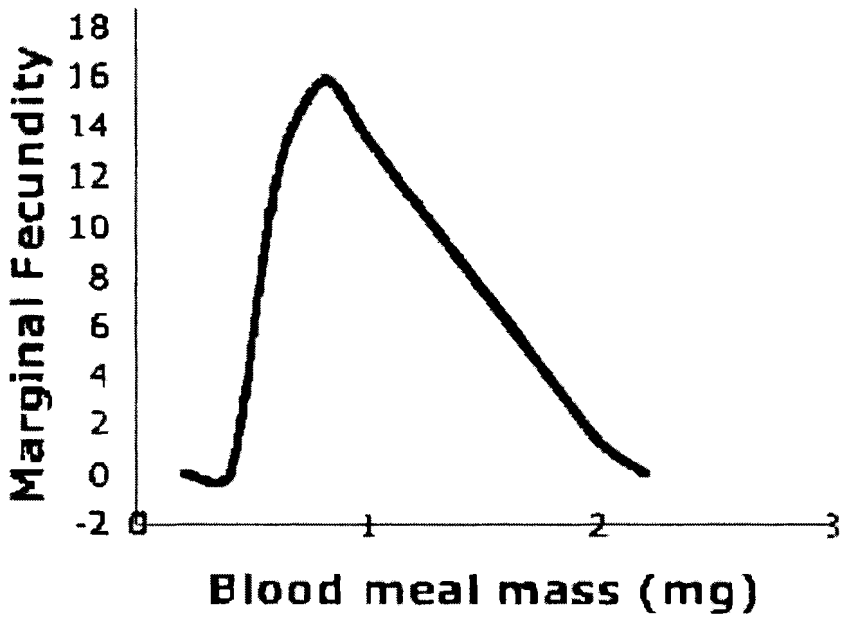

Figure 1.2 The relationship between the size of the blood meal (as measured by weighing the mosquito before and after blood feeding) and marginal fecundity returns in *Anopheles gambiae*. (Modified with permission from Roitberg and Gordon 2005).

1.8. Research Objectives

My research objectives were:

1. to ascertain the weight of the blood meal and fecundity as related to feeding duration.
2. to test the hypothesis that blood feeding mosquitoes are more willing to abandon the host when disturbed if they are mostly full rather than partially full. The null hypothesis is that there is no association between blood meal size/feeding duration and mosquito response to disturbance.

2. MATERIALS AND METHODS

2.1. Mosquitoes

Laboratory colonies of *Anopheles gambiae sensu stricto* (Ifkara strain) and *Aedes aegypti* (black-eyed Liverpool strain) were used in this study.

2.1.1. *Anopheles gambiae* Colony

An. gambiae was reared from a colony originating from Njag, Tanzania in 1997 and has been in laboratory culture at Simon Fraser University for four years. Two hundred to three hundred larvae were reared in plastic pans (30 X 60 X 6 cm) filled with distilled water to a depth of 3cm. Larvae were fed Nutrafin™ fish food daily for 5-7 days until pupation occurred. Food build-up on the bottom of each pan was periodically siphoned out and water was added to maintain a constant depth. A plastic pipette was used to transfer pupae to glass bowls which were then placed inside cages constructed of Plexiglas® and screen (20 X 20 X 20 cm), with a cotton sleeve attached to the front panel for internal access.

Each cage contained 50-150 adult males and females and these were maintained on a 5% solution of sucrose supplied *ad libitum* through braided cotton rolls. Distilled water replaced sucrose 24hr before experiments to encourage blood feeding during experiments. Mating occurred in the cages within two days of eclosion and the mosquitoes were blood fed on the arms of volunteers once each week for 20 minutes. On the day following blood feeding, a glass bowl filled with distilled water was set inside the cage and a 9cm d(iam.) filter paper was placed on the water's surface as an oviposition substrate. Eggs were transferred from the filter paper to distilled water in the

glass bowl and allowed to incubate for two days before being transferred to the plastic rearing pans where they hatched into larvae. Adults and larvae were reared in a Conviron™ 5440 walk-in environmental growth chamber maintained at 28 ± 3 ° C and 70 ± 8 % RH, and a 12:12 L:D photoperiod (9am:9pm).

2.1.2. *Aedes aegypti* Colony

Aedes aegypti rearing procedures are described by Christensen and Sutherland (1984). The established laboratory colony has been maintained at Simon Fraser University for two years.

Adult males and females were kept in a metal and screen cage (20 X 20 X 20 cm). Once per week females were allowed to blood feed through the mesh on the arm of a volunteer. A dish, half filled with distilled water and lined with moist paper towel was placed in the cage as an oviposition substrate. The paper towel was dried to a damp condition and the strip of eggs was placed in a zip loc bag for storage until needed.

Eggs were hatched by cutting a small strip of paper towel (containing approximately 1,200 eggs) and placing it into a 500ml Mason jar filled with 250 ml of autoclaved (low oxygen) water. Larvae were transferred to white enamel pans (40 cm x 25 cm x 8 cm) (200 larvae/pan) and were fed 1 ml of finely ground Tetramin® fish food daily. Pupae were collected with a plastic pipette, separated by sex (based on size) and transferred to 12 oz. cardboard Sweetheart Flexstyle Food Cups 12 oz. (50-100 adults/cup) filled with 100 ml of distilled water. After emergence, about 200 females and 100 males were transferred to a Plexiglas®-screen cage provided with cotton balls saturated with 10 % sucrose solution.

Adults and larvae were reared in a Goldstream™ walk-in environmental chamber maintained at 27 ± 2 °C and a 14:10 L:D photoperiod (7am:9pm).

2.2. Artificial Feeder and Experiment Cage

For both mosquito species, trials were conducted in a 30 x 30 x 30cm Plexiglas® cage with one open side (Fig.2.1). The open side was fastened to a plywood board (62cm X 42 cm) to create a base for the cage. Internal access was provided by a 9cm/d(iam.) hole cut out of the Plexiglas®. An artificial membrane feeder (Lillie Glassblowers) (6 ml capacity) used for blood feeding, was mounted onto the wooden base so it would be contained within the cage. The feeder was attached to the wooden base of the cage by O-rings tightly fastened around the Nalgene® tubing (8mm/d(iam.). (Fig.2.2). The feeder consisted of a glass chamber surrounded by a water jacket fed by Nalgene® tubing connected to a circulating water bath set at 37 °C (Fig.2.3). The opening of the glass feeder was covered with a small section (1.5cm^2) of dried sheep cecum which is easily penetrated by feeding mosquitoes. The membrane was stretched tightly over the opening and held in place with an elastic band. Then 5ml of blood was added with a pipette through an opening in the bottom of the feeder. A small stopper made of plasticine wrapped in Parafilm® was inserted into the hole to retain the blood. The stopper was inserted slowly to avoid trapping air bubbles within the blood/membrane barrier. This type of feeder is usually set in place with the membrane facing downward, letting gravity hold the blood against the membrane, and the mosquitoes feed in an inverted position. Blood feeding mosquitoes increase in weight as they ingest more blood. Therefore, in this study, the feeder was turned so the cecum membrane faced upwards to avoid the confounding factor of gravity on host-leaving decisions. This arrangement also made it an active process for mosquitoes to leave the membrane.

In the experiment, I attempted to disturb feeding mosquitoes in a consistent manner. This was accomplished as follows:

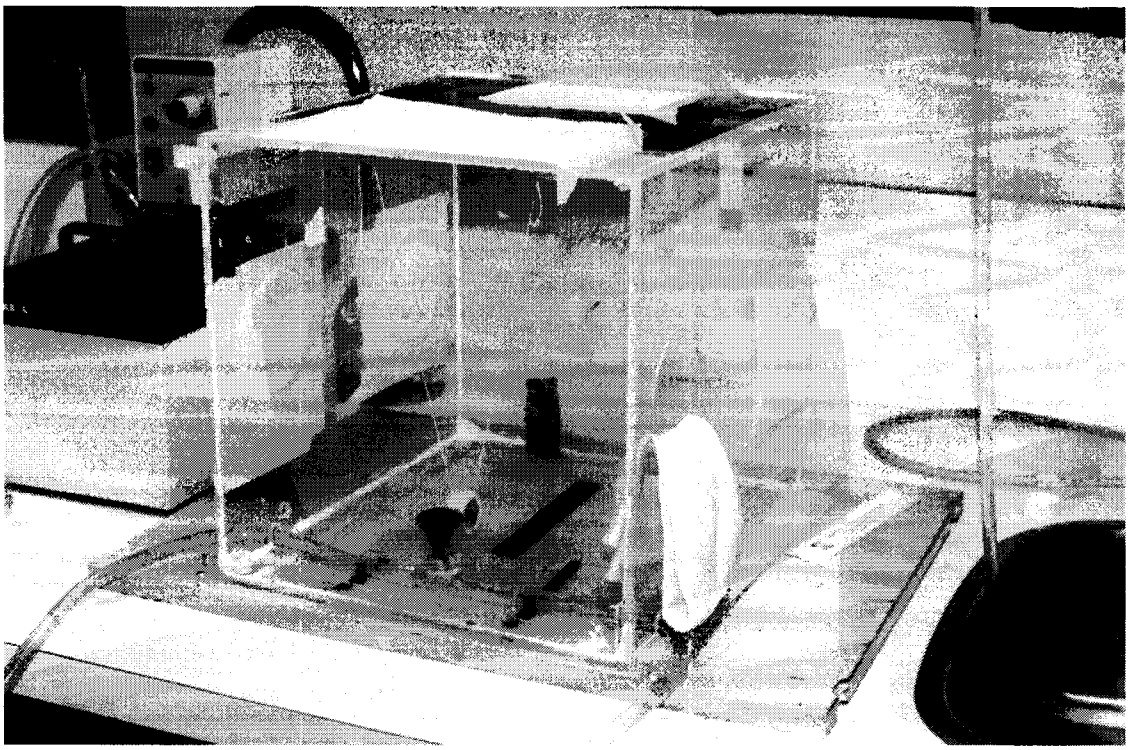

Figure 2.1 Photo of the Plexiglas® experiment cage showing the membrane feeder, Nalgene® tubing, pendulum and circulating water bath.

A brass ring (2 cm d(iam.), 1 cm width) wrapped in thin foam was suspended from the top of the cage so that, when at rest, it would hang alongside the membrane feeder. To disturb each mosquito while blood feeding, the brass ring was pulled back like a pendulum to a distance (12.5 cm) marked by a strip of Plexiglas® (Fig. 2.4A) and released, allowing it to strike the feeder once with a constant force for each trial (Fig. 2.4B). This represents a consistent disturbance for each trial. The motion of the pendulum caused the feeder to spring backward from its original position by 1cm. The elastic Nalgene® tubing repositioned the feeder after was displaced by the pendulum.

2.3. Blood Supply

Human blood for experiments was taken from the author by B.C. Biomedical personnel at Simon Fraser University. Blood was stored in the laboratory refrigerator in plastic cuvettes with sodium citrate (9:1) to prevent coagulation for a maximum of fourteen days before use in experiments.

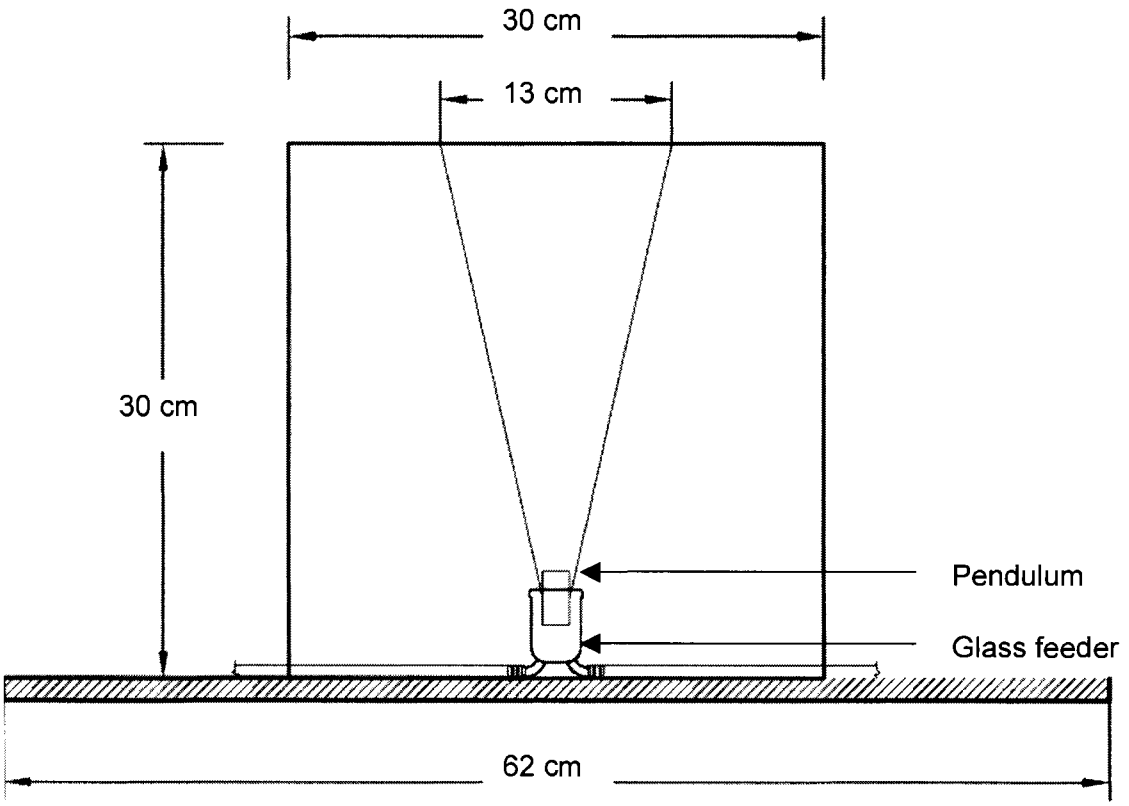

Figure 2.2 Front view of artificial membrane feeder shown attached to Nalgene® tubing.

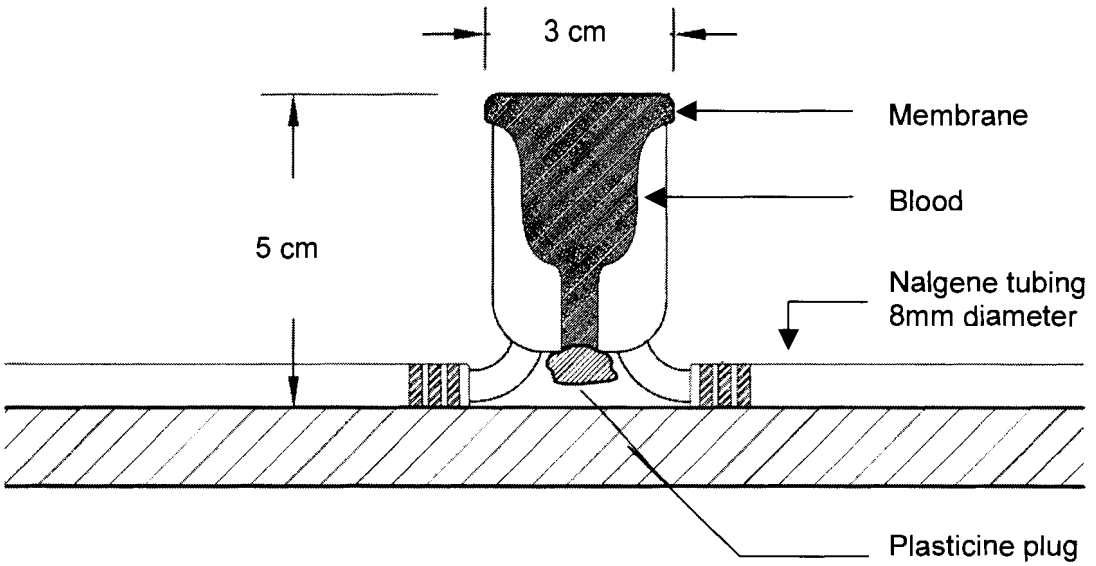

Figure 2.3 Close up of glass membrane feeder shown attached to Nalgene® tubing.

2.4. Blood Feeding

All experiments were conducted in the laboratory at 26-28° C and 30-70% ambient relative humidity. A small portable heater was placed near the experimental cage to regulate temperature. When working with *An. gambiae*, to mimic a dusk-like setting, all lights were turned off except for one fluorescent light at the opposite end of the room. All trials were performed between 1200h and 1900h, which coincided with the peak landing and biting times for both species, based on the light schedules in the chambers.

Previous studies with *Anopheles* have shown that the first blood meal may be used for the synthesis of reserves, whereas subsequent blood meals may be used for egg development (Briegel and Rezzonico 1985). Thus, in order to measure fecundity, *An. gambiae* were allowed to blood feed and lay eggs once prior to use in trials. (*Ae. aegypti* did not blood feed prior to trials). *Anopheles gambiae* used in the trials were 9-21 days old, *Ae. aegypti* were between 7-15 days old. All females were deprived of sucrose for 24h beforehand. Female mosquitoes were collected from the cage by inserting an arm through the sleeve and placing a 20ml glass scintillation vial over individual mosquitoes as they landed in preparation to feed. Before the mosquito could feed the vial was capped with a rubber stopper and the mosquito was immobilized by chilling for 1.5 minutes at 5 °C. Immobilized mosquitoes were weighed to the nearest 0.001 mg on a CAHN^{TM} balance, then returned to the vials and placed in the environmental chamber to warm for 40 minutes. After the warming period, mosquitoes in their vials were introduced into the experimental cage. In experiments with *An. gambiae,* a small amount of Limburger cheese was previously rubbed onto the membrane as an olfactory stimulus. Limburger cheese is an effective attractant for this species because the blend of volatiles from this cheese is very similar to foot odour (Knols and de Jong 1996) and

the foot is a preferred biting site for *An. gambiae*. As a feeding stimulus for both species one human breath was blown into to the cage while holding the inverted vial over the membrane. Once the mosquito landed on the membrane and began probing, the vial was removed. Mosquitoes were considered to have probed when the proboscis penetrated the membrane. Blood feeding time was measured with a stopwatch from the initiation of penetration to the withdrawal of the proboscis. In order to prevent the blood from settling between trials, the stopper was removed and the blood was agitated.

Mosquitoes were disturbed at four different stages for *An. gambiae* (1) at penetration of the membrane (probing stage), in which the female was disturbed when the proboscis entered the membrane. (2) After one minute of feeding. (3) After two minutes of feeding and (4) after four minutes of feeding. Three stages of incomplete feeding were tested for *Ae. aegypti* (1) Probing stage. (2) After twenty seconds of feeding and (3) after one minute of feeding. Feeding stages were selected after preliminary evaluation had indicated that the average times for *An. gambiae* and *Ae. aegypti* to feed to repletion were four minutes eleven seconds and one minute twenty one seconds respectively (n =25 for each species).

In feeding trials with individual mosquitoes, each length-of-feed was randomly predetermined by drawing marked coins. Each female was disturbed at the desired feeding stage by releasing the pendulum to strike the feeder. Individual responses to the disturbance during feeding were recorded as (A) remaining on the membrane or (B) leaving the membrane. If, during any of the trials, two minutes elapsed without any attempt to probe, the female was discarded and another was chosen. Individuals that finished feeding before the predetermined feeding period were not included. Individuals that did not leave the membrane when disturbed were immediately removed and all mosquitoes that fed successfully were chilled and reweighed as previously described.

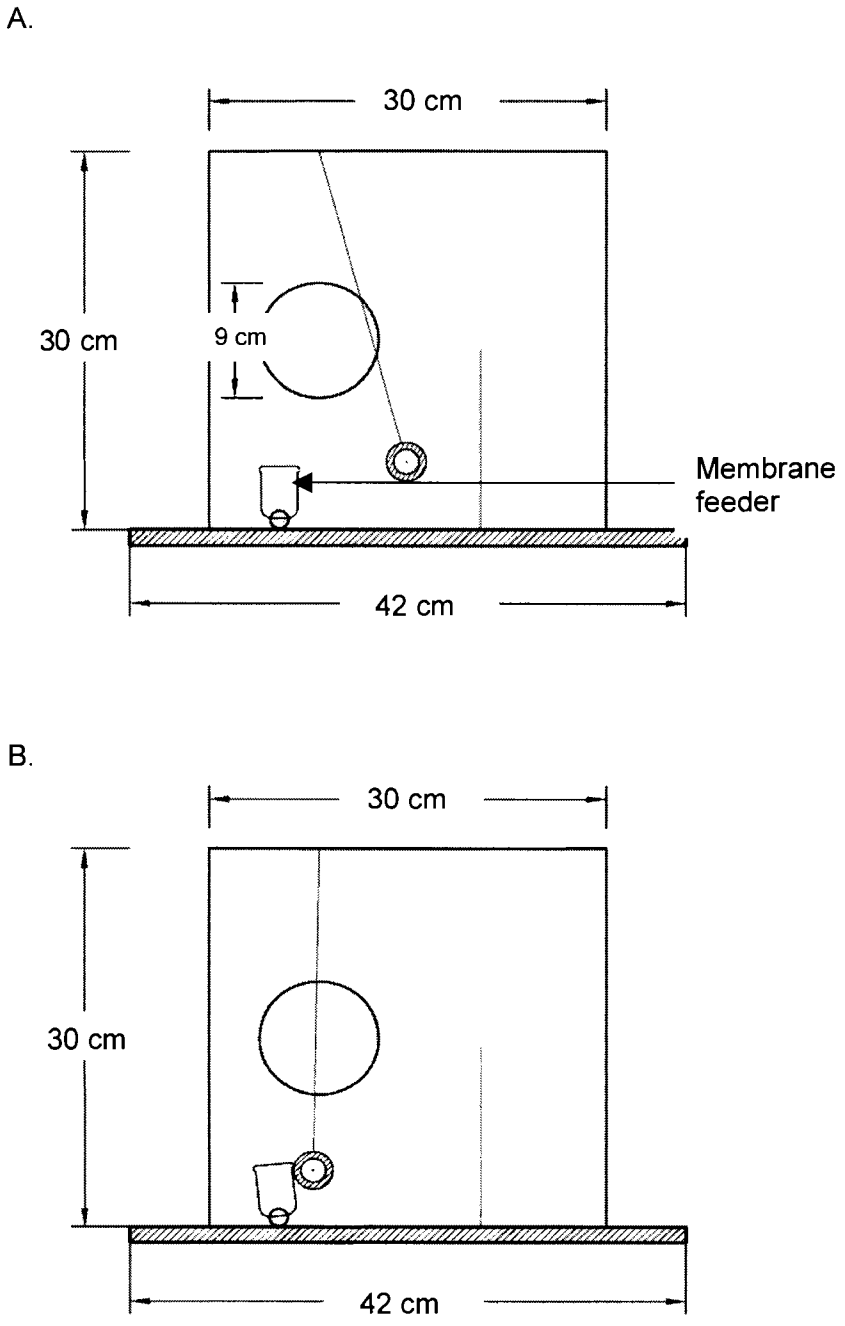

Figure 2.4 Side view of experiment cage showing pendulum in raised position (A) and in contact position (B).

Each mosquito was then placed in an individual Plexiglas® and screen cage (14 X 14 X 14cm) supplied with sucrose solution and an oviposition site. Cages were returned to the environmental chamber until oviposition occurred. Eggs were counted with a dissecting microscope.

Measurement of blood meals in anopheline mosquitoes is complicated by the concentration of erythrocytes in the midgut during feeding, since large amounts of urine and some cells are expelled. It has been suggested that, for species that exhibit prediuresis, protein intake should be assessed by measuring hemoglobin intake or by measuring its digested product, hematin (Hurd 2003). However, for convenience in this study, and since feeding decisions were also evaluated in terms of feeding duration, blood meal weight was taken as the difference between pre-fed and post-fed mosquitoes.

2.5. Comparison of Blood Sources

In the disturbance experiments for *Ae. aegypti*, only 60% of females produced eggs after feeding for 60 s, therefore one concern with this experiment was that egg production and fecundity might have been affected by the use of citrated blood. To determine if citrated blood affected egg development, an additional study was conducted to compare blood sources. Female *Ae. aegypti* were allowed to feed on the membrane (citrated blood) and from my arm until they voluntarily withdrew their proboscis (to repletion). Ten individuals were tested for each blood source. Females were used only once. Eggs were counted under a dissecting microscope.

2.6. Statistical Analysis

Proportions of mosquitoes disturbed during blood feeding experiments were compared using a logistic regression to determine whether observed responses differed significantly from responses under the null hypothesis, that there is no association between 1) feeding duration and the tendency to leave the host and 2) the weight of the blood meal and the tendency to leave the host. Other data were subjected to a *t*-test, Wilcoxon Rank Sums test (where results were not normally distributed) or an analysis of variance (one-way ANOVA) to compare means within each of the categories. That is, weight of blood meal, feeding duration and fecundity. A level of significance of P=0.05 was used in all analyses.

3. RESULTS

Preliminary tests determined the average time for *An. gambiae* and *Ae. aegypti* to feed to repletion and determined appropriate times to disturb their blood feeding. Twenty-five individuals were tested for each species. *Anopheles gambiae* took longer (251.05 s) to ingest a full blood meal than *Ae. aegypti* (81.08 s) (Fig. 3.1). The mean time for *An. gambiae* to initiate prediuresis (expel the first droplet of liquid) was 94.43 s (Fig. 3.1). *Ae. aegypti* did not exhibit prediuresis.

One-way ANOVA results showed that an increase in feeding duration produces an increase in blood meal weight for *An. gambiae* (P= 0.0004) (Fig. 3.2). After feeding for 60 s, 120 s and 240 s, the blood meal weights were 0.883mg, 1.025mg and 1.527mg, respectively. For *Ae. aegypti, t*-test results also showed that the blood meal weight increased significantly with feeding duration (P=0.0001). The weight of the blood meal increased from 0.529 mg after feeding for 20s to 1.317 mg after feeding for 60s (Fig. 3.3).

Regression analysis showed a statistically significant positive correlation between the feeding duration and the female's tendency to leave the membrane for both *An. gambiae* (P = 0.0021) and *Ae. aegpyti* (P= 0.048). For *An. gambiae,* 85%, 30%, 55% and 80% of females left the membrane when disturbed after feeding for 0s (probe phase), 60s, 120s and 240s respectively (Fig.3.4). For *Ae. aegypti,* 30%, 15% and 75% of females left the membrane when disturbed after feeding for 0s (probe phase), 20s and 60s respectively (Fig.3.5).

A (marginally) significant positive correlation was found between the weight of the blood meal and the tendency to leave the membrane for *Ae. aegypti* (P= 0.059) however, no such relationship was found for *An. gambiae* (P = 0.512)

Seventy-five percent, (15/20), 55% (11/20) and 85% (17/20) of female *An. gambiae* produced eggs after feeding for 60s, 120s and 240s respectively. The mean number of eggs laid was higher in females that fed for 240s (mean = 82.6) than those that fed for either 120s (mean = 72.9) or 60s (mean = 52.7) (P = 0.016). (One-way ANOVA) (Fig.3.6). Female *Ae. aegypti* also showed an increase in fecundity with feeding duration (P= 0.043) (Wilcoxon Test). Only 15% (3/20) of those fed for 20s produced eggs (mean = 13), and 60% of those fed for 60s produced eggs (mean = 38.3) (Fig.3.7). Zero values were excluded in fecundity analyses for both species because it was not clear why some females did not produce any eggs.

In the experiment comparing the fecundity of *Ae. aegypti* after feeding from the two blood sources: 1) artificial feeder and 2) human arm, 40% of female *Ae. aegypti* that fed to repletion from the artificial feeder produced eggs and 50% of those that fed to repletion on the human arm produced eggs. The mean number of eggs produced from a replete meal from the artificial feeder was 33.5, which was not significantly different (P = 0.94) when females fed from the human arm (32.8) (Fig. 3.8) (t-test).

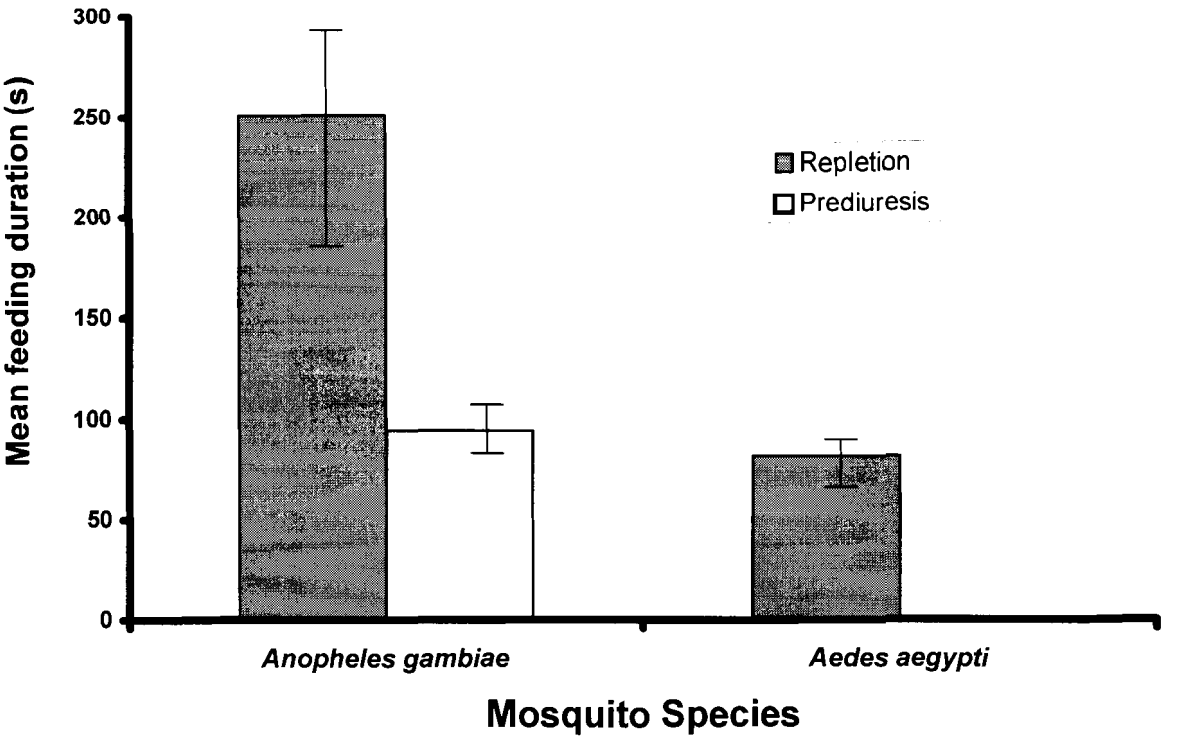

Figure 3.1 **Time taken by undisturbed *Anopheles gambiae* and *Aedes aegypti* mosquitoes to ingest a full blood meal from an artificial membrane feeder. The mean time for *Anopheles gambiae* to initiate prediuresis (expel the first droplet of liquid) is shown. *Aedes aegypti* does not exhibit prediuresis. (n = 25). Bars represent standard error of the mean.**

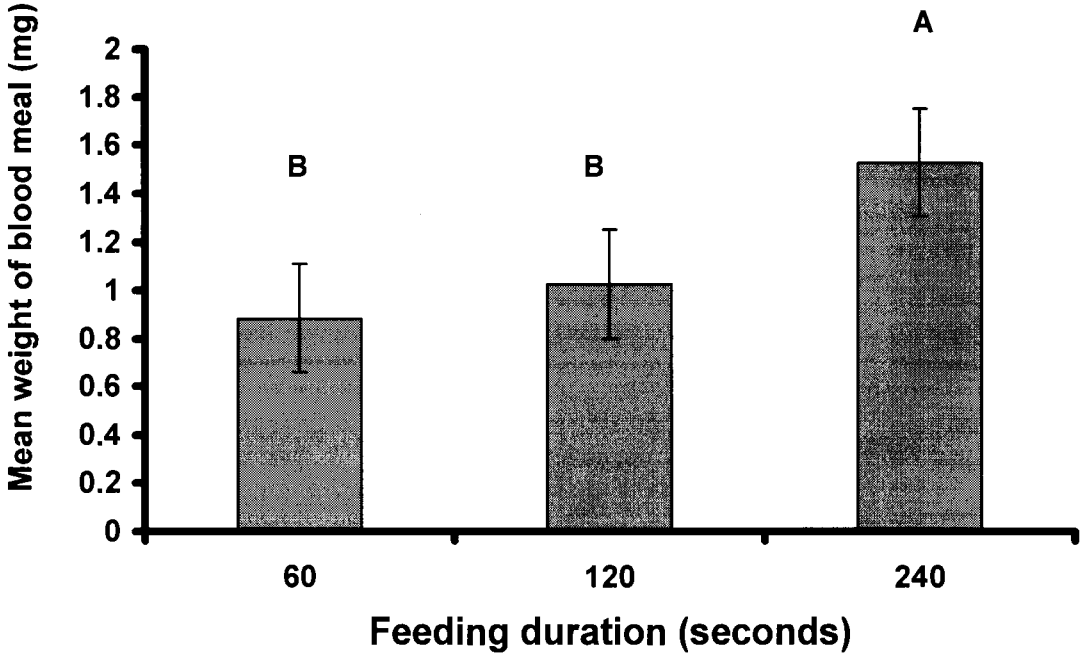

Figure 3.2 **Mean weight of blood meal ingested by *Anopheles gambiae* interrupted after blood feeding for different durations. (n = 20). Bars represent standard error of the mean. Letters above the bars indicate statistically significant differences. ($P < 0.05$, One-way ANOVA).**

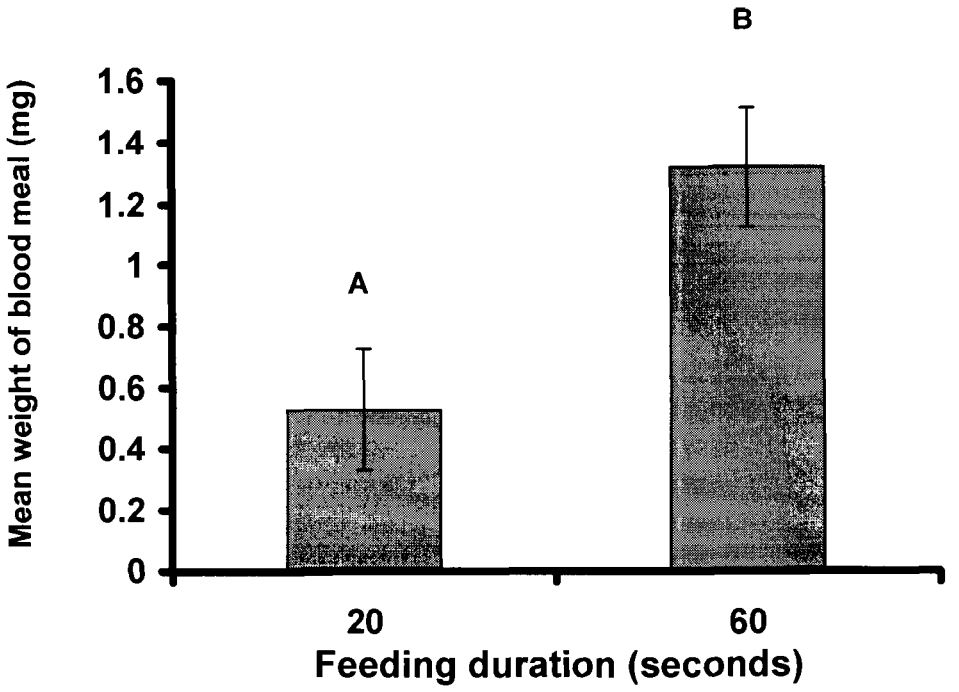

Figure 3.3 **Mean weight of blood meal ingested by *Aedes aegypti* interrupted after feeding for different durations. (n = 20). Bars represent standard error of the mean. Letters above the bars indicate statistically significant differences. ($P < 0.05$, t-test).**

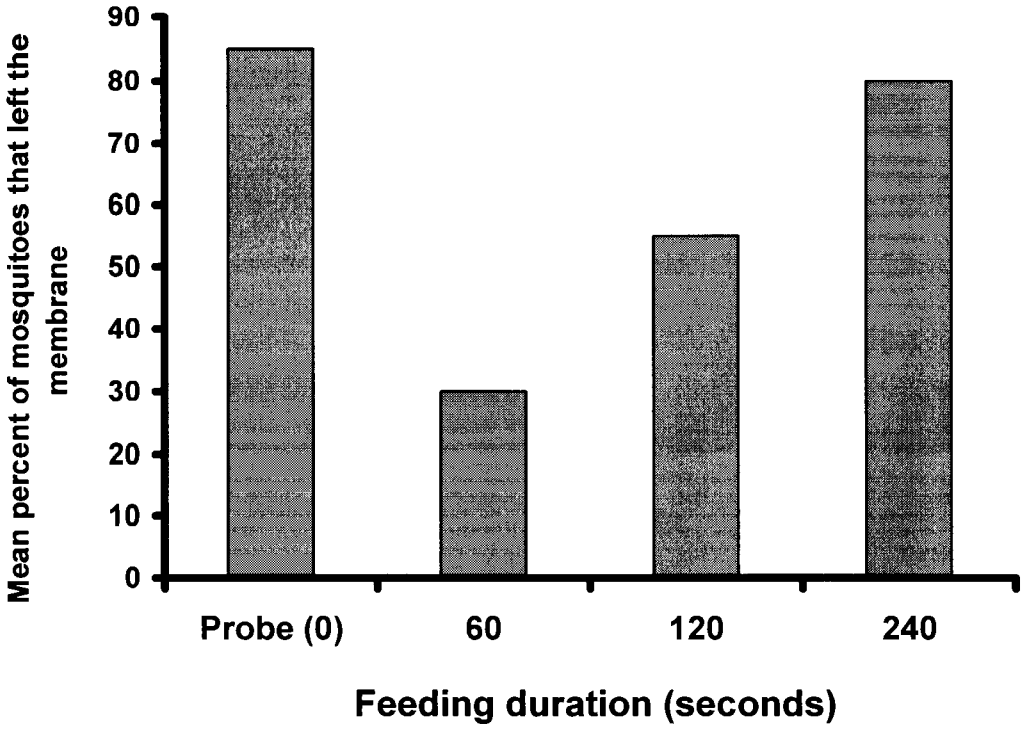

Figure 3.4 **Response of *Anopheles gambiae* to disturbance while blood feeding on an artificial membrane feeder (see text for definition of disturbance). Results are expressed as mean percent of individuals that left the membrane when hit by the pendulum. (n = 20).**

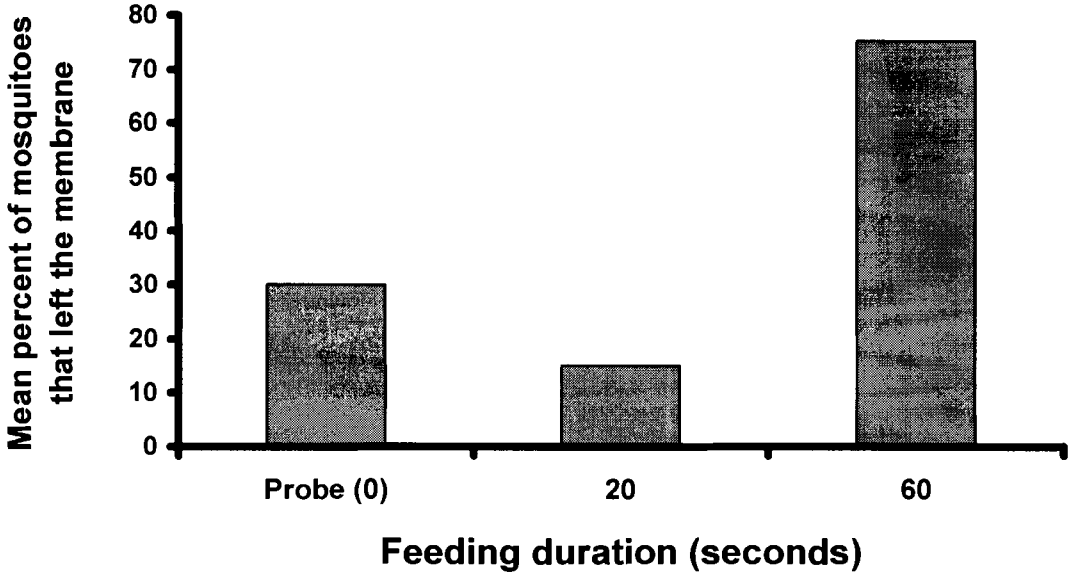

Figure 3.5 **Response of *Aedes aegypti* to disturbance while blood feeding on an artificial membrane feeder (see text for definition of disturbance). Results are expressed as mean percent of individuals that left the membrane when hit by the pendulum. (n = 20).**

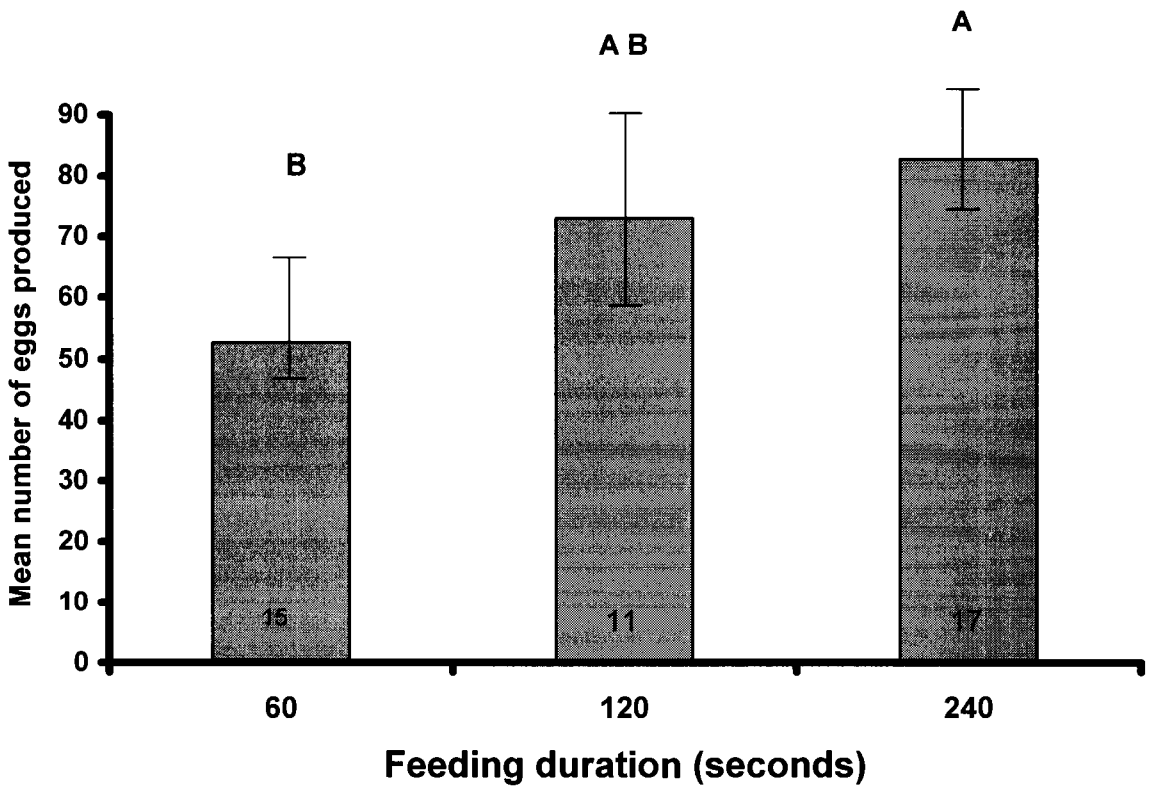

Figure 3.6 **Mean number of eggs laid by *Anopheles gambiae* after blood feeding for different durations. Bars represent standard error of the mean. Values within the bars indicate the numbers of mosquitoes that produced eggs. Letters above the bars indicate statistically significant differences. (n = 20). (P < 0.05, One-way ANOVA). Zero values were excluded in analysis.**

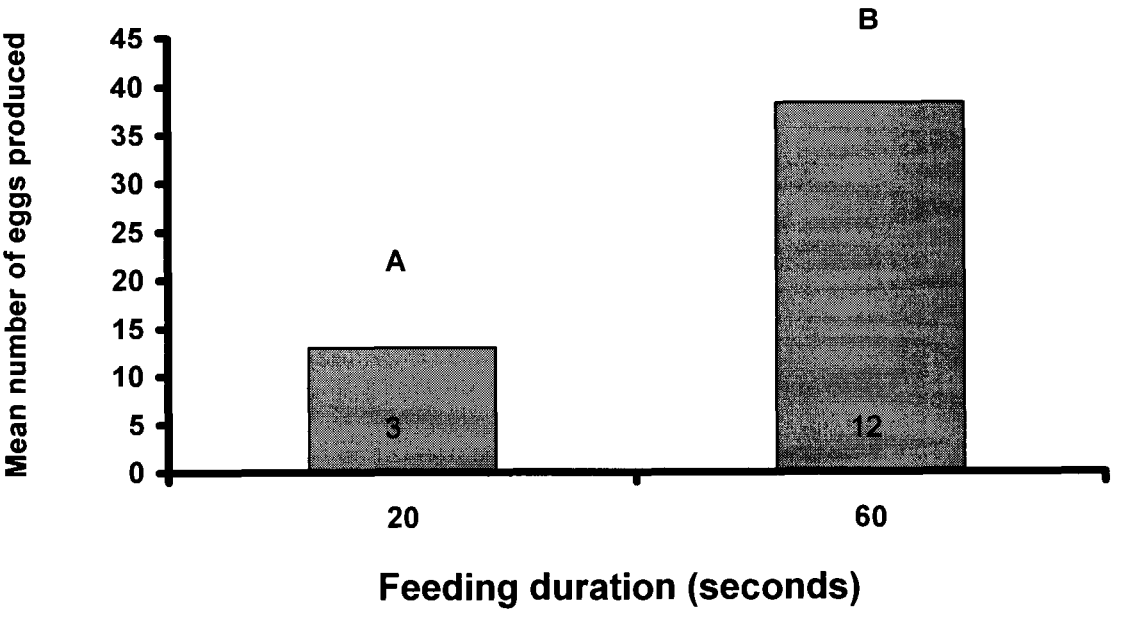

Figure 3.7 **Mean number of eggs laid by *Aedes aegypti* after blood feeding for different durations. Values within the bars indicate the numbers of mosquitoes that produced eggs. Letters above the bars indicate statistically significant differences. (n = 20). ($P < 0.05$, Wilcoxon Rank Sums test). Zero values were excluded in analysis.**

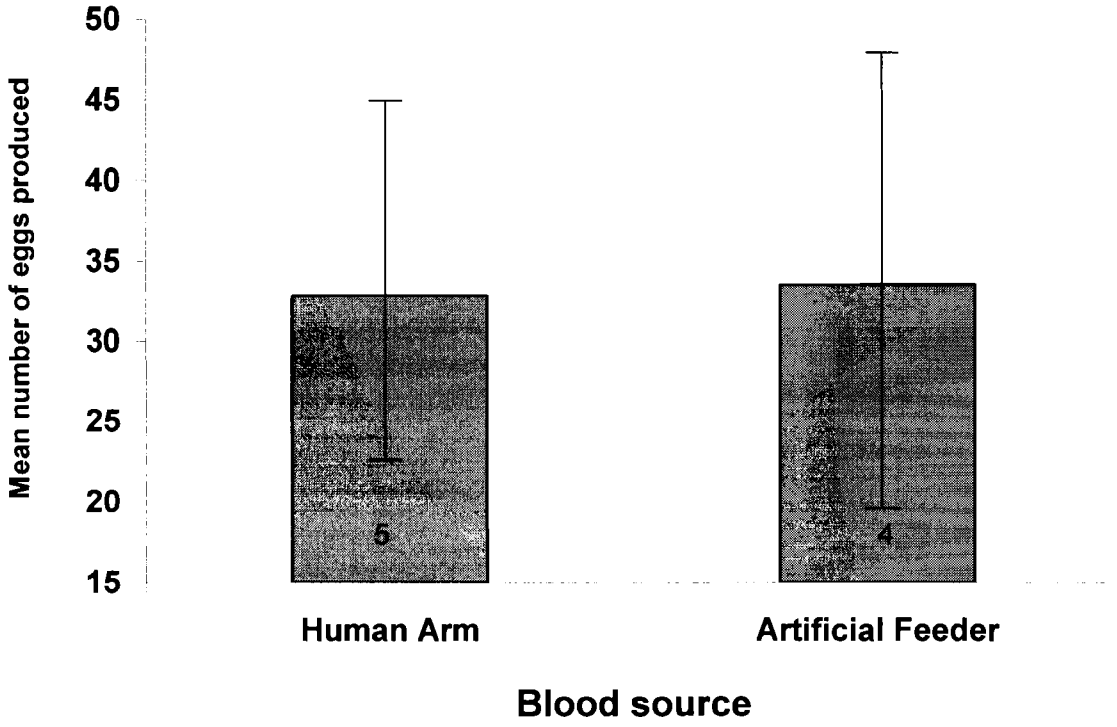

Figure 3.8 **Mean number of eggs produced by *Aedes aegypti* after ingesting a full blood meal from two different blood sources: 1) artificial feeder and 2) human arm. Bars represent standard error of the mean. Values within the bars indicate the numbers of mosquitoes that produced eggs. (n = 10). ($P < 0.05$, Wilcoxon Rank Sums test). Zero values have been excluded.**

4. DISCUSSION

Previous studies have shown that mosquito fecundity increases with increasing blood meal size (Colless and Chellapah 1960, Edman *et al.* 1975, Lea *et al.* 1978). This trend is confirmed in the present study wherein *An. gambiae* and *Ae. aegypti* produced significantly more eggs with increased feeding duration and blood meal volume. It was not clear why only 60 % of female *Ae. aegypti* produced eggs after feeding for 60 s, nor why their fecundity was low compared to other studies (Woke *et al.* 1956, Day *et al.* 1994). Some females may not have been inseminated. Also, the nutritional status of individual females might influence the fate of small blood meals (Bellamy and Bracken 1971). Blood can be used either for egg development or for body maintenance and recent data suggests that the proportion of a full blood meal directed to egg development may vary with the nutritional condition of the females (Harrington *et al.* 2001). Results from the blood source comparison experiment with *Ae. aegypti,* showed that fecundity was unaffected by citrating the blood. However, it should be noted that this only applies to my blood since it was the only type and since only ten individual mosquitoes were used in each category, additional studies should be conducted to confirm that citrated blood has no effect on fecundity.

Recently, Roitberg and Gordon (2005) demonstrated that mosquito fecundity does not increase linearly with blood meal size but levels off with increasing amounts of blood. They proposed that the tendency of blood feeding females to leave the host when disturbed should increase with the size of the blood meal. Due to the risk of injury or death while blood feeding, females should leave the host readily once they have ingested a blood meal volume that will produce the maximum number of eggs in one batch even though the midgut may not be filled to capacity. In this study the weight of

the blood meal and the duration of blood feeding influenced the response of *An. gambiae* and *Ae. aegypti* to disturbance while blood feeding. The results demonstrate that *An. gambiae* and *Ae. aegypti* are more willing to leave the host as their feeding duration increases and/or as their blood meal size increases. This conclusion was reached based on a logistic regression that showed a significant positive correlation between the duration of blood feeding and the female's tendency to leave the host (in both species) and a significant positive correlation between the weight of the blood meal and tendency to leave the host (in *Ae. aegypti*).

The cost of reproduction is a central concept in life history theory in blood feeding insects. In the case of mosquitoes, reproductive costs arise as a consequence of host defensive behaviour, which can result in death, smaller batches of eggs or both. How then, do female mosquitoes assess the optimal amount of time to spend blood feeding on a defensive host? One hypothesis is that mosquitoes assess their current blood meal state and integrate this information with relevant information from the environment pertaining to the likelihood of escape, such as host species, reaction speed, persistence and physiological state. The biological rationale for this is that mosquitoes can assess the volume of blood ingested (Gwadz 1969). Gwadz (1969) first investigated the role played by the central nervous system in regulating blood meal size. In an experiment with *Ae. aegypti,* he cut the ventral nerve cord at various sites along its length. When the cord was cut anterior to the second abdominal ganglion, massive hyperphagia resulted, many females ruptured, and blood uptake was more than 4 times that of the untreated controls. As the site of the operation was moved posteriorly, leaving more ganglia connected to the brain via the ventral nerve cord the degree of hyperphagia and quantity of ingested blood was reduced. He concluded that termination of feeding was initiated by

segmental abdominal stretch receptors that act in concert and signal the presence of optimal blood meal volume to the brain.

Roitberg *et al.* (2003) stated that mosquitoes should be able to accurately assess its blood volume, whereas the likelihood of escape is a complex variable that depends on a number of probabilistic variables such as host species, reaction speed, site of feeding, persistence and physiological state. This reasoning implies that mosquitoes, in common with many organisms, are able to adjust their investment in reproduction based on present risk and physiological conditions. There is a growing body of evidence that insects do indeed possess the ability to (i) assess the risk of predation and (ii) incorporate this risk into their decision making (Lima and Dill 1990, Feener 1988).

For mosquitoes, the most important aspect of integrating current blood meal state with the likelihood of escape is likely to be defensive host behaviour. In the wild, the principal reason mosquitoes leave their host before ingesting a full blood meal is believed to be interruption of feeding caused by host movement or defensive behaviour (Klowden and Lea 1979). Defensive behaviour is a strong selective force (Edman and Scott 1987) and might be one of the selective pressures in the evolution of blood feeding behaviour. In this study, both *An. gambiae* and *Ae. aegypti* were persistent (i.e. remained on the membrane) during the gut-filling stage despite disturbance. Gut-filling is distinct from the probing phase and host defensive behaviour occurs primarily during the landing and probing stage (Burkot 1988). Females engaged in gut-filling benefit from persistent behaviour by increasing their fecundity, whereas females that have just begun to probe have no fitness gains to lose and much to gain by leaving a defensive host and attempting to feed on a different one. Most mosquito species therefore, would be expected to demonstrate persistent behaviour (although to varying degrees) during the gut-filling stage and greater differences in decisions to abandon hosts might be seen

during the probing and landing phase. Not all vertebrates respond equally to blood feeding mosquitoes. The intensity of host defensive behaviour will contribute to differences in the degree of feeding persistence among mosquito species. The intensity of host behaviour, while related to certain mosquito biting characteristics (e.g. biting density), varies according to several factors such as their species, and body size (Klowden and Lea 1979). For example, the intensity of antimosquito behaviour elicited by avian hosts will be significantly greater than bovine hosts due to the birds' predatory nature and level of normal body maintenance behaviour.

Individuals of the same host species will not consistently interrupt feeding with the same intensity. Species-specific defensive behaviour has been shown to influence mosquito engorgement success. Factors such as health, age and activity patterns will also influence the response of a host to blood feeding mosquitoes. For example, Day and Edman (1983) and Day *et al.* (1983) showed that mice experimentally infected with *Plasmodium yoelii* were less defensive due to the disease and consequently were preferentially fed upon by mosquitoes.

Mosquito species also vary in their biological traits such as biting rate, persistence and host preference (Harrington *et al.* 2001, Takken *et al.* 1998). Variation in the degree to which individual mosquitoes tolerate host disturbance may be a significant factor that determines inter- and intraspecific differences in feeding persistence levels. If host behaviour has contributed to the evolution of these responses, variation in tolerance to host behaviour would be expected in species that differ in host preference such as ornithophilic, zoophilic and anthropophilic species.

A mosquito adapted to feeding on avian hosts (e.g. *Cx. pipiens*) or human hosts (e.g. *Ae. aegypti*), which generally display intense antimosquito behaviour, face a high degree of risk when blood feeding. During the probing phase, mosquito species that preferentially feed on actively defensive hosts might be more persistent than species

that feed on tolerant hosts because for species that feed on actively defensive hosts, the probability of locating another host that elicits a lower degree of defensive behaviour is minimal. Whereas mosquito species that feed on primarily tolerant hosts might occasionally come across an unusually defensive host. In this instance, the mosquito would have an increased likelihood of encountering another host with a significantly lower degree of defensive behaviour. Generalist feeders will encounter a variety of defensive behaviours and may not be well adapted to certain host species. For example, *Cx. quinquefasciatus* has a strong tendency to orniphagy and appears to have only recently adapted to mammals (Ribeiro 2000).

In this study, two anthropophilic species were investigated. *Anopheles gambiae* was relatively less tolerant of host disturbance during the probing phase than *Ae. aegypti.* Perhaps *Ae. aegypti* has evolved a feeding strategy such that they are more likely to ingest a full blood meal from one host despite defensive movement so they do not have to find another equally defensive host. *Ae. aegypti* is a diurnal host seeker in which the mortality risk associated with landing and probing is likely to be quite high compared to that of anophelines, which are primarily crepuscular and feed when humans are least active. Sleeping humans during the nocturnal feeding period could shape the feeding behaviour of *An. gambiae* such that the female is inclined to leave a host while probing at the first sign of defensive behaviour in search of one more tolerant.

Feeding duration might also influence host abandonment decisions. Tremendous interspecific differences in feeding duration have been documented (Chadee and Beier 1996, Ribeiro 2000) and fast feeding (< 2 min) species such as *Ae. aegypti* might be more willing to remain on a host despite disturbance because a full blood meal can be ingested quickly. Prolonged feeding durations have been documented most notably in

species that exhibit prediuresis, so similar behavioural responses might be seen among some anophelines.

Anopheles gambiae did not show a significant positive correlation between the mean weight of blood meal and tendency to leave the membrane. A possible explanation for this is that mosquitoes assess the bloodmeal-fecundity curve exclusively in terms of time allocated to blood feeding. Indeed there is interspecific variation in blood feeding kinetics (Chadee and Beier 1996) and gut-filling tends to be less variable among species. However, previous reports have shown that smaller females, resulting from marginal larval diets feed for shorter times (Klowden and Lea 1978, Briegel 1990), indicating that termination is not related to time allocation, but is related to the size of the abdomen.

Furthermore, the lack of a positive correlation between blood meal size and tendency to leave in *An. gambiae* may be explained by prediuresis in this species (Briegel and Rezzonico 1985). As in many hematophagous insects (Sadlova *et al.* 1998), prediuresis begins in many anopheline species shortly after ingestion of blood. The mechanical process of prediuresis makes my gravimetric measurement of blood meals more complicated in *Anopheles* species than in *Aedes* species. In a study with *An. stephensi, An. albimanus* and *An. quadrimaculatus,* the average hemoglobin content of the midgut at the end of feeding was at least double that determined by weighing (Briegel and Rezzonico 1985). Additionally, Vaughan *et al.* (1991) demonstrated that, as a result of prediuresis during feeding, *An. gambiae* can concentrate the blood meal by 1.89 times. Thus, weighing mosquitoes can substantially underestimate blood consumption values.

The mean time for *An. gambiae* to expel fluid (initiate prediuresis) in this study was 94.52 s. Based on evidence from previous reports (Briegel and Rezzonico 1985,

Vaughan *et al.* 1991), measurements of blood meal weight beyond this time would have been underestimated. Therefore, I also used feeding duration to measure gut-filling. *Aedes aegypti* does not exhibit prediuresis and in this study, these mosquitoes did demonstrate a positive correlation between the mean weight of blood meal and tendency to leave when disturbed.

To avoid underestimating the blood meal weight due to prediuresis, the HiCN procedure developed by Briegel *et al.* (1978) could be used to measure the volume of blood ingested. It is based on the conversion of hemoglobin in the blood meal to hemiglobincyanide (HiCN), which is evaluated spectrophotometrically and compared to a standard curve of the optical densities of different host blood volumes. This method would allow the blood volume to be measured without weighing individual mosquitoes before and after feeding. However, the insect must be killed, eliminating the opportunity to measure fecundity.

It should be noted that laboratory colony conditions, i.e. using an unresponsive host (human arm), may have reduced selective pressures for feeding persistence resulting in behaviour not representative of that observed in nature. The use of field-collected females for blood feeding studies may represent an ideal way to obtain information on natural behaviours in the absence of biases associated with laboratory colonization.

Concepts proposed by Roitberg and Gordon (2005) regarding mosquito response to host behaviour are elaborated in the present study, and some important parameters that might mediate the trade-off between leaving and staying on the host are identified, however only two mosquito species were investigated. It is difficult to predict mosquito response to host behaviour while blood feeding as it applies to other mosquito genera because of the inherent differences in feeding behaviour that influence host

contact including innate host preferences, blood feeding frequency (Anderson and Brust 1995, Burkot *et al.* 1988), duration (Chadee and Beier 1996) and blood meal volume.

The relevance of my results and predictions as they apply to other mosquito species or genera should be confirmed with further studies of mosquitoes with physiological, ecological and behavioural differences. The methods described in this work provide a novel system for future studies on disturbance behaviour in that it provides a repeatable, constant level of disturbance to blood feeding mosquitoes.

It has become increasingly clear that the blood feeding habits of female mosquitoes are of critical importance in understanding disease transmission. The size of the blood meal has important implications for the vector potential in natural populations (Albuquerque *et al.* 1999). McGreevy *et al.* (1982) found that for a variety of *Culex, Aedes* and *Anopheles* species, variations in microfilariae uptake reflected the mean volume of blood ingested. Albuquerque *et al.* (1999) also noted that, compared to *Ae. aegypti, Cx. quinquefasciatus* always ingested more microfilariae from the host blood and attributed this to its ability to take up twice as much blood.

Here, *An. gambiae* readily left the membrane when disturbed during the probing phase. Probing without ingestion of sufficient blood to initiate completion of ovarian development increases the incidence of multiple feedings (Klowden and Lea 1979, Golenda *et al.* 1995). Multiple feedings during a single gonotrophic cycle can increase the chances of acquiring parasites from an infected host, as well as spreading patent infections to uninfected hosts (Klowden and Lea 1979, Putnam and Scott 1995). In contrast, *Ae. aegypti* did not readily leave the membrane when disturbed while probing. A consequence of this behaviour is reduced number of host contacts in a single gonotrophic cycle.

Behavioural components of vector competence are important factors in mathematical models that estimate transmission rate by vector species by making the models more realistic. Thus the parameters of blood feeding measured in this study are relevant to understanding the dynamics of pathogen transmission by mosquitoes.

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