THE EFFECTS OF VARIOUS CARBOHYDRATE SOURCES ON LONGEVITY AND NUTRITIONAL RESERVES OF *Culex quinquefasciatus* SAY, *Culex nigripalpus* THEOBALD AND *Culex salinarius* COQUILLETT

By

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To my mom and dad, Jocelyn Paul and Jeffrey Vrzal. I would also like to dedicate this to all of my friends and family who have provided love and support throughout my life and my educational career. Without you, this would not have been possible.

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Abstract of Thesis Presented to the Graduate School
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Culex species are important vectors of West Nile virus, Eastern Equine Encephalitis virus, St. Louis Encephalitis virus and lymphatic filariasis. While these mosquitoes feed on a wide range of nectar sources consisting of varying concentrations and amounts of carbohydrates and amino acids, little is known about the utilization of these different carbohydrates, their accompanying amino acids and their effect on longevity and deposition of nutritional stores in different species of mosquitoes. In this thesis, male and female *Culex nigripalpus*, *Culex quinquefasciatus* and *Culex salinarius* were fed a variety of single sugars, including monosaccharides (glucose, fructose, sorbose and mannose), disaccharides (sucrose and trehalose) and trisaccharides (melezitose and raffinose), as well as a water control and a *Lantana camara* nectar mimic with and without amino acids, all of which have been reported to support varying levels of survival. The effects of feeding these single carbohydrates as 5% (w/v) solutions or nectar mixtures (with or without amino acids) and water controls *ad libitum* had on 50% survival of each species was determined. Nutritional deposition in *Culex quinquefasciatus* males and females was also examined by feeding 5% (w/v) solutions of sorbose, mannose, melezitose and sucrose only. Overall, females lived longest on nectar sugars sucrose, glucose and fructose, but

survival on melezitose, a honeydew sugar allowed accumulation of large amounts of lipid and glycogen and enhanced survival in *Culex quinquefasciatus* as well as the nectar sugars. Male survival was enhanced by raffinose, another honeydew sugar, and they also displayed increased survival when fed common nectar sugars with nutritional reserves of *Cx. quinquefasciatus* increasing as a result of feeding on nectar and honeydew sugars sucrose and melezitose. The addition of amino acids into the adult diet of female *Cx. quinquefasciatus* increased survival, but only when fed a low nutrient food diet as larvae. These results underscore the importance of considering larval nutritional conditions, and recognizing their potential to result in decreased nutritional reserves upon emergence, decreased adult survival and dependence on a well-nutritioned adult diet to overcome larval deficits if larval conditions are poor.

CHAPTER 1
INTRODUCTION

Mosquitoes

Taxonomy

The genus *Culex* Linnaeus is in the family Culicidae with 29 species in North America north of Mexico and 15 species occurring in Florida (Darsie and Ward 2005). There are three subgenera, *Culex, Melanoconion* and *Neoculex*. This thesis uses mosquitoes that are included in the subgenus *Culex,* that are found in the subtropical and tropical regions of the world. The species used in this thesis are *Culex nigripalpus* Theobald, *Culex quinquefasciatus* Say and *Culex salinarius* Coquillett.

The three *Culex* species mentioned are the most common in Florida and have been previously grouped together as simply "*Culex* spp." (Provost 1969). There have since been clear distinctions made between these species; including variable habitats, behaviors and identifiable characteristics. *Culex quinquefasciatus* is a part of the *Culex pipiens* complex and was formerly known as *Culex fatigans* (Foster and Walker 2002). It lives in the southern range of the complex and readily hybridizes with *Cx. pipiens* (which has a northern range) in the mid-range of the U.S. where both species occur simultaneously.

Biology and Distribution

Culex nigripalpus display crepuscular behaviors, feeding early in the morning and at dusk, as do many *Culex* spp., and are known to feed primarily on avian hosts during the dry winter months and very opportunistically on everything from birds in dry winter months to horses, cows and armadillos to humans during wet summer months in Florida (Day 1997). *Culex quinquefasciatus* are also crepuscular mosquitoes and feed primarily on avian hosts in the U.S., but will feed on humans (Zinser et al. 2004). However, they are thought to be mostly

anthropophilic in Africa and India, thriving in urban areas where breeding conditions are optimal and human populations are dense (Chandler et al. 1975, Subra 1970, 1981). Similarly, *Culex salinarius* are also active at dawn and dusk and are very opportunistic when it comes to host feeding (Edman 1974, Molaei et al. 2004). All of these species lay their eggs in the form of rafts on the top of water, but *Culex quinquefasciatus* prefer polluted oviposition sites, whereas *Cx. nigripalpus* and *Cx. salinarius* prefer to lay their eggs in fresher water such as roadside pools. Additionally, *Culex salinarius* may sometimes prefer brackish water (Murphey 1961).

Culex nigripalpus are found in Central and South America, Mexico the Caribbean Islands and the southeastern United States from Texas to Kentucky and down into Florida, where they are most abundant in the southern part of the state (Provost 1969, Day 1997). *Culex quinquefasciatus* are widely distributed in the tropical and subtropical areas of the world, including Africa, Asia and in southern Japan and the United States (Subra 1981). In the U.S., it is restricted to the southern part of the country. *Culex salinarius* is most abundant along the Atlantic and Gulf Coasts of the United States where it is a severe nuisance species.

Vectoring Capabilities

Culex nigripalpus is the primary vector of St. Louis encephalitis (SLE) in Florida (Chamberlain et al. 1964, Dow et al. 1964, Day 1997) and Eastern Equine encephalitis (EEE) has been frequently isolated in this mosquito (Wellings et al. 1972). *Culex quinquefasciatus* are the main vectors of *Wuchereria bancrofti*, the cause of bancroftian filariasis (Subra 1981) in the neotropics and Asia (Hawking 1973), India (Samuel et al. 2004), Australia (Chow 1973), East Africa (Nelson et al. 1962) and reportedly in rural West Africa (Dossou-yovo 1995). *Culex quinquefasciatus* is also a known vector of SLE (Jones et al. 2002, Foster and Walker 2002) and West Nile virus (WNV) (Sardelis et al. 2001) in the southeastern United States. *Culex salinarius* is a potential vector of EEE (Scott and Weaver 1989) and has been implicated an important

bridge vector of WNV to humans in the Northeastern U.S. due to its opportunistic feeding behaviors (Molaei et al. 2004, Zyzak et al. 2002).

Longevity

Survival in insects is often studied due to its connection with the ability of those insects to transmit disease. This is especially true in mosquitoes where early studies of longevity in relation to malaria vectors showed that longer lived *Anopheline* mosquitoes tended to be vectors of the disease in particular areas where other, shorter lived *Anophelines* were not (Treillard 1938). In a field setting longevity is influenced by many factors, including larval and adult diets, mating status, temperature, humidity, and predation (Smith 1975). It has thus far been difficult to study survival of mosquitoes in field settings, though Reisen et al. (1991), Muir and Kay (1998) and others, have attempted mark-release-recapture studies, they are only successful at looking at daily survival times. Many studies have focused on longevity in the laboratory as a means for understanding the relationship between survival and vectoring capability of various species (Galun and Fraenkel 1957, Nayar and Sauerman 1971a, 1975, Eischen and Foster 1983, Briegel et al. 2001). Despite the many factors involved in the survival of mosquitoes in the field, the only way to answer many of these questions about vectoring capabilities are to conduct laboratory studies mimicking as many of the factors as possible. *Culex* mosquitoes are particularly long-lived mosquitoes which allow them greater opportunities to contact multiple hosts, including humans as well as the ability to vector diseases which require a longer life-span.

Sugar Feeding

Many have recognized sugar feeding as an activity in which both males and females partake and a critical factor to increase survival and dispersal to the point of vectoring capability (Day 1997, Foster 1995, Bidlingmayer and Hem 1973, Nayar and Sauerman 1971a, 1971b and 1975). Feeding on sugar leads to the accumulation of lipid and glycogen, which are used for

flight energy, survival and fecundity far beyond what would be possible from teneral reserves alone (Van Handel 1965b).

Nectars are considered the primary source for sugar feeding (Foster 1995), although alternative sources such as honeydew, extrafloral nectaries, rotting fruit and even plant tissues are suitable (Joseph 1970, Grimstad and DeFoliart 1974, Muller and Schlein 2005, Foster 1995). Generally, there have been some observations made of mosquitoes feeding on particular nectar and honeydew sources (Haeger 1955, Grimstad and DeFoliart 1974, Nayar 1982) and some identification of particular sugar in the crops of mosquitoes (Burkett et al. 1998, Shaefer and Muira 1972), but field observation is difficult, especially considering that feeding habits are crepuscular in most *Culex* mosquitoes (Magnarelli 1979). The primary sugars in nectar are sucrose, glucose and fructose, which are available in various combinations and concentrations depending on the source (Baker and Baker 1983a, 1983b, 1975). Traces of mannose are also found in nectar, but are not a primary sugar (Wackers 2001). Honeydew contains many sugars known to sustain insect life including common nectar sugars sucrose, glucose and fructose as well as sugars more specific to honeydew including melezitose, raffinose and trehalose (Volkl et al. 1999, Baker and Baker 1983b).

Studies have shown that some mosquitoes will not even begin host seeking until they have obtained their first sugar meal (Foster 1995), although some species of sugar fed mosquitoes seem to be less avid host-seekers (Foster and Eischen 1984). Despite sugar feeding deterring host seeking, Walker and Edman (1985) found that sugar fed mosquitoes are more persistent when they are host seeking, which combined with a long-life may allow them more successful blood meals. These findings raise important questions about the effect of sugar feeding on behaviors such as host seeking and biting persistence, which are directly related to vector

competence. These highly specialized behaviors play an important role in mosquitoes as dangerous disease vectors and these factors are critical to why laboratory studies of longevity in many insects are so important and well studied.

Nutritional Reserves and Body Size

Nutritional reserves have been directly correlated with larval diet and body size upon emergence in mosquitoes (Telang and Wells 2004). Glycogen is stored in the thoracic muscles and fat body and used for immediate energy when sugar is not available in the crop (Clements 1955, Nayar and Sauerman 1971a, 1971b), whereas lipids are used for long-term energy and survival (Foster 1995, Nayar 1982). Nayar and Pierce (1977) inferred that emergence triglycerides were used as a source for survival by connecting 50% survival times to rate of decline of lipids and that a poor larval diet resulted in low triglyceride levels and decreased 50% survival time. Briegel et al. (2001) also were interested in the effects of body size and teneral reserves on longevity and recognized that larger mosquitoes contained more teneral lipid and glycogen and survived longer on a water diet than smaller mosquitoes. It was also determined that 50% survival times were higher for larger mosquitoes when fed a similar sugar diet as smaller mosquitoes. Additionally, small mosquitoes, which are common in nature due to frequent poor larval nutritional conditions, emerge with not only minimal nutritional reserves but also underdeveloped follicles, which without sugar feeding cannot accumulate yolk after bloodfeeding (Feinsod and Spielman 1980).

Mating Status

Mating is suspected to have an effect on longevity of both male and female mosquitoes. For males and females, the ingestion of sugar is integral in their increased survival and ability to mate (Gary and Foster 2004). Without sugar their lifespans are not long enough to allow time for mating. Feeding on nectar or other carbohydrate sources is a sufficient resource to extend

life to the point of mating capability. Liles and DeLong (1960) observed that when maintained together, males lived less time and females lived more than if reared separately. They concluded that mating likely reduced the lifespan in male *Aedes aegypti,* whereas females benefitted from the association with males by an increased life span. Increased mating instances have been implicated in reduced survival in male mosquitoes, and furthermore increased mating competition and number of matings can be a result of optimal larval rearing (Ng'habi et al. 2005, 2008). Typically, males reared under optimal larval conditions would live longer than those provided less optimal diets as larvae; however, this may not always be the case when taking mating into consideration.

Amino Acids in Nectar Sources

Since the discovery that nectar sources contained resources other than sugar, and that amino acids were second most abundant component of nectars, there has been a focus on understanding the role amino acids play in insect life history (Baker and Baker 1973, 1978). Most butterflies do not obtain large amounts of protein as adults, thus many of these studies have focused on butterflies and the effect small amounts of proteins from their adult food sources, such as nectar, dung and fruit or pollen feeding may have on fitness (Alm et al. 1990, Mevi-Shutz and Erhardt 2003a, 2005). However, mosquitoes also require protein for egg production and usually rely on those obtained from blood feeding. Their need to feed on sugar sources for survival makes nectar a relevant potential source of amino acids alternate to a blood source, especially during times when a host is not present or in between blood feeding events.

Preference for nectars mixtures containing amino acids by honeybees and cabbage white butterflies has been shown (Alm et al. 1990) and feeding on amino acids in nectar has been known to increase longevity and fecundity in the map butterfly (Mevi-Shutz and Erhardt 2005). Pollen fed in a sugar solution to *Aedes aegypti* increased the longevity and fecundity of that

particular mosquito species (Eischen and Foster 1983). However, the findings that longevity and fecundity were increased in the map butterfly were only valid for larvae maintained on poor diets. Butterflies that were not challenged in larval life did not seem to need the added nutrition of amino acids in their adult diets. Wild mosquitoes are often nutritionally challenged as larvae, and since nectar feeding is also an important part of their adult life amino acids contained within may increase life span and fecundity in these insects as well.

CHAPTER 2
EFFECT OF CARBOHYDRATE SOURCE ON NUTRITIONAL RESERVES OF *Culex quinquefasciatus*

Introduction

Many adult insects rely on sugar as a source that can be used immediately for energy or stored as glycogen and lipids that will be used for energy, survival and fecundity (Chapman 1998, Heimpel et al. 2004, Xue et al. 2008). Most mosquitoes require sugar as a source of energy for flight prior to host seeking, and they likely depend on teneral reserves for the initial energy needed to locate a carbohydrate source. The body size, teneral reserves, survivorship and flight potential of adult mosquitoes depend highly on nutritional conditions available to larvae such as amount and type of accessible food (Nayar and Sauerman 1970, Briegel et al. 2001). Females from nutritionally-deprived larvae are smaller, contain fewer teneral reserves, less fecund and live less time than females from larvae that were reared under more optimal larval conditions (Nayar and Pierce 1977, Telang and Wells 2004, Feinsod and Spielman 1980).

Adult mosquitoes feed on floral nectar, fruit and honeydew as sources of sugar, which can be metabolized for use as immediate energy for flight (Nayar and Van Handel 1971) or converted into glycogen and lipid and used for long-term energy and maintenance, survival and fecundity (Foster 1995, Nayar and Sauerman 1971a, 1971b, 1974). Without feeding on sugars, newly-emerged mosquitoes typically deplete their teneral reserves and die within 2-4 days (Nayar and Sauerman 1975, Nayar 1982, Van Handel 1965b). There is a direct correlation between 90% depletion of lipids and 50% mortality (Nayar 1982). Both *Aedes aegypti* and *Anopheles gambiae* have been reported to have increased survival feeding solely on human blood without feeding on sugar (Costero et al. 1998, Harrington et al. 2001), however, there are other reports of increased survival when these species feed on sugar (Gary and Foster 2004, Impoinvil et al. 2004). Excess sugars, from nectar feeding or from teneral reserves, are stored as

lipid and glycogen within the mosquito fat body and glycogen in the flight muscle (Clements 1992). Lipid stores may be used to provision the initial batch of eggs in anautogenous females (Briegel et al. 2002).

Fructose, glucose and sucrose have been identified as the main components of nectar (Percival 1961, Baker and Baker 1975, 1983a, 1983b) and are also present in honeydew (Wackers 2001). Other sugars, such as melezitose and raffinose are more specifically honeydew sugars and have been identified in the mosquito crop (Burkett et al. 1999, Wackers 2001). Mannose is less common, but is present in trace amounts in nectar and has been used, like sorbose, to examine the effects of various types of sugars on survival and deposition of lipids and glycogen when fed to mosquitoes (Galun and Fraenkel 1957, Ozalp and Emre 2001). Sorbose is a sugar that is found in the mountain ash berry and is a byproduct of the breakdown of sugars (US Government 2008), but it has also been used experimentally and found to be very unsupportive of life in *Aedes aegypti* (Galun and Fraenkel 1957).

Nectar is thought the be the primary source of carbohydrates for mosquitoes (Baker and Baker 1983a, 1983b), though identification of honeydew sugars in the crops of mosquitoes and increased survival of some species when fed these sugars suggests that feeding on honeydew is also very important (Foster 1995, Burkett et al. 1999, Galun and Fraenkel 1957, Vrzal Chapter 3 of this thesis). Questions about the importance of nectar sugars remain because field observation of mosquitoes feeding on carbohydrates sources have been difficult, although various authors have witnessed mosquitoes feeding on nectar sources and even *Cx. nigripalpus* feeding on homopteran honeydew (Haeger 1955, McCrae et al. 1969, Nayar 1982, Grimstad and DeFoliart 1974).

The objective of this study was to examine the effect of different sugars on the accumulation of adult reserves of male and female *Cx. quinquefasciatus*. We chose to evaluate a range of naturally-occurring sugars including nectar and honeydew sugars that occur more commonly (sucrose and melezitose) and some sugars which are less common (mannose and sorbose). Reserves of lipid and glycogen were quantified through the first 15 days of adulthood to examine the effects of different sugars on reserve accumulation.

Materials and Methods

Larval Rearing

The *Culex quinquefasciatus* colony was established from a Gainesville, FL collection in 1995. Adults were maintained at 27 ± 1.0 ºC, ~80% relative humidity and a 14:10 light: dark photoperiod. Adults were blood fed on defibrinated bovine blood and continuously provided a 5% sucrose solution. Larvae were reared in plastic trays (35.5 cm x 48.3 cm x 6.4 cm) containing 2.5 liters of well water. Approximately 10 egg rafts were set in each pan. Larval food was provided as a slurry of 25 ml of a 3:2 (g/ml of water) bovine liver powder: brewer's yeast on day 1 and a finely ground 2% hog chow (Purina Mills, LLC, St. Louis, Missouri) slurry (50 ml on days 2 through 5, and 75 ml on day 6) until pupation.

Mosquito Dry Weights

Dry mass measurements were obtained from samples of mosquitoes 24 ± 2 hours post emergence. Mosquitoes were sampled prior to being sugar-fed and these measurements provided the initial (emergence) levels for lipid and glycogen deposits. Experiments conducted using sorbose, sucrose or mannose/melezitose treatments used mosquitoes from different days. Therefore, it was necessary to establish a baseline measurement to confirm that these mosquitoes began at similar nutritional levels.

Groups of 10 males or 10 females were placed in microcentrifuge tubes, frozen (-20°C/ -80°C) and then freeze-dried for approximately 48 hours to remove all moisture. Immediately after freeze-drying, the samples were placed in a dessicator, weighed on a microbalance (Sartorius CP2T) and again stored at -20°C until biochemical analysis.

Nutritional Cage Experiment

Upon adult emergence, also referred to as day 1, 15 male and 15 female *Culex quinquefasciatus* were placed in 0.47 L paper containers (Solo Cup Company, Highland Park, IL) modified with screened tops to allow viewing and air movement. No sugar was provided prior to being set in cages. Half of a cotton dental wick (Richmond Dental, Charlotte, NC) measuring 3.8 cm in length was placed in a 1.5 ml microcentrifuge tube. The wick was saturated with either 1.0 ml of treatment solution. The tube containing the cotton wick was inserted through a hole (1.0 cm in diameter) in the side of the cage (15.0 cm from the bottom) to allow access to the solution on the moist cotton. They were provided with a 5% (w/v) solution of D-(+)-mannose, D-(+) melezitose, sucrose, or D-(+)-sorbose (Sigma-Aldrich, St. Louis, MO) *ad libitum* and the cotton wicks were changed daily to reduce fungal growth. To describe the accumulation of lipid and glycogen, groups of 10 males and females were sampled on days 1, 3, 5, 10 and 15 post-eclosion. There were five replications per treatment and control. The groups of mosquitoes were frozen at -20°C for later analysis using the sulphosphovanillin and hot anthrone assays.

Glycogen and Lipid Analyses

Initial nutritional reserves of the mosquitoes at the time of pupal emergence were determined by measuring the levels of glycogen and triglyceride. The hot anthrone (glycogen) (Van Handel 1965a, 1985) and sulphosphovanillin (lipid) (Van Handel 1965a, 1985, Hahn 2005) assays were conducted following the methods provided in detail in Appendix A. Three to five

replicates were completed for each sex, larval rearing regime (low and high food diets) and day (1, 3, 5, 10 and 15).

All of the samples that were previously freeze-dried, weighed and refrozen were analyzed for glycogen and lipid levels using both the hot anthrone assay for glycogen and the sulphosphovanillin assays for lipids. Briefly, (full protocol is presented in the Appendix), the dried mosquitoes were homogenized in 100 µl of saturated sodium sulfate, 200 µl of methanol, 100 µl ultrapure water and 500 µl of 1:1 chloroform: methanol in the microcentrifuge tube. The glycogen and lipid samples were measured using the methods of Van Handel (1965a, 1985), as modified by Hahn (2005), including a chromatographic step using silicic acids to remove polar lipids. A spectrophotometer was used to measure glycogen and triglyceride levels at 625 and 525 nm, respectively using standard curves.

Statistical Analyses

Dry weight measurements of 10 mosquitoes for each of the 5 replicates were used to obtain average weights of individual mosquitoes in each treatment group. Glycogen and triglyceride levels were calculated for 3-5 replicates of 10 individuals fed each sugar. Mannose and melezitose are from the same batch of mosquitoes, so they are considered together for initial dry weights and lipid and glycogen analyses. Sorbose did not support adequate survival beyond 2 days, so these samples were excluded from all statistical analyses. Teneral (day 1) dry weights and nutritional contents for all treatments (mannose/melezitose, sucrose and sorbose) were compared with Minitab 15 (Minitab, Inc) using a one-way ANOVA and Tukey's HSD to separate the means ($P<0.05$). Separate analyses of lipid and glycogen contents throughout time (days 1-15) of males and females were performed with JMP (SAS, Inc) using multivariable ANOVAs with diet, age and their interaction term as factors.

Results

Comparisons of Initial Dry Weight, Glycogen and Lipid Content

Glycogen and lipid contents of newly emerged females used for sucrose, mannose/melezitose and sorbose experiments did not differ significantly, nor did glycogen contents of males ($F_{3,19}$= 2.68, P=0.082; $F_{3,18}$=2.24, *P*=0.126; $F_{3,17}$=0.38, *P*=0.769, respectively) (Tables 2-1, 2-2). However, initial lipid contents of males used for the sorbose experiment were different from the other batches ($F_{3,17}$=5.08, *P*=0.014). Dry weights of males or females used the different treatments were significantly different ($F_{3,18}$=60.63, *P*<0.001; $F_{3,19}$=90.01, *P*<0.001) (Table 2-1 and 2-2).

Glycogen Content

Sorbose-fed adults lived for only 2 days and glycogen content of males decreased from 27.86 ± 5.26 µg/mg initially to 6.06 ± 2.03 µg/mg by day 2. Females contained 6.36 ± 0.49 µg/mg of reserves initially and were reduced to 2.62 ± 0.68 µg/mg glycogen by day 2. Because of their short survival times, they were excluded from further analysis due to their short lifespans.

Analysis of the remainder of the sugar diet treatments for days 1, 3, 5, 10 and 15 revealed that the most significant effect on glycogen accumulation by males and females was age not diet (Table 2-3). For both males and females, with diets pooled, there was a significant overall increase in glycogen content from teneral levels at day 1 to day 3 with a slight decrease until day 10 where the reserves increased again on day 15, though the increase was not significant for females (Figures 2-1a, 2-1b).

The interaction between age and diet was also significant for males and females (Table 2-3), with both increasing their glycogen levels significantly beyond teneral reserves with all sugar diets (Figures 2-1c, 2-1d). Feeding on melezitose and sucrose by males and females resulted in

significant increases in the amount of glycogen by days 3 and 5, with levels remaining above teneral levels for the period of the study. (Figures 2-1c, 2-1d). Levels of glycogen for melezitose-fed males were significantly greater at day 15 than day 3 (Figure 2-1c). Females fed melezitose had glycogen levels that remained high until day 15, with a slight drop at day 10 (Figure 2-1d). By days 10 and 15, the glycogen content of sucrose-fed males and females were significantly lower than days 3 and 5 (Figure 2-1c). Mannose-fed glycogen reserves of males never increased significantly above teneral levels (Figure 2-1b), although females fed mannose had glycogen levels that increased above teneral levels on all day except on day 10, when they decreased to teneral level amounts (Figure 2-1d).

Lipid Content

Sorbose-fed adults lived for only 2 days lipid content of males decreased from 68.20 ± 7.29 μg/mg initially to 47.75 ± 5.47 μg/mg by day 2. Females contained 52.05 ± 7.26 μg/mg initially which was reduced to 42.87 ± 6.70 μg/mg by day 2.

Analysis of the remainder of the sugar diets indicated that age had the most significant affect on lipid accumulation in males and females (Table 2-4). However, males and females displayed opposite trends, with overall lipid content in males continuously decreasing through time from the teneral reserves (Figure 2-2a) and females slowly deposited more lipid over time (Figure 2-2b). The interaction between age and diet was also significant for both males and female for lipid content (Table 2-4). Mannose-fed males contained more lipid reserves on emergence than for days 3-15 (Figure 2-2c) In contrast, mannose-fed females did not differ in lipid levels, except at day 10 (Figure 2-2d). Melezitose-fed males did not differ in their lipid content from teneral reserves throughout the study period (Figure 2-2c), however females fed this diet had reserves that increased significantly on day 3 and remained steadily high for the entire 15 days (Figure 2-2d). For sucrose-fed males, there was no difference in lipid deposits

between any of the ages tested (Figure 2-2c), however lipid reserves in females slowly increased from days 3 to 15, resulting in large accumulations by day 15 (Figure 2-1d).

Discussion

Age of male and female *Cx. quinquefasciatus* had a significant effect on glycogen and lipid accumulation in this study. Nutritional reserves are critical to survival and flight stamina, which greatly impact the ability of mosquitoes to locate hosts and transmit disease. However, age alone was not the only factor affecting glycogen or lipid content. The interaction of adult diet with age also played a role in whether glycogen or lipid increased or remained steadily low throughout the 15 day study.

Briegel et al. (2002) showed with *Aedes aegypti,* that continuous feeding on sugar in between gonotrophic cycles allowed triglyceride levels to continue to increase for >40 days, even with the addition of some of the triglycerides synthesized going to egg production. Nayar and Pierce (1977) showed a correlation between depleting lipid level and death, and concluded that three different species had 50% survival times that corresponded with 90% decrease of teneral lipid reserves. Additionally, death occurred in *Cx. nigripalpus* when their lipid reserves were completely used up (Nayar 1982). Without feeding on a sugar source that supports survival, such as those sugars contained in nectar and honeydew, teneral reserves are depleted in 2-4 days followed by mosquito death (Nayar and Sauerman 1975, Nayar 1982, Van Handel 1965b). We fed these male and female *Cx. quinquefasciatus* sorbose, mannose, melezitose and sucrose, which are derived from different sources and known to support very low to maximum survival (Galun and Fraenkel 1957, Wackers 2001).

Sorbose, a monosaccharide, did not support survival beyond 2 days in males or females. Lipid and glycogen decreased from teneral reserves by day 2, and based on the findings of Nayar and Pierce (1977), we believe that reserves would be depleted completely by day 3, though we

did not measure the contents of the dead mosquitoes due to inaccuracies associated with breakdown of nutritional reserves after death. These results are consistent with the findings of Galun and Fraenkel (1957) when they fed this sugar to *Ae. aegypti*, although we found no evidence that it is toxic. According to the U.S. Government's Code of Federal Regulations (2008) and Gardner (1943) this sugar is found in the mountain ash berry and is a byproduct of the breakdown of some sugars, indicating that it is possible for mosquitoes to encounter this sugar in a field setting. There is also evidence that this sugar is palatable and readily ingested by the ant *Lasius niger* (Tinti and Nofre 2001) and the blow fly, but did not support adequate survival in the blow fly (Fraenkel 1940), or in *Aedes aegypti,* where survival was less than or equal to survival on water (Galun and Fraenkel 1957). Because it is so unsupportive of survival but is readily ingested by other insects, it may be a good candidate as an attracticide for mosquitoes.

Mannose, another monosaccharide, increased glycogen content of females slightly beyond teneral reserves, but the glycogen content of males and lipid contents of males and females were all reduced from teneral reserves. Longevity recorded in various mosquito species feeding on mannose, which is found as traces in nectar (Wackers 2001), have indicated that it is a fairly poor source of food leading to decreased survival when compared to more adequate nectar or honeydew sugars like sucrose and melezitose (Galun and Fraenkel 1957, Vrzal Chapter 3 of this thesis). Because this supports low survival and does not increase lipids and glycogen very far beyond teneral reserves and it is found only as traces in nectar, it would be interesting to find whether feeding on other, better sources of carbohydrates would counteract the negative affects of this sugar. *Culex tarsalis, Ae. aegypti* and other dipterans have been found to have salivary carbohydrases that seem to differ between family and even species (Gooding 1975, Marinotti and

James 1990, Schaefer and Muira 1972). These are shunted to the crop upon ingestion of carbohydrates (Gooding 1975) and it is possible that if an enzyme is lacking to break down this particular sugar, the mosquito imbibing it will not be able to use it for nutrition and survival.

Melezitose, a trisaccharide, and sucrose, a disaccharide, allowed for the accumulation of moderate to large amount of lipid and glycogen in males and females. Melezitose-fed adults maintained high levels of glycogen throughout the study period, whereas sucrose-fed adult content tapered off on days 10 and 15. Lipid levels remained above teneral reserves in females when fed both diets, and male reserves remained steady and equal to teneral reserves for all 15 days. Both of these sugars have been found to enhance survival significantly in mosquitoes (Galun and Fraenkel 1957, Vrzal, Chapter 3 of this thesis), with melezitose supporting survival as well or better than sucrose. Sucrose is a nectar sugar, but also occurs in honeydew and is often used for maintenance of insect colonies that sugar feed (Baker and Baker 1983a, 1983b Wackers 2001). Melezitose, on the other hand, is more specifically a honeydew sugar (Burkett et al. 1999, Wackers 2001). Melezitase is a specific carbohydrase that was found in *Cx. tarsalis* (Schaefer and Muira 1972) and may provide an explanation for why mosquitoes are able to utilize this sugar so well. Burkett et al. (1999) postulated that analysis of mosquito crops testing positive for fructose, a byproduct of the breakdown of melezitose, may be missing that these insects had been feeding on honeydew. Because of the increased survival on this sugar as well as the identification of honeydew sugars in the crops of various mosquito species (Burkett et al. 1999, Galun and Fraenkel 1957, Vrzal Chapter 3 of this thesis) and the variety of sugars that are unique to homopteran honeydew (Wei et al. 1996, Yee et al. 1996). Therefore, it may be possible that mosquitoes prefer specific honeydews and that they contain the carbohydrases to utilize them to their full advantage.

The accumulations of lipid and glycogen stores are important for increased longevity in mosquitoes. Without feeding on them, mosquitoes would not be able to seek hosts or have the opportunity for multiple feedings and ultimately transmit disease. Some sugars found in nature that are palatable, but do not support life (Fraenkel 1940). The basis for why some sugars increase longevity and some do not remains understudied. The identification of species-specific carbohydrases may be an important step to assist in determining species-specific attracticides or finding attractive honeydew sugars to use for population monitoring.

Table 2-1. Initial (day 1) glycogen, lipid and dry weights of unfed individual male *Cx. quinquefasciatus*.

Treatments	Initial nutritional status measurements				
	Glycogen (μg/mg)[1]	N	Lipid (μg/mg)[1]	N	Dry Weight (mg)[2]
Sucrose	29.58 ± 3.81a	5	73.19 ± 10.80ab	3	0.32 ± 0.02a
Mannose/Melezitose	33.38 ± 4.25a	4	113.00 ± 12.04a	5	0.45 ± 0.01b
Sorbose	27.86 ± 5.26a	5	68.20 ± 7.29b	5	0.34 ± 0.00a

Similar letters within a column indicated no significant differences (Tukey's HSD, $P<0.05$).
[1]Means of groups of 10 mosquitoes/dry weights of 10 mosquitoes.
[2]Dry weights = approximate weights of individual mosquitoes.

Table 2-2. Initial (day 1) glycogen, lipid and dry weights of unfed individual female *Cx. quinquefasciatus*.

Treatments	Initial nutritional status measurements				
	Glycogen (μg/mg)[1]	N	Lipid (μg/mg)[1]	N	Dry Weight (mg)[2]
Sucrose	11.64 ± 2.13a	5	39.96 ± 7.81a	4	0.51 ± 0.01a
Mannose/Melezitose	11.61 ± 1.66a	5	60.58 ± 4.98a	5	0.68 ± 0.01b
Sorbose	6.36 ± 0.49a	5	52.05 ± 7.06a	5	0.50 ± 0.01a

Similar letters within a column indicated no significant differences (Tukey's HSD, $P<0.05$).
[1]Means of groups of 10 mosquitoes/dry weights of 10 mosquitoes.
[2]Dry weights = approximate weights of individual mosquitoes.

Table 2-3. Results of ANOVA on glycogen levels displaying the effects of sugar diet, age and their interaction on male or female *Culex quinquefasciatus*.

Trait	**Source**	**df**	***F***	***P***
Males	Whole model	14	48.57	<0.001*
	Diet	2	0.05	0.952
	Age	4	42.28	<0.001*
	Diet x age	8	21.32	<0.001*
	Error	57		
	Total	71		
Females	Whole model	14	52.07	<0.001*
	Diet	2	0.00	1.000
	Age	4	110.23	<0.001*
	Diet x age	8	8.85	<0.001*
	Error	60		
	Total	74		

*Indicates significant term ($P<0.05$).

Table 2-4. Results of ANOVA on lipid levels displaying the effects of sugar diet, age and their interaction on male or female *Culex quinquefasciatus*.

Trait	**Source**	**df**	***F***	***P***
Males	Whole model	14	13.39	<0.001*
	Diet	2	5.08	0.009*
	Age	4	9.38	<0.001*
	Diet x age	8	5.92	<0.001*
	Error	56		
	Total	70		
Females	Whole model	14	29.39	<0.001*
	Diet	2	1.77	0.180
	Age	4	16.53	<0.001*
	Diet x age	8	15.79	<0.001*
	Error	56		
	Total	70		

*Indicates significant term ($P<0.05$).

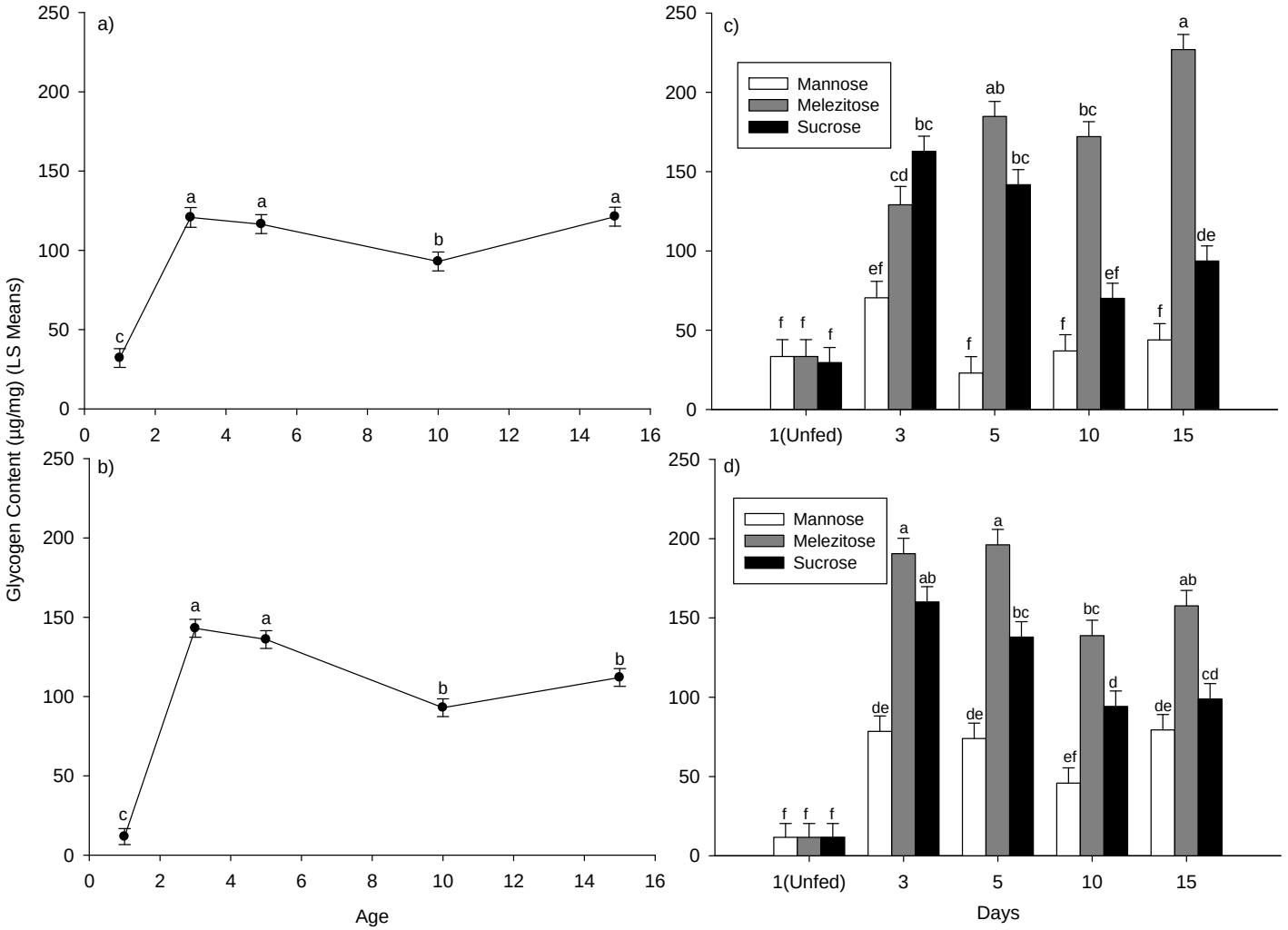

Figure 2-1. The effect of age of males (a) and females (b) and the interaction between sugar diet and age on male (c) and female (d) *Cx. quinquefasciatus* on glycogen content (µg/mg) (±SE) (LS Means). Treatments with similar letters are not significantly different (Tukey's HSD, $P<0.05$).

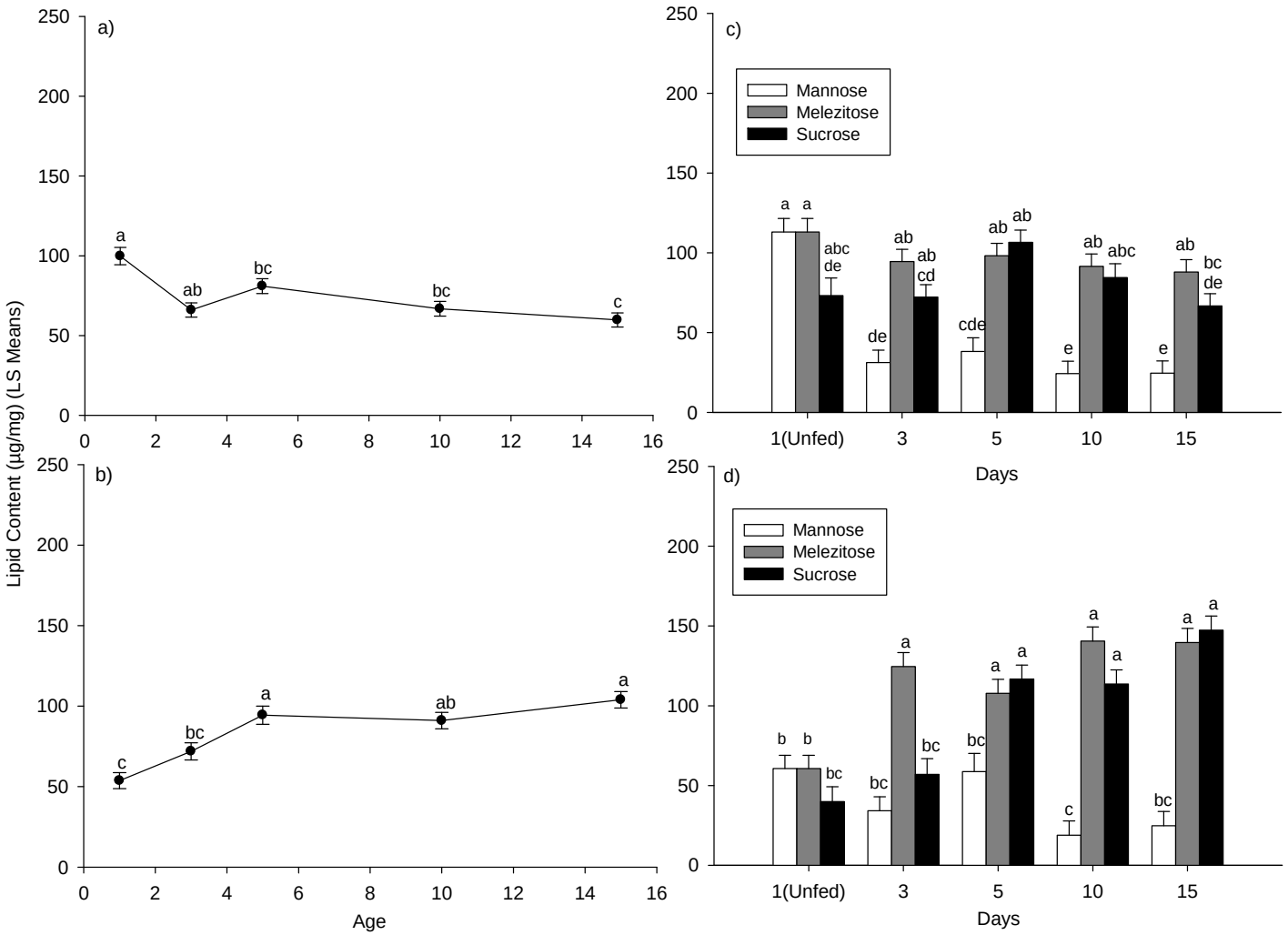

Figure 2-2. The effect of age of males (a) and females (b) and the interaction between sugar diet and age of male (c) and female (d) *Cx. quinquefasciatus* on lipid content (µg/mg) (±SE) (LS Means). Treatments with similar letters are not significantly different (Tukey's HSD, $P<0.05$).

CHAPTER 3
EFFECTS OF VARIOUS CARBOHYDRATE SOURCES ON THE LONGEVITY OF *Culex nigripalpus*, *Culex quinquefasciatus* AND *Culex salinarius*

Introduction

Adult male and female mosquitoes feed on carbohydrates, which are converted to glycogen for immediate energy (Nayar and Van Handel 1971) and to lipids for long-term energetic needs and survival (Clements 1955, Nayar and Sauerman 1971a, 1971b, 1975). Nectar is considered the primary source of carbohydrates (Foster 1995, Yuval 1992, Grimstad and DeFoliart 1974, Haeger 1955), but Magnarelli (1983) found very few mosquitoes actually feeding on floral nectaries compared to the number found with sugar in their crops. This suggests that other sources may be used to meet carbohydrate needs, but how frequently these sources are used is unknown. Additional sources include honeydew (Burkett et al. 1998, Haeger; unpublished in Nayar 1982, Haeger 1955), extrafloral nectaries (Foster 1995), and even plant tissues (Müller and Schlein 2005).

Floral nectar is often composed primarily of sucrose, fructose and glucose (Baker and Baker 1983a, 1983b), while traces of mannose may also be present (Wackers 2001) it is considered a primary source of carbohydrates for mosquitoes (Foster 1995). As well as sucrose, glucose and fructose, honeydew can contain other unique sugars such as raffinose, melezitose (Volkl et al. 1999) and trehalose (Baker and Baker 1983a), all of which have been identified in the crops of mosquitoes (Burkett et al. 1999). Identification of sugars in mosquito crops proves that mosquitoes are encountering these in nature and likely using them for survival. Sorbose is rare in nature (Gardner 1943), but is found in mountain ash berries and can result from microbial breakdown of sugars (United States Government 2008, Gardner 1943). Previously it was evaluated in longevity studies with *Ae. aegypti* (Galun and Fraenkel 1957). Sorbose has been implicated as having deleterious effects on survival and may possibly be toxic (Galun and

Fraenkel 1957). In the laboratory, mosquitoes are often maintained on a 5-10% sucrose solution, which is sufficient to maintain survival for long periods of time, such as greater than 2 months for *Cx. nigripalpus* (Nayar and Sauerman 1973). Physiological and behavioral studies, as well as studies relating to mosquito disease transmission, require prolonged survival in the laboratory.

Culex species play an important role in the transmission of vector-borne disease in Florida, throughout the United States, and around the world. *Culex nigripalpus* are considered the main vectors of West Nile virus (WNV) and St. Louis encephalitis virus (SLE) (Day 1997) in Florida. In Asia, Africa, the Western Pacific and South America, *Culex quinquefasciatus* are urban vectors of lymphatic filariasis caused by *Wuchereria bancrofti* (Subra 1981). *Culex quinquefasciatus* are also known vectors of SLE (Jones et al. 2002) and competent and potential vectors of WNV (Sardelis et al. 2001, Molaei et al. 2007) in the southeastern United States. *Culex salinarius* are potential vectors of Eastern Equine encephalitis virus (EEE) (Scott and Weaver 1989) and considered a possible bridge vector of WNV to humans in the northeastern U.S., mainly due to its opportunistic feeding behaviors (Molaei et al. 2004).

Transmission of mosquito-borne diseases relies heavily on the survival of mosquitoes and the ability of the pathogen to survive and replicate in the field (Patz et al. 1998, Craig et al. 1999). Factors affecting mosquito longevity are critical as the longer lived the mosquito, the greater the opportunity to obtain multiple blood meals and to acquire and transmit disease. Sugar feeding is imperative to the extended survival of mosquitoes and adult feeding on nectar, honeydew and other carbohydrate sources is necessary for disease transmission. *Culex* species differ in their life history parameters and may potentially utilize carbohydrate sources differently. The objective of this study was to examine the effect of various sugars on the longevity of *Cx.*

nigripalpus, *Cx. quinquefasciatus* and *Cx. salinarius*. Additionally, comparisons were made to determine sex-specific patterns of survival on various carbohydrate sources.

Materials and Methods

Larval Rearing

Mosquitoes used in this study were from colonies maintained at the USDA, ARS (Gainesville, FL) and included *Culex nigripalpus* (Vero Beach, 1999), *Culex quinquefasciatus* (Gainesville, 1995) and *Culex salinarius* (Hartford, 2001). All colonies were maintained at 27 ± 1.0ºC, ~80% relative humidity and a 14:10 light: dark photoperiod. Adults were blood-fed on manually defibrinated bovine blood or chicken blood (University of Florida IACUC D469). Adults were provided continuous access to a 5% sucrose solution on saturated cotton balls. All larvae were reared in plastic trays (35.5 cm x 48.3 cm x 6.4 cm) containing 2.5 liters of well water. The rearing of *Cx. nigripalpus* entailed placing 4 egg rafts in each tray and the subsequent larvae were fed a 0.08 g (on day 1) and 0.24 g (from day 2 until pupation) portion of dry food mixture (1:6 bovine liver powder: brewer's yeast) (MP Biomedicals, Solon, OH)) daily for 6 days until pupation. An increased number of egg rafts resulted in higher larval density and increased death or incomplete pupation. *Culex quinquefasciatus* and *Cx. salinarius* were reared with 10 egg rafts per tray and were fed a slurry of 25 ml of a 3:2 bovine liver powder: brewer's yeast (g/ml of water) on day 1 and a 2% finely ground hog chow (Purina Mills, LLC, St. Louis, Missouri) slurry (50 ml on days 2 through 5 and 75 ml on day 6) until pupation.

Longevity Assays

For longevity assays, mosquitoes were allowed to emerge into a cage with no access to sugar. Within 24 hours post-emergence mosquitoes were chilled, and 35 males and 35 females were placed into 0.47 L paper containers (Solo Cup Company, Highland Park, IL). Container lids were modified with transparent tulle fabric to allow viewing inside the cages. An access

hole (1.0 cm) was cut in the side of the container approximately 15.0 cm from the bottom. A 1.5 ml microcentrifuge tube, containing a cotton dental wick (3.8 cm long) (Richmond Dental, Charlotte, NC) saturated with 1.0 ml of a 5% sugar solution, was inserted into the hole to allow the mosquitoes to feed *ad libitum*. The mosquitoes were provided with new wicks saturated with a 5% sugar solution or water control every other day. Sugars tested include monosaccharides; D-(-)-fructose, D-(+)-glucose, D-(+)-mannose and D-(+)-sorbose, disaccharides; sucrose and D-(+)-trehalose, and trisaccharides; D-(+)-melezitose and D-(+)-raffinose (Sigma, St. Louis, MO). Between six and ten replicates of each treatment were conducted for each species, depending on the availability of mosquitoes. The cages were maintained in incubators at 28 ± 1.0ºC and 80 ± 0.44 % RH with a 14:10 light: dark photoperiod. The dead mosquitoes were counted daily and recorded. For each container, 50% mortality was determined to be the time (day) at which 50% of the mosquitoes in the cage were dead.

Statistical Analyses

Statistical analyses were conducted using JMP (SAS, Inc. Cary, NC). Kaplan-Meier survival analysis using log-rank was used to create survivorship curves. Pairwise comparisons using log-rank tests were made of the survival curves for each species and sex to determine if the curves differed. Additionally, the number of days to 50% mortality was determined for each cage (Briegel et al. 2001, Nayar 1986, Nayar and Sauerman 1975) and these averaged for each treatment. Analyses were conducted for males and females separately because they are known to differ in their survival (Liles and DeLong 1960, Briegel and Kaiser 1973) as well as for water and sorbose treatments and all other treatments due to very low survival and variances when fed water and sorbose (Galun and Fraenkel 1957). Multivariable ANOVA models were constructed with species and sugar as fixed effects and their interaction as factors. The time to 50% mortality for each species was examined separately using a one-way ANOVA or Kruskall-Wallis

nonparametric analysis when necessary and then means were separated using Tukey's HSD (P<0.05) in all analyses. Assumptions of normality and homogeneity of variance were confirmed using graphical representations of the data and Levene's tests.

Results

Male Survivorship Curves

Culex nigripalpus. Feeding different sugars to males had a significant effect on survival curves (χ^2=2987.62, df=8, P<0.001) (Figure 3-1). Pairwise comparisons allowed for the separation of the survival curves (P<0.05). Survival curves of males feeding on melezitose and raffinose indicated significantly longer survivorship than any of the other sugars. Males feeding on trehalose and fructose fed males produced curves that indicated high survivorship, both supporting longer survivorship than sucrose. Whereas, mannose and glucose fed adults lived a very short period of time compared to the other treatments thus far. The only diets that were less supportive than mannose and glucose were sorbose and the water control. The sorbose curve indicated a significantly shorter lifespan resulted from feeding on this treatment than when feeding on the water control.

Culex quinquefasciatus. Males fed different sugars differed in their survival significantly (χ^2=3803.29, df=8, P<0.001) (Figure 3-1). For this species, fructose and raffinose curves indicated the greatest survivorship, followed by survival on glucose. Moderate survival was indicated by the curves of melezitose, sucrose and trehalose. Very poor survival was obtained by those males fed mannose. Again, however, water and sorbose were the poorest diets, with sorbose fed adults living less time that those fed the water control.

Culex salinarius. Feeding different sugars led to significantly different survivals of the males feeding on them (χ^2=3050.15, df=8, P<0.001) (Figure 3-1). Greatest survival was achieved by adult males fed raffinose, with fructose, glucose, melezitose and sucrose-fed adults

surviving almost as well. Mannose and trehalose-fed males did not survive as long as the other treatments, but they did increase survival over that of water and sorbose. For this species, sorbose-fed males lived longer than those fed the water control.

Female Survivorship Curves

Culex nigripalpus. Survival differed significantly depending on the diet fed to female Cx. nigripalpus (χ^2=3378.61, df=8, P<0.001) (Figure 3-2). Glucose-fed females survived significantly longer than females fed any other diet. Females fed fructose, melezitose, trehalose and raffinose were moderately long-lived in comparison. Sucrose and mannose provided the nutrition that allowed for only moderately low survival, although the mosquitoes fed these treatments did live significantly longer than those fed sorbose and the water control. Females fed sorbose again lived a shorter amount of time than those fed the water control.

Culex quinquefasciatus. Females fed different sugars survived significantly different amounts of time depending on treatment (χ^2=4607.95, df=8, P<0.001) (Figure 3-2). Fructose-fed females lived longer than females fed any other treatment, with glucose and melezitose-fed females living significantly less time. Females fed sucrose and raffinose lived moderately long lives. However, trehalose and mannose-fed adults did not survive as long as any of the previous treatments. They did outlive sorbose and water-fed adults, but survival was fairly low. Water-fed adults lived longer than those fed sorbose diets.

Culex salinarius. Varying sugar treatments resulted in differing survival rates of female *Cx. salinarius* (χ^2=3491.58, df=8, P<0.001) (Figure 3-2). Females fed fructose and glucose surpassed the rest of the females in survivorship. Melezitose, sucrose and raffinose-fed adults clustered behind them and had long lives as well. Supporting only moderately low survival, trehalose and mannose-fed females did live longer than those fed the sorbose and water treatments. Water did not extend life beyond that of sorbose.

Comparison of Male Days to 50% Mortality

Sorbose and water supported minimal survival and were partitioned from other sugars in analysis. For males fed on water and sorbose, there were significant effects of sugars and species with a significant sugar x species interaction (Table 3-1). A similar pattern was seen for males fed the other sugar treatments (Table 3-1). Overall, males differed in the time to 50% mortality based on species with *Cx. quinquefasciatus* males living the longest, followed by *Cx. salinarius* and the shortest lived were *Cx. nigripalpus* (Figure 3-3a). The longest-living males were fed on raffinose and fructose followed by glucose and sucrose, then melezitose (Figure 3-3b). Trehalose and mannose supported poor survival, though they did support survival significantly longer than water controls. Males that were fed sorbose lived less time that those fed the water control (Figure 3-3b).

Survival of *Cx. nigripalpus* males was similar when fed fructose, glucose, melezitose, raffinose, sucrose and trehalose with significantly lower survival on mannose (Figure 3-4). Male *Cx. quinquefasciatus* lived longest on raffinose, fructose, glucose, followed by sucrose then melezitose and trehalose. Mannose supported low survival. Survival of male *Cx. salinarius* were supported the longest on raffinose, sucrose, fructose, glucose and mannose, with trehalose-fed males living the least amount of time. Water and sorbose both supported poor survival for *Cx. quinquefasciatus* and *Cx. salinarius* and survival length was not different (t=0.12, df=15, P=0.905). Survival of *Cx. nigripalpus* and *Cx. quinquefasciatus* males, however was less when fed sorbose than water (t=707.11, df=18, P<0.001; t=2.44, df=8, P=0.040, respectively) (Figure 3-4).

Comparison of Female Days to 50% Mortality

Similar to males, all species of females maintained very low survival on water and sorbose and multivariable ANOVAs were conducted on water and sorbose-fed females

separately from those fed the other sugars (Table 3-2). For females fed water and sorbose, there was a significant effect of species and species x sugar interaction, but not of sugar alone (Table 3-2). Females fed on other sugars displayed significant effects of species, sugars and the interaction of species and sugars (Table 3-2). Overall, female *Cx. nigripalpus* and *Cx. salinarius* had similar lifespans and *Cx. nigripalpus* lived significantly longer than *Cx. quinquefasciatus* (Figure 3-5a). With species pooled, fructose, glucose and sucrose supported the greatest survival (Figure 3-5b). Raffinose and melezitose fed females lived as long as those fed sucrose, which is commonly used to rear laboratory adults (Figure 3-5b). Again, as seen in males, trehalose and mannose supported only moderate survival, but significantly higher than the water- or sorbose-fed adults. Water- and sorbose-fed females did not differ in their survival times.

The longest survival of each species was achieved on a different sugar, *Cx. quinquefasciatus* on fructose, *Cx. nigripalpus* on glucose and *Cx. salinarius* on sucrose (Figure 3-6). Survival of *Cx. nigripalpus* females was greatest on glucose, fructose, melezitose, raffinose, sucrose and trehalose, with moderate survival on mannose. Female *Cx. quinquefasciatus* survived the longest feeding on sucrose, fructose, glucose and raffinose, with moderate to poor survival on trehalose and mannose. *Culex salinarius* females lived the longest when fed sucrose, fructose, glucose and raffinose, with moderate to low survival on trehalose and mannose (Figure 3-6). Variation in survival was seen on all the sugars, each supporting longevity better or worse depending on the species. However, longevities were not significantly different when provided any of the sugars, except for trehalose, which *Cx. nigripalpus* lived significantly longer than the other two species and fructose, which allowed *Cx. quinquefasciatus* to live significantly longer than *Cx. nigripalpus*. Female *Cx. salinarius* fed water and sorbose lived less time on water (t=4.49, df=15, P<0.001), but *Cx. nigripalpus* and *Cx. quinquefasciatus*

females lived less time on sorbose or showed no difference in survival between the two treatments, respectively (t=8.99, df=18, P<0.001; t=0.00, df=8, P=1.000) (Figure 3-6).

Discussion

The length of adult *Culex* survivorship was clearly affected by sex, dietary sugar and species. Much research has been dedicated to examining the main sugar components of nectars present in common trees and shrubs (Percival 1961, Baker and Baker 1975, 1983a, 1983b). Nectar is widely known to be fed upon by mosquitoes (Grimstad and DeFoliart 1974, Foster 1995) and consists primarily of sucrose, glucose and fructose (Baker and Baker 1975), with mannose found in trace amounts (Wackers 2001). Honeydew as a source of carbohydrates for mosquitoes contains several unique sugars, including melezitose, raffinose and trehalose (Wackers 2001, Foster 1995), which were also examined. Water was fed to mosquitoes as a control and sorbose is a sugar that has been used in previous studies and has been implicated as having toxic effects on the insects that feed on it. The effect of various sugars on longevity for insects such as mosquitoes and flies (Galun and Fraenkel 1957, Liles and DeLong 1960, Nayar and Sauerman 1971a, 1971b and 1975), parasitoids (Jacob and Evans 2000), phorid flies (Fadamiro et al. 2005) and butterflies (Hill and Pierce 1989, Mevi-Shutz and Erhardt 2003a, 2005) have been widely studied. Other complex sugar sources for mosquitoes that are suspected to provide nutrition include rotting fruit, plant tissues (Muller and Schlein 2005) and aphid honeydew (Burkett et al. 1998). The role of honeydew sugars on adult survival has been investigated with *An. gambiae* (Gary and Foster 2004) and with parasitoids (Lee et al. 2004, Jacob and Evans 2000).

Male and female survival curves in all species were mostly reverse sigmoidal in shape, similar to curves produced when *Ae. aegypti* were fed a 10% sucrose solution (Liles and DeLong 1960) or pollen (Eischen and Foster 1983) and a phorid fly, *Pseudoacteon tricuspis* was fed

various sucrose solutions (Fadamiro et al. 2005). Males and females of all species in this study showed similar trends when fed the common nectar sugar components. Survival was increased far beyond that seen in individual provided only water, and in most cases, they were among the most suitable sugars provided. These data correspond with many of the studies of this type with various insects (Galun and Fraenkel 1957, Wackers 2001) and support the findings that nectar sugars play an important role in the survival of mosquitoes and other insects.

However, feeding sorbose, mannose and trehalose did not follow the same pattern of survival and were unsuitable diets and that did not increase survival much above the water control. For both males and females, water and sorbose did not support survival over 4 days. Male and female *Cx. nigripalpus* lived significantly less time when fed sorbose than when fed the water control, while *Cx. salinarius* females lived longer when fed sorbose. All others lived as long on sorbose as on the water control, but this sugar was very unsupportive of survival when compared to any of the other treatments. Galun and Fraenkel (1957) postulated that sorbose may have possible toxic effects to mosquitoes, which may explain the low survival seen in *Cx. nigripalpus* males and females when fed that sugar alone.

Mannose is a sugar that is found in nature as traces in plant nectar (Wackers 2001). Mannose was found previously to be moderately supportive of longevity in the parasitoid *Cotesia glomerata* (Wackers 2001) and barely increased survival beyond that of sorbose in *Ae. aegypti* (Galun and Fraenkel 1957). In this study, survival of male *Cx. quinquefasciatus* and *Cx. nigripalpus* males maintained on mannose was low, but slightly higher than the water control. *Culex salinarius* survival, however, was as long on mannose as when fed fructose, glucose or melezitose. Clearly differences exist in the utilization of this sugar between species.

Finally, trehalose, a known component of aphid honeydew sugar (Baker and Baker 1983a), only supported moderately low survival of *Culex*. Similar trends were seen in the survival of the parasitoids *Cotesia glomerata* (Wackers 2001) and *Bathyplectes curculionis* (Jacob and Evans 2000) and the mosquito *Ae. aegypti* (Galun and Fraenkel 1957). In contrast, *Cx. nigripalpus* males and females maintained on this sugar had equivalent survival when fed another disaccharide, sucrose.

Honeydew is a carbohydrate-rich product of aphids feeding on plant phloem (Holldobler and Wilson 1990). Composition and amount of honeydew produced by aphids is variable depending on species (Volkl et al. 1999). Honeydew sugars include common nectar sugars sucrose, fructose and glucose, but also contain a variety of other sugars including xylose, maltose, melezitose, raffinose (Volkl et al. 1999) and trehalose (Baker and Baker 1983a). Sugars associated with honeydew have been identified in the crops of many field-caught insects such as parasitoids (Heimpel et al. 2004), sandflies (MacVicker et al. 1990) and mosquitoes, *Culiseta melanura* and *Anopheles quadrimaculatus* (Burkett et al. 1999). Additionally, feeding on honeydew from the green aphid (Haeger 1955) and from *Coccus viridis,* by *Cx. nigripalpus* in the field has been documented (Nayar 1982). Feeding on honeydew has been implicated as a possible alternative source for mosquitoes to obtain sugars not found in nectar or found in different concentrations from nectar sources (Foster 1995). Because mosquitoes are known to feed on honeydew (Burkett et al. 1999), but observation of this is complicated (Foster 1995), survival on the associated sugars equaling the survival seen in nectar sugars in our study suggests that the importance of honeydew as a sugar source for *Culex* mosquitoes has been greatly underappreciated.

Our results reveal a trend of increased survival on sugars commonly found in aphid honeydew, with the exception of trehalose, which was only a moderate diet for male and female *Cx. nigripalpus*. Males of all three species survived as long on raffinose as on the three nectar sugars mentioned previously, which is the main sugar component of bahiagrass and which *Cx. nigripalpus* has been observed feeding on around homes in Florida (Burkett et al. 1999). Melezitose was moderately supportive for males, increased survival significantly beyond that of water and sorbose, and displayed similar survival to that of *Cx. quinquefasciatus* fed sucrose and *Cx. salinarius* fed glucose and fructose. In addition, females fed raffinose and melezitose also survived a similar amount of time to those fed the three nectar sugars. Raffinose did not increase survival over that of water when fed to *Cotesia glomerata* (Wackers 2001) and induced only moderate survival in *Ae. aegypti* over nectar sugars fed to that species (Galun and Fraenkel 1957). The different type and amount of sugars present in honeydew may benefit different insect species according to their efficiency in utilization of these sugars.

Because particular species survived overall longer than others when treatments were pooled, there was an interest in comparing the different effects that a similar diet would have on each of the three species. For example, male *Cx. quinquefasciatus* lived the longest on fructose, glucose and raffinose, *Cx. salinarius* on mannose, and there were no significant differences in survival between species when males were maintained on melezitose or sorbose. Females also differed in survival depending on species and diet. Female *Cx salinarius* survived the longest on sucrose and trehalose fed mosquitoes displayed significant differences in survival among all three species, with *Cx. nigripalpus* living the longest and *Cx. quinquefasciatus* the shortest. These differences may be explained by a separation in space and time providing one species of male or female with the resources allowing them to survive better on one diet than another.

Culex nigripalpus occur throughout the year in the extreme southern portion of the U.S. (Carpenter and LaCasse 1955), but peak season is from late summer into the fall. In collections of larvae from an oviposition site in Florida, all three *Culex* species in this study were found in association with each other throughout the year (Nayar 1982). Seasonal abundances of the species were similar for *Cx. quinquefasciatus* and *Cx. nigripalpus*. *Culex salinarius* has a larger range in the U.S., which extends from the southeast into the northeast and west to Utah and even into Canada, but it is likely most abundant in the Atlantic and Gulf Coasts. In the extreme south, this species can be present all year, but overwinters as adults in the northern range (Carpenter and LaCasse 1955). It can be assumed that different plants are flowering and different homopteran species may be abundant, introducing varying ratios of sucrose: glucose: fructose and different honeydew sugars all together depending on what species is present. These species may differ in their preference for honeydews produced by certain aphids depending on the sugars contained therein.

The relationship between mosquitoes and sugar feeding is critical because of the associated survival (Foster 1995, Nayar 1982, Nayar and Sauerman 1975) and subsequent host-seeking behavior (Nasci 1991, Foster 1995) which relate to vector potential and disease transmission. Although findings that sugar-fed mosquitoes are less avid host-seekers than starved or water-fed mosquitoes (Foster and Eischen 1984), there is evidence that they are more persistent at blood feeding attempts (Walker and Edman 1985), which coupled with long life can be important to vector potential. Studying longevity of *Culex* species in a laboratory setting may provide a better understanding of the potential of these insects to survive on particular sugars found in natural sources as well as the effect of different sugar sources on mosquito physiology, such as deposition of nutritional reserves. Furthermore, sugar feeding studies can be used as a way to

improve species-specific trapping and population monitoring by improving attractants or baiting systems, which is important for anticipating the potential for disease outbreak.

Table 3-1. Results of ANOVA on days to 50% mortality of males fed water and sorbose and males fed the remaining treatments that supported survival.

Treatment	**Source**	**df**	***F***	***P***
Water and Sorbose	Whole model	5	8.67	<0.001*
	Species	2	8.10	0.001*
	Sugars	1	13.19	0.001*
	Species x sugars	2	5.63	0.007*
	Error	41		
	Total	46		
Other sugars	Whole model	20	39.52	<0.001*
	Species	2	44.01	<0.001*
	Sugars	6	71.66	<0.001*
	Species x sugars	12	21.33	<0.001*
	Error	165		
	Total	185		

*Indicates significant terms ($P<0.05$).

Table 3-2. Results of ANOVA on days to 50% mortality of females fed water and sorbose and males fed the remaining treatments that supported survival.

Treatment	**Source**	**df**	***F***	***P***
Water and Sorbose	Whole model	5	28.04	<0.001*
	Species	2	17.75	<0.001*
	Sugars	1	0.39	0.539
	Species x sugars	2	48.88	<0.001*
	Error	41		
	Total	46		
Other sugars	Whole model	20	29.43	<0.001*
	Species	2	4.83	0.001*
	Sugars	6	76.79	<0.001*
	Species x sugars	12	9.67	<0.001*
	Error	167		
	Total	187		

*Indicates significant terms ($P<0.05$).

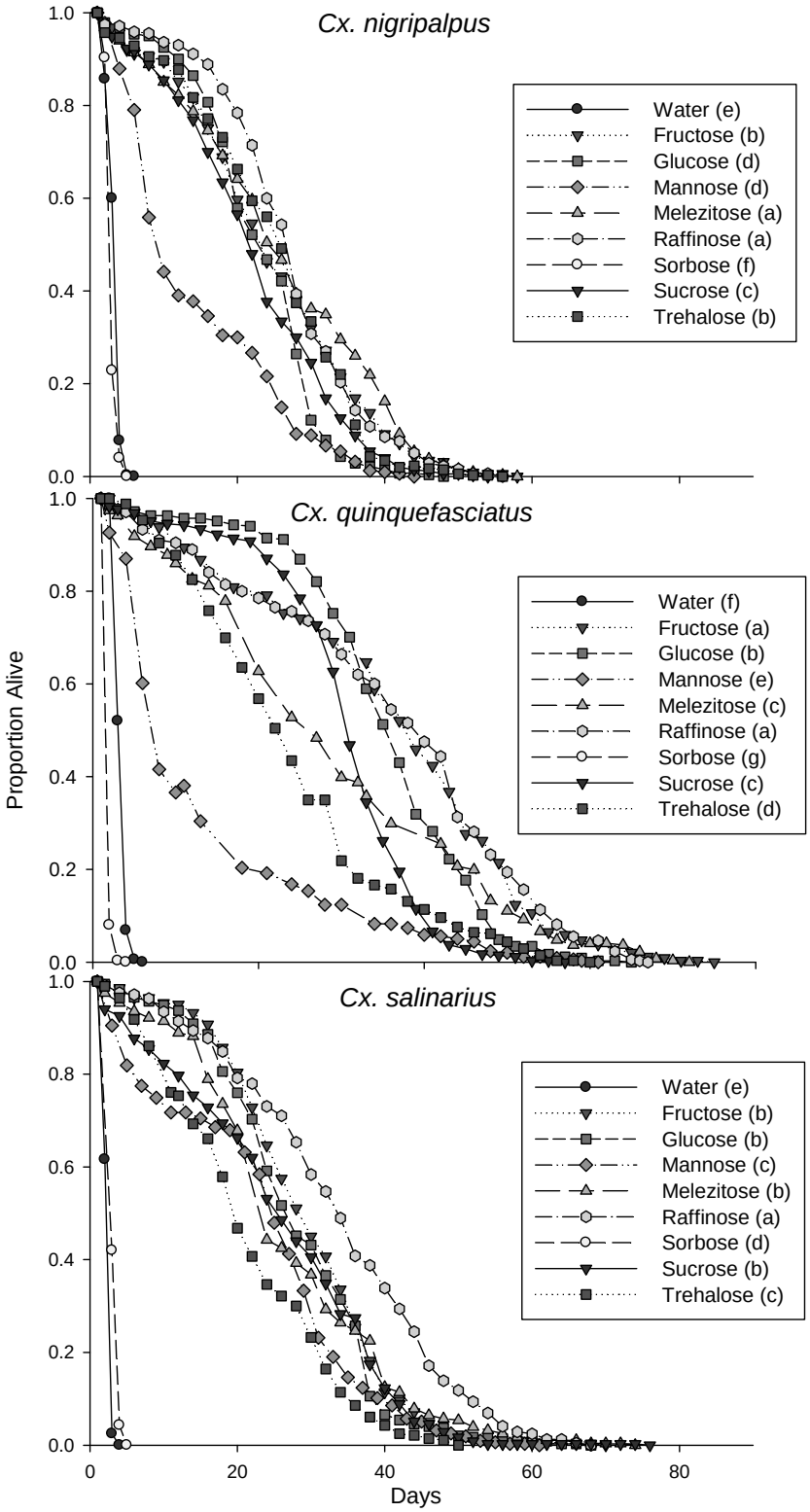

Figure 3-1. Proportion survivorship of *Culex* males maintained on 5% solutions of different sugars. Treatments with similar letters are not significantly different (Kaplan-Meier Survival Curve, pairwise tests, $P<0.05$).

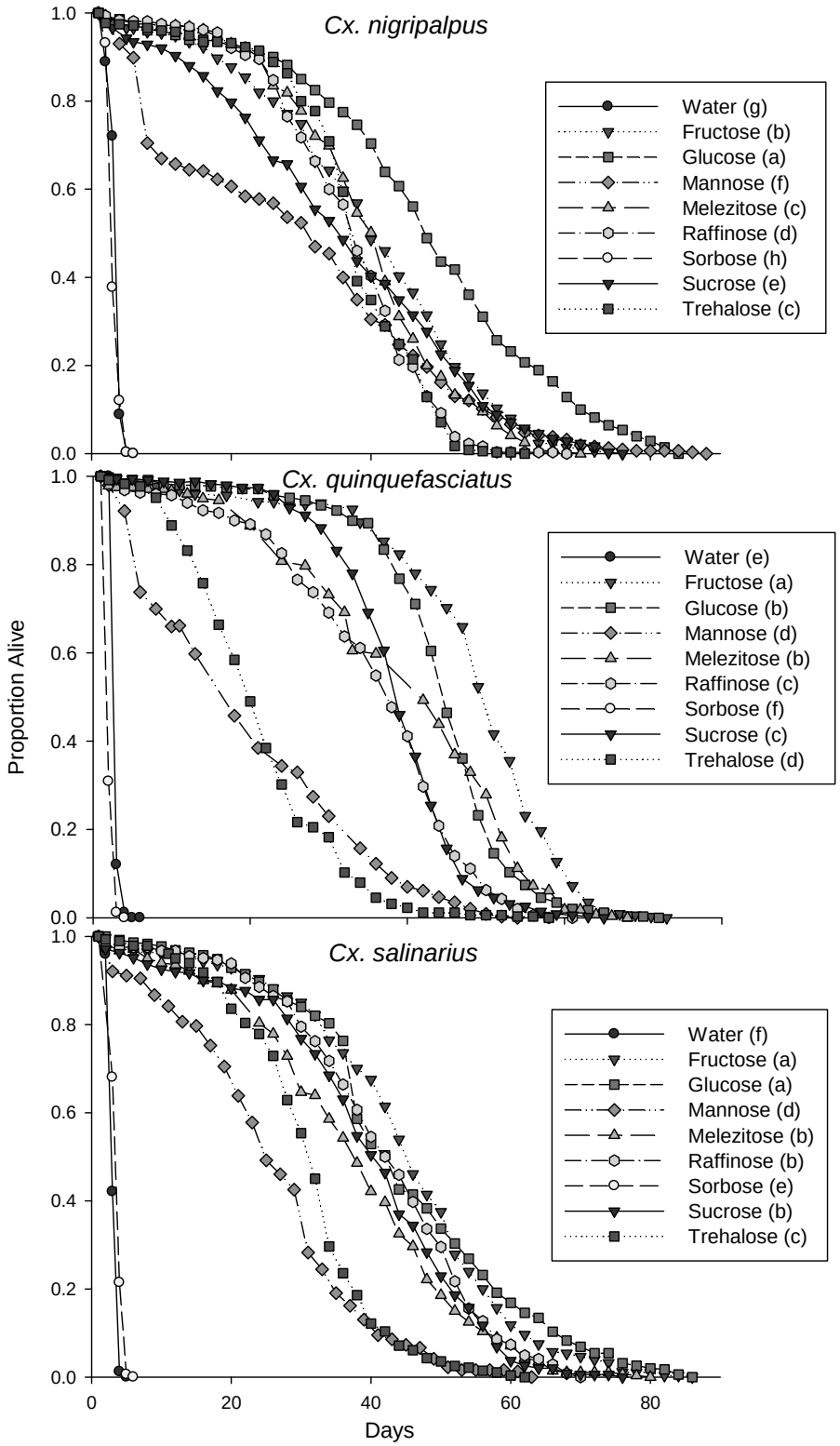

Figure 3-2. Proportion survivorship of *Culex* females maintained on 5% solutions of different sugars. Treatments with similar letters are not significantly different (Kaplan-Meier Survival Curve, pairwise tests, $P<0.05$).

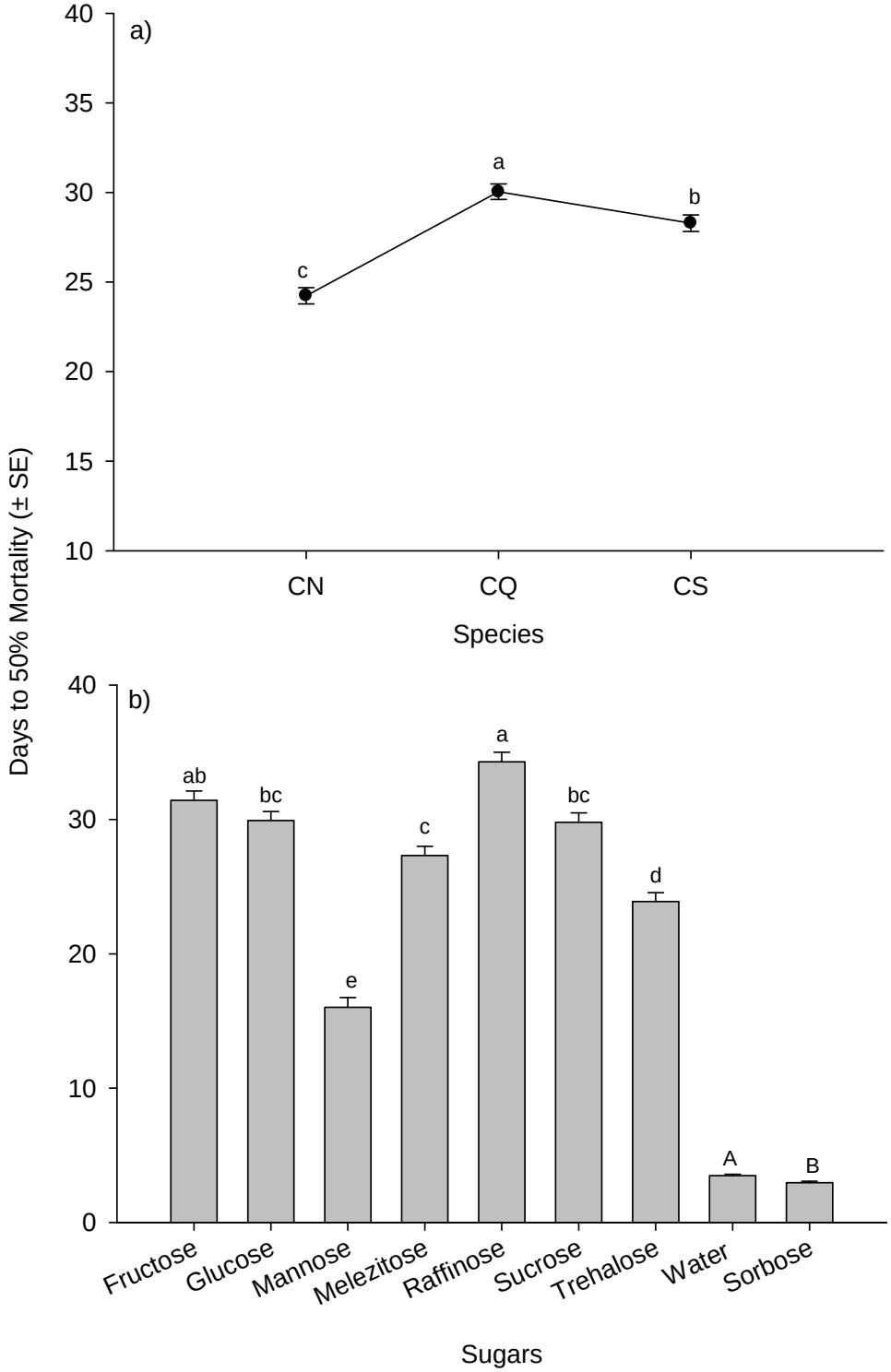

Figure 3-3. Effect of species (a) and sugar (b) on days to 50% mortality (± SE) (LS Means) of males. Columns with similar lowercase (Tukey's HSD, $P<0.05$) or uppercase (*t*-tests, $P<0.05$) letters are not significantly different.

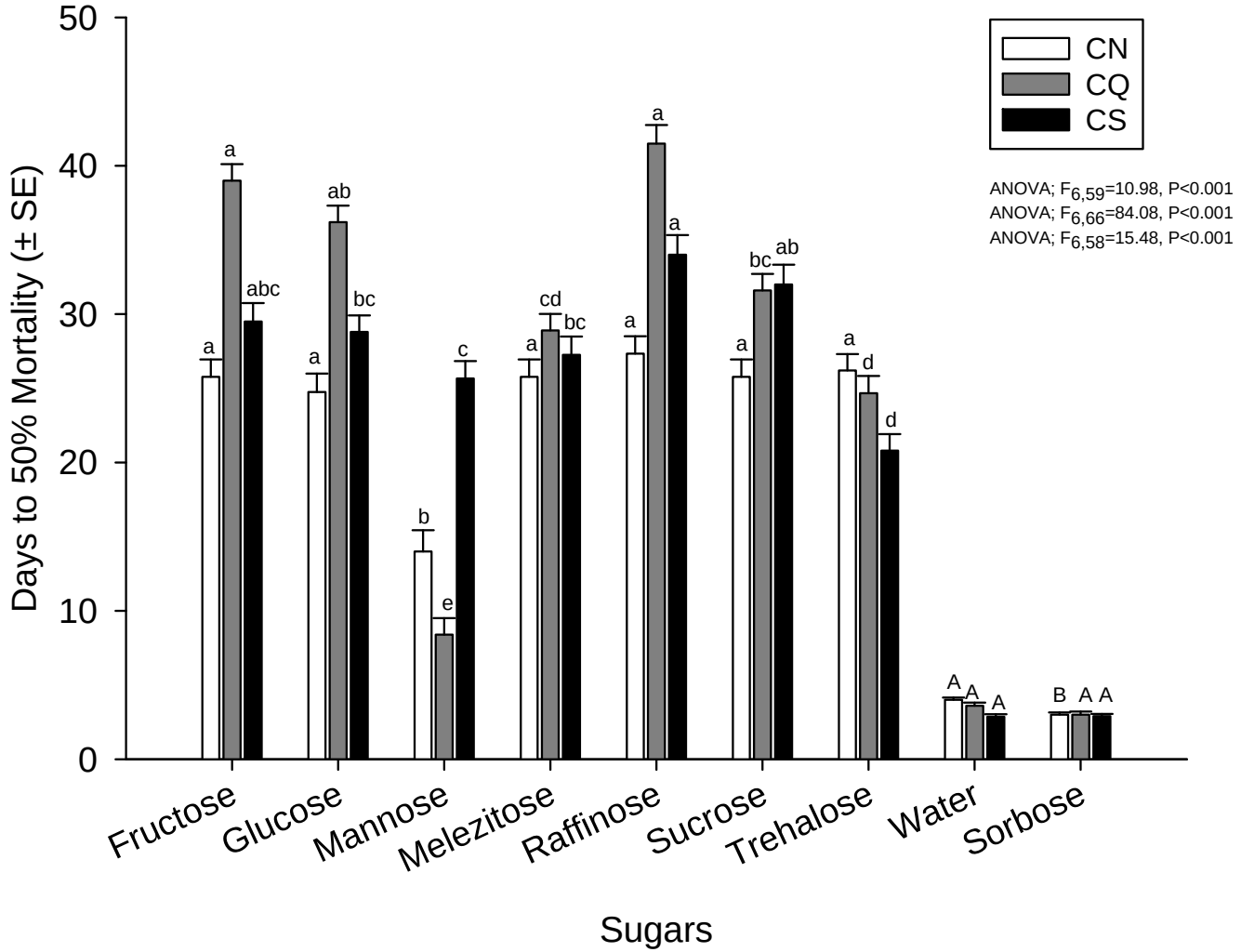

Figure 3-4. Comparison of days to 50% mortality of *Cx. nigripalpus* (CN), *Cx. quinquefasciatus* (CQ) and *Cx. salinarius* (CS) males maintained on 5% solutions of similar sugars. Columns for each species with similar letters are not significantly different (Tukey's HSD, $P<0.05$).

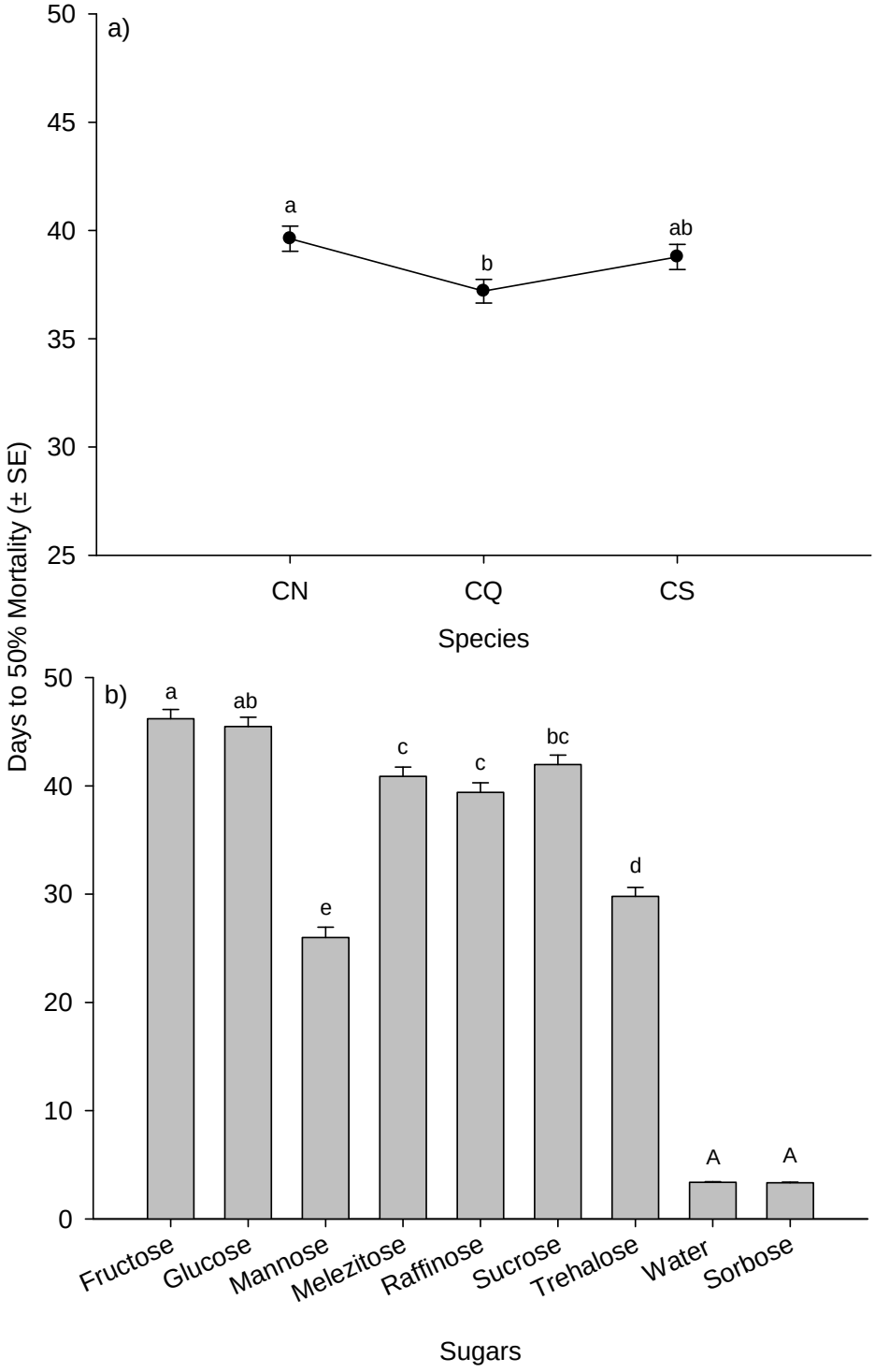

Figure 3-5. Effect of species (a) and sugar (b) on days to 50% mortality (± SE) (LS Means) of females. Columns with similar lowercase (Tukey's HSD, $P<0.05$) or uppercase (*t*-tests, $P<0.05$) letters are not significantly different.

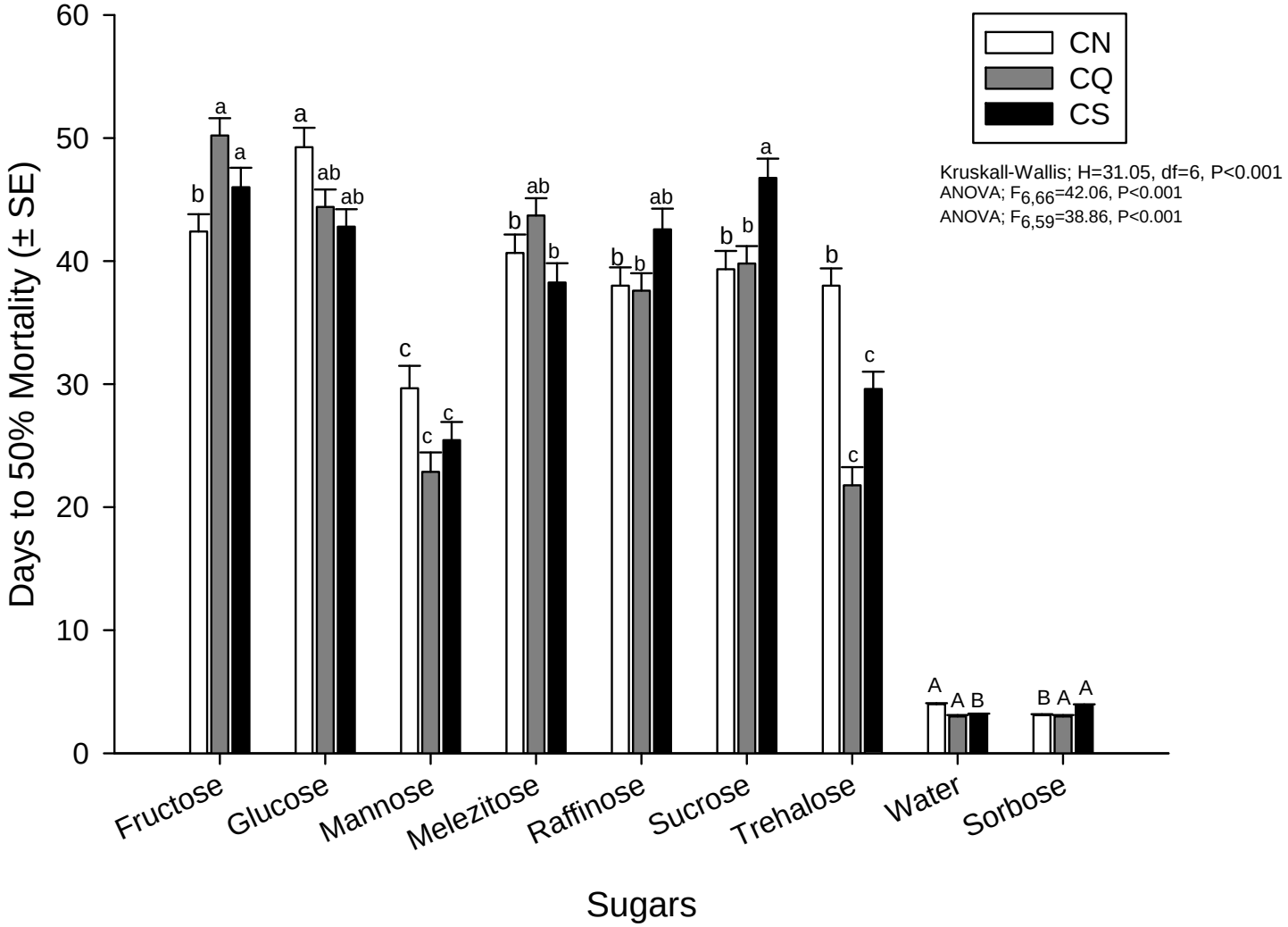

Figure 3-6. Comparison of days to 50% mortality of *Cx. nigripalpus* (CN), *Cx. quinquefasciatus* (CQ) and *Cx. salinarius* (CS) females maintained on 5% solutions of similar sugars. Columns for each species with similar letters are not significantly different (Tukey's HSD, $P<0.05$).

CHAPTER 4
EFFECT OF NUTRITIONAL STATUS AND PRESENCE OF AMINO ACIDS TO A SUGAR MIXTURE ON LONGEVITY OF C*ulex quinquefasciatus*

Introduction

Since the discovery that amino acids are the second most abundant component of nectars, there has been a keen interest in the role that they play in the fitness of the insects that feed on them (Baker and Baker 1973). Some insects rely on nectar as a primary source for amino acids, but others feed on fruit, dung, pollen or even blood to obtain supplements. Ingestion of amino acids in sugar solution or by feeding on pollen has been reported to enhance longevity and fecundity in females and males of several of species of butterflies (O'Brien et al. 2003, Mevi-Shutz and Erhardt 2005, Beck 2007). Providing pollen in sugar solutions to *Aedes aegypti* also increased longevity and fecundity in that species (Eischen and Foster 1983).

Mosquitoes feed on nectar and honeydew as a source of flight energy as well as to enhance longevity (Nayar and Sauerman 1971a, 1971b, 1973, 1975; Gary and Foster 2004). When not feeding on nectar, anautogenous species may also feed on blood as a source of amino acids, which are used to make vitellogenin for egg production (O'Meara 1987). Eischen and Foster (1983) demonstrated some egg production from anautogenous *Ae. aegypti* fed only pollen. However, this is not typical, and in the absence of a sufficient amino acid source, most anautogenous mosquitoes will not be able to obtain enough provisions for egg production. In most cases, blood feeding is the primary source of amino acids; however, when vertebrate hosts are unavailable, amino acids from nectar or pollen could enhance longevity and fecundity.

Many physiological attributes can affect the longevity of mosquitoes in the field and mosquitoes reared in the laboratory. Larvae collected from the field can be exposed to a wide variety of nutritional conditions, and those from suboptimal conditions may result in smaller body sizes (McCann 2006). Field-collected mosquitoes from natural rearing conditions may

contain nutritional reserves that are much smaller than their counterparts reared in a laboratory under optimal conditions (Day and Van Handel 1986). Adult mosquitoes maintained in the laboratory solely on sucrose can attain high levels of lipid and glycogen deposition (O'Meara 1987; Day and Van Handel 1986), with levels typically greater than field caught individuals (Day and Van Handel 1986).

Culex quinquefasciatus are the primary vector of *Wuchereria bancrofti* in Asia, Africa, the Western Pacific and sporadically in the Americas (Samuel et al. 2004). Lymphatic filariasis is caused by *W. bancrofti*, and is a debilitating disease associated with a large number of deaths each year. It may even be a contributing factor in maintaining poor economic conditions in areas where it is endemic (WHO 1994). In the United States, *Cx. quinquefasciatus* is also a known vector of St. Louis encephalitis virus (SLE) (Jones et al. 2002) and a competent and potential vector of West Nile virus (WNV) (Sardelis et al. 2001, Molaei et al. 2007) in the southeastern United States. The ability of these mosquitoes to vector such diseases relies heavily on their length of life, potential to feed on multiple hosts, and successful incubation of the various pathogens. Amino acids in nectar could be an important source of energy and survival with potential to optimize the mosquito's ability to survive and, thus, to vector disease more effectively.

Levels of amino acids in nectar have been used to broadly classify flowers into different groups based on the preferences of their potential pollinators (*e.g.* butterfly flowers, bee flowers) (Baker and Baker 1973). Butterfly flowers contain high levels of amino acids, and have been studied as a potential critical source for proteins contributing to reproduction (Baker and Baker 1973). Common lantana (*Lantana camara* L. (Verbenaceae)) is an example of a butterfly flower that contains a high amino acid concentration (16 μmoles/ml) as well as the sugars, fructose,

glucose and sucrose (Alm et al. 1990). *Lantana camara* is a widely used ornamental in many temperate regions (Morton 1994), including Florida and extending throughout the southeastern United States. Nectar mimics of this species have been used in various feeding preference studies with butterflies (Mevi-Shutz and Erhardt 2003b, Alm et al. 1990) and are a known attractant for the dipteran vector, tsetse fly, in Africa (Syed and Guerin 2004). Additionally, Haeger (unpublished data, cited in Nayar 1982) has observed *Cx. nigripalpus* feeding on *Lantana* in the field, so a critical question is whether mosquitoes feeding on nectar amino acids such as those present in *Lantana* nectar will also display enhanced survival.

The objective of this study was to evaluate the effect of amino acids in nectar on survival of adult mosquitoes. To determine if amino acids alone enhanced longevity, the mosquitoes were not blood fed at any time. In the field, mosquito larvae may experience stressful conditions such as overcrowding or poorly nutritioned diets. Rearing insects on high and low food diets as larvae mimic conditions mosquitoes may encounter in the laboratory or nature. Day and Van Handel (1986) determined that individuals reared in the laboratory, presumably under high nutrient conditions, contained increased levels of glycogen and lipid over those that were field-caught. Adults of the map butterfly that were fed poor diets as larvae had longer lives and were more fecund when fed an adult diet containing amino acids (Mevi-Shutz and Erhardt 2005). For this experiment, glycogen and lipid analyses were performed on unfed newly emerged mosquitoes to illustrate differences between deposition of glycogen and lipid in larvae reared on low and high food larval diets. Additionally, measures of winglength and dry weights were obtained to compare body size between rearing regimes. Previous experiments have shown that females outlive males (Liles and DeLong 1960, Briegel and Kaiser 1973) and that a water diet only supports survival for a few days (Nayar 1986, Nayar and Sauerman 1971a, 1971b, 1975). In

our study, females and males from both larval diets were compared separately and treatments were compared as without sugar (water only, water + amino acids) or with sugar (*L. camara* nectar mimic only, *L. camara* nectar mimic + amino acids).

Materials and Methods

Mosquito Rearing

Culex quinquefasciatus were reared at the USDA, CMAVE facility in Gainesville, FL. Larvae were reared in plastic trays (35.5cm x 48.3cm x 6.4cm) with 2.5 liters of water. Approximately 5 egg rafts were set in each pan, resulting in about 500 larvae. The colony was established from a Gainesville light trap collection in 1995. The protocols of Telang and Wells (2004) were followed to differentiate between the negative effects of crowding and to focus on the effect of larval nutrition. Keeping larval density consistent and feeding some larvae more than others allowed us to test specifically for effect of nutrition on larvae and examine those effects on size and nutritional state of adults. When rearing larvae on a high food diet, food was provided as a slurry of 50 ml of a 3:2 bovine liver/brewer's yeast powder (MP Biomedicals, Solon, OH) (added as 40 g to 1.0 L of water) on day 1 and 50 ml of finely ground 2% hog chow slurry (Purina Mills, Gray Summit, MO) thereafter on days 3, 4 and 5 (Table 4-1). When rearing larvae on a low food diet, food was provided as a slurry of 50 ml of a 3:2 bovine liver/brewer's yeast powder on day 1 and 25 ml of 2% hog chow slurry on day 4 only (Table 4-1). The number of days from egg to pupation for the larvae reared on low food diets was equal to that of the larvae reared on high food diets.

Winglength and Dry Weight Measurements

To evaluate the effect of rearing regimes on the size of resulting adults, we quantified winglength and dry weight. Upon pupal emergence, with no prior exposure to sugar, a subsample of 10 males and 10 females were removed from cages and frozen (-20ºC) for later

measurement of winglengths and dry weights. Wings were removed, mounted, viewed under a microscope and photographed using ScopePhoto 1.0 (Scopetek, Hangzhou). Wings were measured from the alular notch to the distal end of the R_2 wingvein, fringehairs excluded (Figure 4-1) (SigmaScan Pro 5.0 (SPSS, Inc., Chicago, IL). The software was calibrated with a 5.0 mm steel pin and the wing measurements based on that calibration.

To measure dry weights, groups of 10 male and female mosquitoes from each larval feeding regime were frozen at -20°C prior to being set up in the longevity experiment. These mosquitoes were stored at -20°C until they were moved to -80°C in preparation for freeze-drying. The groups of mosquitoes were freeze-dried for 48 hours and dry weights were obtained with a microbalance (Sartorius, Germany). The mosquitoes were stored again at -20°C until used for lipid and glycogen analyses.

Glycogen and Lipid Analyses

Initial nutritional reserves of the mosquitoes at the time of pupal emergence were determined by measuring the levels of glycogen and triglyceride. The hot anthrone (glycogen) (Van Handel 1985) and sulphosphovanillin (lipid) (Van Handel 1985, as modified by Hahn 2005) assays were conducted following the methods provided in detail in the Appendix. Five replicates were completed for each sex and larval rearing regime.

All of the samples that were previously freeze dried, weighed and refrozen were analyzed for glycogen and lipid using the hot anthrone assay for glycogen or sulphosphovanillin assays for lipids. The dried mosquitoes were homogenized in microcentrifuge tubes with 100µl of saturated sodium sulfate, 200 µl of methanol, 100 µl ultrapure water and 500 µl of 1:1 chloroform: methanol with a motorized homogenizer. The glycogen and lipid samples were measured using the methods of Van Handel (1985). Lipid solutions were washed through glass

pipette columns of 0.2 g silicic acid and rinsed with 4 mL of chloroform to extract only neutral lipids (Hahn 2005).

Mating Assay

The association of male and female mosquitoes has been reported to shorten the life of males and lengthen the life of female mosquitoes beyond that of females reared alone. Liles and DeLong (1960) reported that mated parous female *Aedes aegypti* lived as long as unmated nulliparous females, indicating a possible benefit in longevity of females from being mated. Additionally, larger male *An. gambiae* have been reported to compete for mates more efficiently than smaller males (Ng'habi et al. 2005). Therefore, it is important to determine whether mating occurred successfully under the experimental conditions. We hypothesized that mating would occur under our conditions, provided the mosquitoes lived long enough to mate. The setup of this assay was similar to that of the longevity assays (described in more detail later), in which the mosquitoes were maintained on a 5% sucrose solution via a cotton wick. Thirty-five male and female mosquitoes were placed in the containers and maintained in an incubator at 28.0 ± 1.0ºC, 81.2 ± 0.1% relative humidity and 14:10 light: dark photoperiod. Five replicates were completed for each larval rearing regime. The tests were conducted for fifteen days, to ensure enough time to complete mating. The mosquitoes were removed and frozen at -20°C until dissection. Upon dissection and examination of the spermathaeca under a microscope, the number of females that contained sperm and those that did not were counted.

Amino Acid Longevity Assay

Upon adult emergence, 35 males and 35 females were placed in 0.47 liter paper containers (Solo Cup Company, Highland Park, IL). The lids were modified with white tulle to allow viewing inside the cages. The cages were also modified by cutting an access hole in the side where a 1.5 ml microcentrifuge tube could be inserted. The tube was filled with 1.0 ml of

treatment solution and a cotton dental wick previously saturated with the same solution (3.8 cm) (Richmond Dental, Charlotte, NC). This allowed the mosquitoes to feed *ad libitum* on the solution provided. Treatments included a *Lantana camara* sugar mixture mimic only, *Lantana camara* mimic with amino acids added, amino acids in water and a water only control. The nectar mimic contained 0.547 M sucrose, 0.282 M D-(+)-glucose, and 0.316 M D-(+)-fructose (Sigma, St. Louis, MO). The nectar mimic with amino acids additionally contained the nonessential amino acids L-alanine (0.718 mM), L-asparagine (0.421 mM), L-glutamic acid (0.326 mM), L-glutamine (0.931 mM), glycine (2.371 mM), L-proline (2.23 mM), and L-serine (1.37 mM) and the essential amino acids L-arginine (0.201 mM), L-threonine (0.672 mM), L-tyrosine (0.221 mM), and L-valine (0.137 mM) (Sigma, St. Louise, MO). This particular nectar mimic mixture has been used in previous studies evaluating effects of amino acids on fitness of various species of butterflies (Alm et al. 1990, Mevi-Shutz and Erhardt 2005). *Lantana camara* is also a known nectar source of the tsetse fly in Africa (Syed and Guerin 2004) and *Culex nigripalpus* in the U.S. (Haegar; in Nayar 1982), and is widely abundant as an ornamental and naturalized plant in many areas of the Southeastern United States, including Florida (Morton 1994). Ten replicates of each treatment and five replicates of the water control were completed. Mosquitoes were maintained at 28.12 ± 1.0ºC, 81.2 ± 0.1% relative humidity and 14:10 day: night photoperiod. The number of dead males and females were counted daily and the day of 50% mortality determined.

Statistical Analyses

All statistical analyses were conducted using Minitab 15.1 (Minitab, Inc., State College, PA) or JMP (SAS Inc., Cary, NC). Assumptions of normality and homogeneity of variance were confirmed using graphical representations of the data and a Levene's test ($P<0.05$).

One-way ANOVAs were used to determine differences in winglength and dry weight among females or males from each larval rearing regime. Mean triglyceride and glycogen weights and their standard errors were obtained for each group of 10 mosquitoes. Nutritional reserve weights were calculated by dividing glycogen or lipid weights by the dry weights of the mosquitoes in order to correct for weight differences. Data for glycogen analyses were Ln transformed as necessary. A one-way ANOVA was used to determine differences in the mean lipid or glycogen weights, and Tukey's HSD ($P<0.05$) was used to locate differences between the means.

Previous experiments have shown that there are differences in the survival of males and females (Liles and DeLong 1960, Briegel and Kaiser 1973) and that providing a water only treatment will not support survival beyond a few days, whereas treatments containing sugar will likely increase survival exponentially beyond that (Nayar and Sauerman 1971a, 1971b, Nayar 1986). Therefore, days to 50% mortality for each replicate were determined for males and females separately and treatments without sugar (water only and water + amino acids) and treatments with sugar (*Lantana camara* nectar mimic and *L. camara* nectar mimic + amino acids) separately and used to build 4 multivariable ANOVA models containing all explanatory variables (adult diet, larval diet and their interaction). Tukey's HSD ($P<0.05$) or two-sample *t*-tests ($P<0.05$) were used to separate the means.

Results

Effect of Larval Nutrition on Adult Size, Nutrient Reserves and Mating

Adult males and females had significantly larger winglengths ($t=6.90$, df=15, $P<0.001$; $t=8.37$, df=12, $P<0.001$, respectively) (Table 4-2, Figure 4-2) and greater body mass ($t=14.02$, df=4, $P<0.001$; $t=27.77$, df=6, $P<0.001$, respectively) (Table 4-2, Figure 4-2) when reared on high food diets than adults than from larvae reared on low food diets (Table 4-2). Feeding a high

food diet resulted in adults that weighed nearly 2 times more in males and were 2.6 times larger in females.

High food diets as larvae resulted in larger glycogen and lipid reserves in both males ($t=6.35$, $df=4$, $P=0.003$; $t=20.64$, $df=5$, $P<0.001$, respectively) and females ($t=16.73$, $df=4$, $P<0.001$; $t=26.24$, $df=7$, $P<0.001$, respectively) (Table 4-3, Figure 4-2). Males contained nearly 9 times more glycogen and 4 times more triglycerides when fed a high food diet than males reared on a low food diet. Females showed a similar trend, accumulating approximately 13 times more glycogen and 3 times more triglycerides when fed a high food diet as larvae as opposed to a low food diet. Adult females that were fed high food and low food diets as larvae were nearly all mated (98% and 96%, respectively).

Survival Analyses

***Culex quinquefasciatus* fed treatments without sugar**

Adult diets lacking sugar, either water only or water + amino acids, did not sufficiently support survival, with all individual living less than 5 days (Table 4-5). Larval diet had the most significant effect on survival; males and females resulting from larvae reared on a high food larval diet lived significantly longer than those adults from larvae reared on a low food larval diet (Table 4-6) (Figures 4-3, 4-4a, 4-4b). Amino acids added to the water did not increase longevity of males or females (Table 4-6) (Figures 4-3, 4-4c, 4-4d).

***Culex quinquefasciatus* fed treatments with sugar**

The addition of sugar to the adult diet increased survival far beyond that achieved by treatments without sugar for both males and females, with all adults living >25 days or 5 times longer than individuals not receiving sugar (Table 4-5) (Figure 4-3). The two sexes differed in the effects of larval diet on adult longevity (Table 4-6) (Figures 4-5a, 4-5b). Adult males fed sugar lived longer when fed a low food diet as larvae (Figure 4-5a). Females that were fed a

high food diet as larvae continued to live longer than those that were fed a low food diet (Figure 4-5b). Adding amino acids to the adult diet of males did not increase longevity (Table 4-5, Figure 4-5c), but there was a slight increase in longevity when females were fed a diet containing amino acids (Table 4-5, Figure 4-5d). The interaction between adult and larval diet of females revealed that those fed a low food diet as larvae benefitted from the addition of amino acids to their adult diet, but females from larvae fed a high food diet did not. They were able to slightly overcome a poor larval diet and live as long as females that were fed a high food larval diet (Figure 4-5d).

Discussion

The addition of amino acids into the adult diet of female *Cx. quinquefasciatus* significantly increased lifespan over females fed only a *L. camara* nectar mimic when larval diets were pooled, but had no effect on male survival. Females, when fed an adult diet containing amino acids, overcame their poor larval diet and survival was equivalent to those females resulting from optimally fed larvae. Although the increase in survival is small, it is statistically significant and is supported by similar research with *Ae. aegypti* fed pollen in their adult diet (Eischen and Foster 1983) and the map butterfly fed amino acids in their adult diets (Mevi-Shutz and Erhardt 2005). Beck (2007) indicated that longevity was increased only in some species of male butterflies, whereas other authors did not find a link between ingestion of amino acids and increased fitness in insects (Liles and DeLong 1960, Mevi-Shutz and Erhardt 2003a, Molleman 2008). Considering the research conducted on the map butterfly and our study, it appears that an important factor that has been overlooked is the effect that amino acids in the adult diet may have on survival of adults that were nutritionally challenged as larvae. Larval insects are exposed to many challenges in nature, including overcrowding and inadequate nutrition, with larval nutritional deprivation common in mosquitoes (Day and Van Handel 1986). Preference

for adult diets containing amino acids compared to those without has also been reported with butterflies (Alm et al. 1990) with stronger effects reported in butterflies nutritionally deprived as larvae (Mevi-Shutz and Erhardt 2003b). Examining the effects of the addition of amino acids to the adult diet of laboratory-reared mosquitoes may mask a resulting increase in longevity if we consider that optimally-reared mosquitoes may contain sufficient nutritional reserves acquired from the larval stage. There are differences reported on the possible effect of amino acids on the adult diets of various insects (Mevi-Shutz and Erhardt 2005, 2003a, Eischen and Foster 1983) and even among species living under similar conditions (Beck 2007). Thus, it is possible that the role of amino acids in nectar may differ between mosquito species and should be taken into consideration.

The effect of a larval diet on adult longevity was the most significant factor in this study for both sexes. Male and female *Cx. quinquefasciatus* both emerged with larger winglengths, dry weights and glycogen and lipid reserves when reared on a high food diet. In studies that have used laboratory-reared mosquitoes, females exposed to high food resources likely have greater glycogen and lipid reserves for flight and increased survival and would more closely resemble those fed high food diets in this study, in contrast to field-caught individuals. However, unlike females, males in our study lived significantly longer when fed a low food diet as larvae. The association of males and females has been shown to positively affect female longevity and negatively impact survival of males (Liles and DeLong 1960). Nearly all of the females in this study were mated, which we think may play a role in their increased longevity. However, males seem to suffer from their association with females, particularly those that were reared on a high food diet as larvae. It has been shown that males that were larger upon emergence, and fed high food diets as larvae, were more likely to be better competitors and to

mate first than those males that were smaller as adults due to nutritional challenges as larvae (Ng'habi et al. 2005). Additionally, increased mating occurrences reduced survival of male *Saltella sphondylli* (Martin and Hosken 2004) and may explain why the more sexually competitive, male *Cx. quinquefasciatus* reared on a high food larval diet in our study survived less time than adults from their low food reared counterparts.

It is important to understand how larval and adult nutrition affect longevity, flight potential (Nayar and Sauerman 1971a, 1971b, 1975), biting persistence (Nasci 1991) and, most importantly, disease transmission. Arboviruses transmitted by these *Culex* species have lengthy amplification periods that require long lifespans (Turell et al. 2005). The survival of mosquitoes is highly dependent on their ability to locate and feed on nectar, honeydew or other sources of carbohydrates and amino acids. We found that teneral reserves are an important predictor of adult life span in male and female *Cx. quinquefasciatus*. Similar to the findings of Mevi-Shutz and Erhardt (2005), amino acids added to the adult diet contributed to the enhanced survival of females reared on poor larval diet regimes. These females were able to survive as long as females resulting from a high food larval diet. Not only are substantial teneral reserves important for increased longevity, but also they are crucial for immediate flight energy which is essential for location of carbohydrate sources when teneral reserves are depleted. There is evidence that some mosquitoes will not even begin host-seeking until they have obtained their first sugar meal (Foster 1995) and that adults with low nutritional reserves are better vectors of WNV due to their reduced ability to avoid infection compared to adults with high nutritional reserves (Vaidyanathan et al. 2008). Because amino acids are the second most abundant component of nectar, and mosquitoes feed on nectar frequently to increase their nutritional reserves, future studies may reveal additional roles that amino acids play in the diet of females.

Future research may examine the effect of larval nutrition on the establishment and transmission of pathogenic agents, and the mitigation of this by adults feeding on diets rich in amino acids.

Table 4-1. Schedule of feeding for *Cx. quinquefasciatus* under a high or low food regime. Larval food = 3% bovine liver powder (LP): 2% Brewer's yeast (BY) (30 g bovine liver powder and 20 g Brewer's yeast in 1L of water), 2% hogchow (36 g finely ground hog chow in 1800 ml of water).

Days After Hatch	Amount of food given per diet level (ml)	
	High	Low
1	50 ml; 3% LP:2% BY	50 ml; 3% LP:2%BY
3	50 ml; 2% hogchow	
4	50 ml; 2% hogchow	25 ml; 2% hogchow
5	50 ml; 2% hogchow	

Table 4-2. Mean winglength (N=5) and dry weight (N=10) measurements for adult *Cx. quinquefasciatus* males and females reared on high or low food diets as larvae.

	Winglength (mm)		Dry Weight (mg)	
	Males	Females	Males	Females
Low food	2.41 ± 0.04a	2.64 ± 0.06a	2.70 ± 0.05a	3.49 ± 0.18a
High food	2.74 ± 0.03b	3.16 ± 0.03b	5.07 ± 0.16b	9.11 ± 0.10b

Means within columns with a similar letter are not significantly different (*t*-test, $P<0.05$).

Table 4-3. Mean glycogen and lipid content (N=5) measurements for adult *Cx. quinquefasciatus* males and females reared on high or low food diets as larvae.

	Glycogen (μg/mg)		Lipid (μg/mg)	
	Males	Females	Males	Females
Low food	9.38 ± 1.67a	7.15 ± 0.56a	42.64 ± 2.83a	33.84 ± 3.71a
High food	82.61 ± 9.70b	96.44 ± 5.12b	113.31 ± 1.43b	108.37 ± 2.41b

Means within columns with a similar letter are not significantly different (t-test, $P<0.05$).

Table 4-4. Percent mated female *Cx. quinquefasciatus* when reared on high or low food diets as larvae and maintained under conditions of survival assay.

Larval Feeding Regime	N	Percent Mated Females
High food	N=55	98%
Low food	N=50	96%

Table 4-5. Days to 50% mortality (LS Means ± SE) of male and female *Cx. quinquefasciatus* fed high or low food diets as larvae. Adult diets were no sugar treatments (water only, water + amino acids) and sugar treatments (*Lantana camara* nectar mimic and *L. camara* mimic + amino acids).

Adult Diet with sugar	Larval diet	Adult diet	Days to 50% Mortality (±SE) (LS Means)[a]					
			♂	N	No. of mosquitoes	♀	N	No. of mosquitoes
No	Low	Water only	2.20 ± 0.14	5	175	3.00 ± 0.12	5	175
No		Water + amino acids	2.33 ± 0.13	6	210	3.00 ± 0.11	6	210
No	High	Water only	5.00 ± 0.10	5	175	4.80 ± 0.12	5	175
No		Water + amino acids	5.00 ± 0.14	10	350	4.90 ± 0.09	10	350
Yes	Low	*L. camara* nectar mimic	30.00 ± 1.04	7	245	32.71 ± 0.98	7	245
Yes		*L. camara* mimic + amino acids	32.00 ± 1.12	8	280	35.25 ± 0.92	8	280
Yes	High	*L. camara* nectar mimic	27.33 ± 0.99	10	350	36.70 ± 0.82	10	350
Yes		*L. camara* mimic + amino acids	27.60 ± 0.94	10	350	38.00 ± 0.82	10	350

[a]All LS Means derived from *full models* (adult diet, larval diet and their interaction).

Table 4-6. Results of ANOVA on the effect of larval diet, adult diet and their interaction on survival of male or female *Cx. quinquefasciatus*.

Treatment	Sex	Source	df	*F*	*P*
No sugar	Males	Whole model	2	251.43	<0.001*
		Larval diet	1	492.37	<0.001*
		Adult diet	1	0.23	0.634
		Error	23		
		Total	25		
No sugar	Females	Whole model	2	148.40	<0.001*
		Larval diet	1	289.98	<0.001*
		Adult diet	1	0.25	0.625
		Error	23		
		Total	25		
Sugar	Males	Whole model	2	52.44	0.067*
		Larval diet	1	11.33	0.002*
		Adult diet	1	0.51	0.478
		Error	31		
		Total	33		
Sugar	Females	Whole model	2	9.16	0.001*
		Larval diet	1	14.43	0.001*
		Adult diet	1	4.40	0.044*
		Error	31		
		Total	34		

* Indicates a significant effect ($P<0.05$).

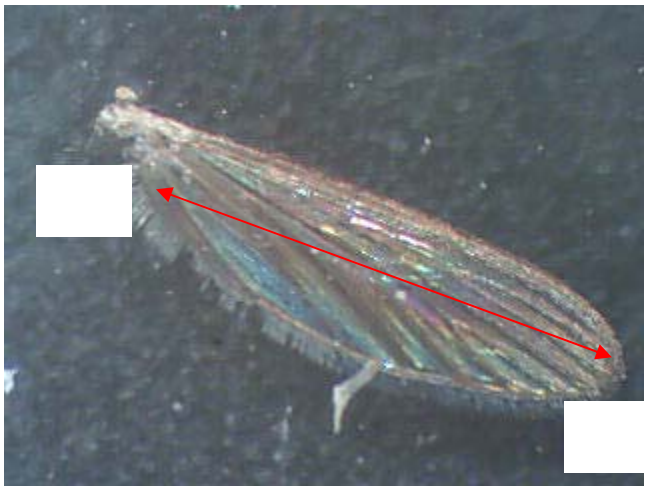

Figure 4-1. Photograph of mosquito wing with arrow indicating measurements taken from the alular notch (A1) to the distal end of wing vein R_2 and used to calculate mean wing lengths.

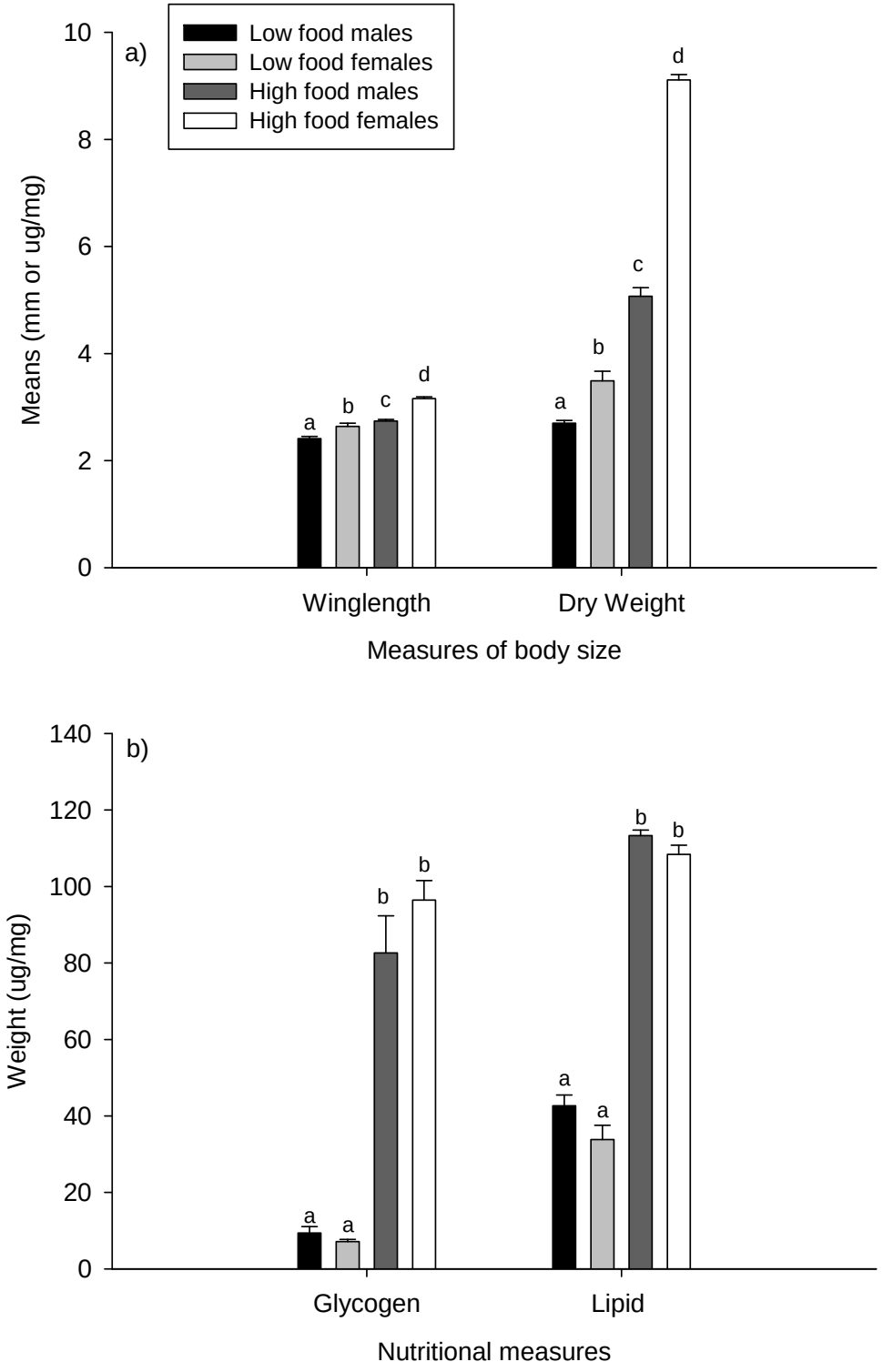

Figure 4-2. Mean winglength (mm), dry weight (mg) (a) and glycogen and lipid weights (µg/mg) (b) of male and female *Cx. quinquefasciatus*. Treatments with similar letters are not significantly different (Tukey's HSD, $P<0.05$).

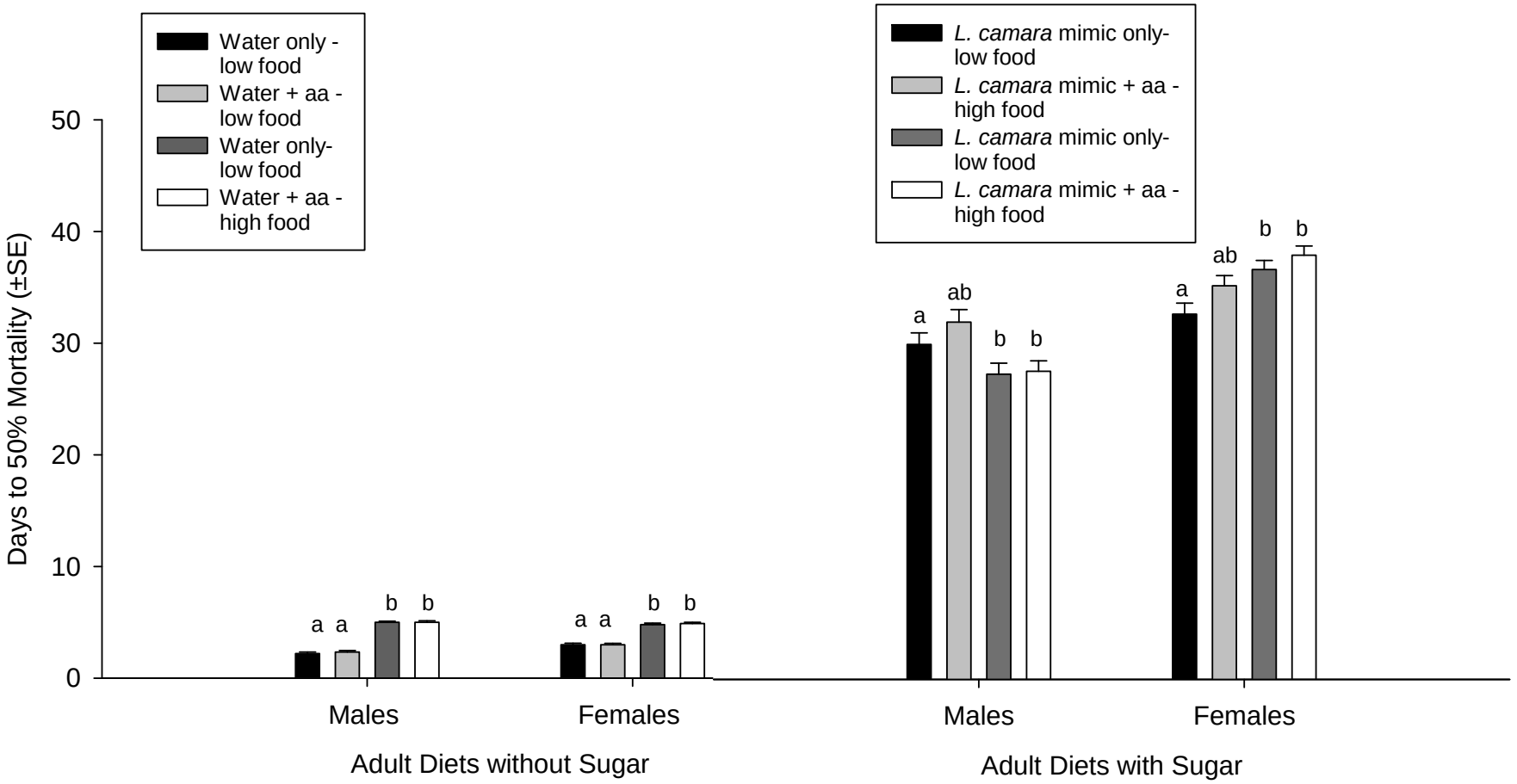

Figure 4-3. Days to 50% mortality of males and females fed low or high food diets as larvae and diets with or without sugar as adults. Letters within a sex and adult diet` that are similar are not significantly different (Tukey's HSD, $P<0.05$).

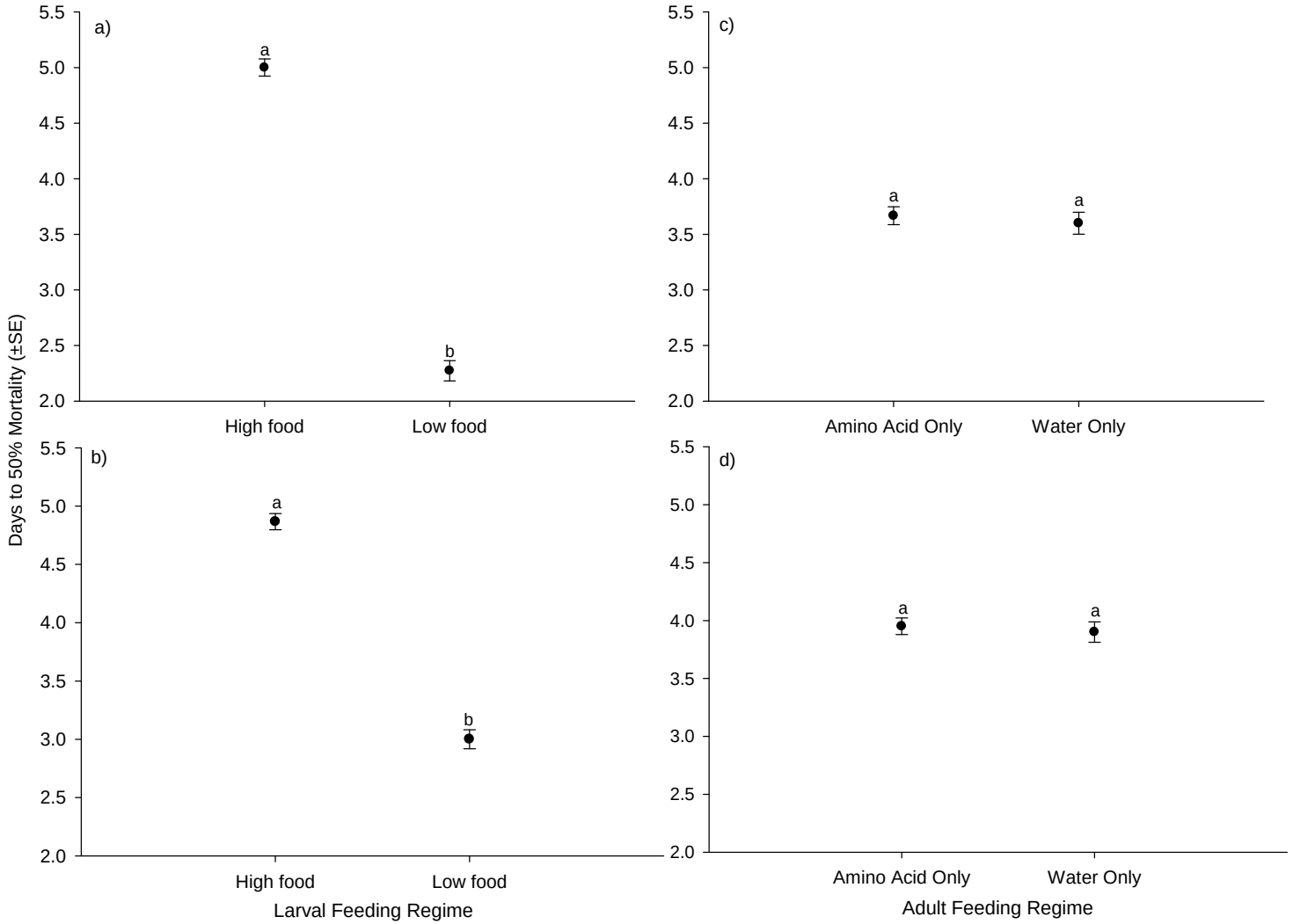

Figure 4-4. The effect of larval diet on days to 50% mortality (LS Means) of males (a) and females (b) and adult diet on males (c) and females (d) fed treatments without sugar. Treatments with similar letters are not significantly different (*t*-tests, $P<0.05$).

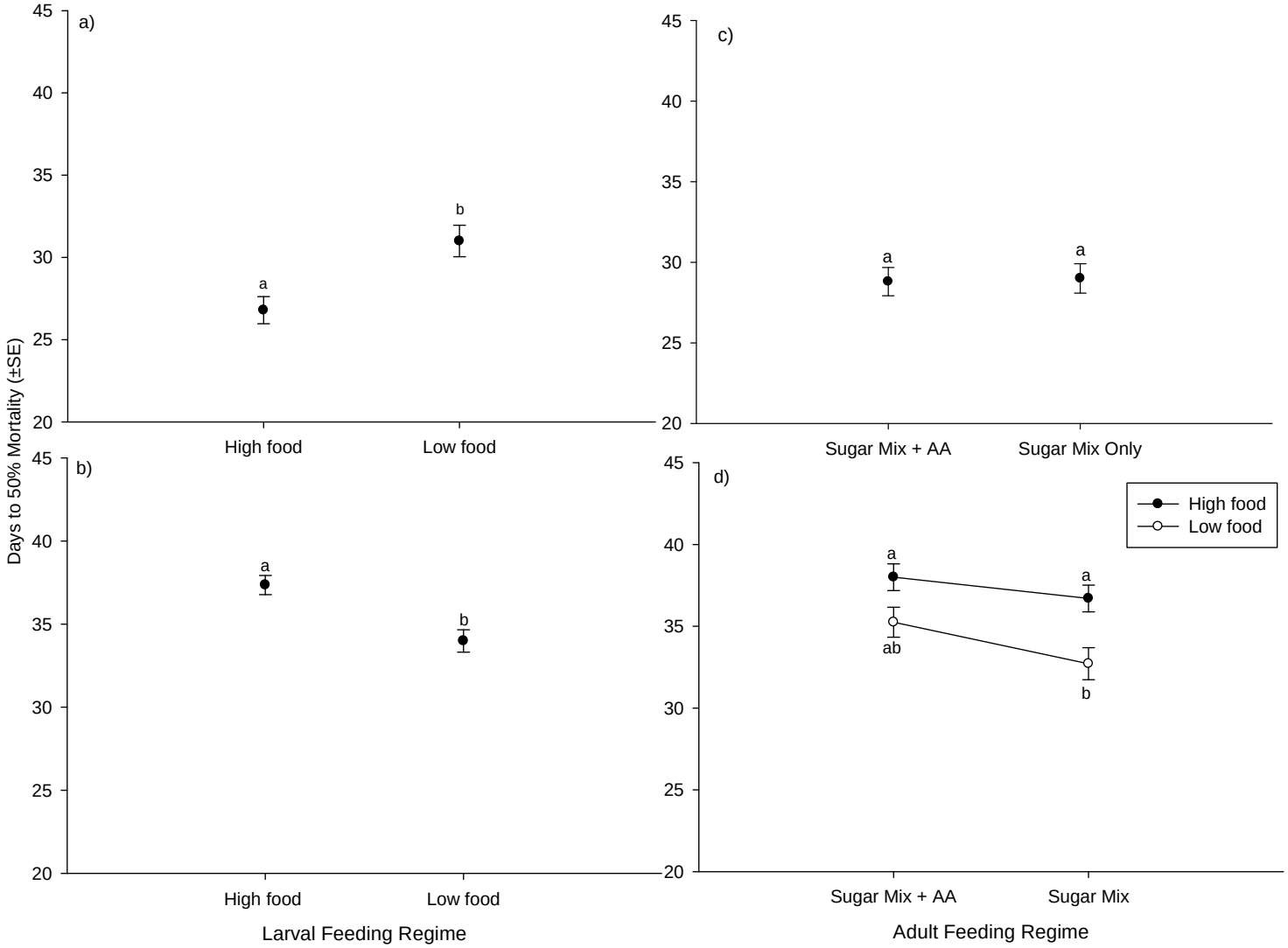

Figure 4-5. The effect of larval diet on days to 50% mortality (LS Means) of males (a) and females (b) and adult diet on males (c) and females (d) fed treatments with sugar. Treatments with similar letters are not significantly different (*t*-tests or Tukey's HSD, $P<0.05$).

CHAPTER 5
CONCLUSIONS AND FUTURE RESEARCH

Sugar feeding is an important behavior of *Culex* mosquitoes resulting in acquisition of carbohydrates and amino acids, which increase nutritional stores after emergence and potentially enhance longevity and fecundity (Mevi-Shutz and Erhardt 2005, Beck 2007). The potential to disperse, host seek and become deadly disease vectors increases with sugar feeding (Nayar and Sauerman 1971b). Without sugar feeding, all species in these studies died within 4 days, which corresponds with previous studies where various mosquito species fed only water died rapidly (Nayar 1982, Galun and Fraenkel 1957, Gary and Foster 2004). Previous studies in the laboratory focusing on longevity of mosquitoes are numerous, however, most of them have focused on *An. gambiae, Ae. aegypti* or *Ae. taeniorhynchus* (Galun and Fraenkel 1957, Eischen and Foster 1983, Nayar and Sauerman 1971a, 1971b, 1974, 1975, Briegel et al. 2001, Liles and DeLong 1960) with only some including *Culex* species (Briegel and Kaiser 1973, Nayar and Sauerman 1973, Nayar 1986, Nayar and Pierce 1977). Few studies examined survival of males and females (Liles and DeLong 1960, Briegel and Kaiser 1973, Nayar 1986) and only Galun and Fraenkel (1957) have fed mosquitoes various sugars that appear in all types of natural conditions, including nectars, fruits and honeydew.

Culex mosquitoes transmit West Nile virus (WNV) (Sardelis et al. 2001, Molaei et al. 2004), St. Louis Encephalitis virus (SLE) (Dow et al. 1964, Day 1997, Jones et al. 2002, Foster and Walker 2002), Eastern Equine Encephalitis virus (EEE) (Wellings et al. 1972, Scott and Weaver 1989) lymphatic filariasis (LF) (Subra 1981, Hawking 1973, Chow 1973, Nelson et al. 1962, Dossou-yovo 1995), dog heartworm (Villavaso and Steelman 1970) and many more. Because they are important disease vectors in Florida and throughout the world, understanding their biology and physiology is important to prevent disease outbreaks by working to improve

population monitoring or more effective baiting systems. Because sugar feeding involves both males and females and is critical for increasing energy reserves which make possible, dispersal, increased survival and vectoring capability, understanding this behavior and how it may affect the different sexes and species is important.

Longevity and Sugar Feeding

Often overlooked in favor of research on effects of blood feeding, sugar feeding is an important activity for male and female mosquitoes. Feeding on carbohydrate sources increases longevity far beyond what would be possible without obtaining the additional carbohydrates provided by nectar, rotting fruit, honeydew and other identified sources. The main components of nectar are sucrose, glucose and fructose, which also are found in honeydew along with unique sugars such as melezitose, raffinose and trehalose. Sorbose is also a naturally occurring sugar (US Government 2008), which we used in these studies and has been previously found to be unsupportive of life in mosquitoes and other dipterans (Galun and Fraenkel 1957).

These studies focused on the effect that sugar feeding had on the longevity of male and female *Culex nigripalpus, Culex quinquefasciatus* and *Culex salinarius.* Survival was variable depending on species and adult sugar diet; however, some overall trends occurred. Sugars that are commonly found in nectar and honeydew (sucrose, fructose and glucose) supported long-life, in males and females of all three species. Additionally, sugars that are exclusively found in honeydew (melezitose and raffinose) were as supportive as nectar sugars commonly thought to support the greatest life span in these mosquitoes. Other sugars such as mannose and trehalose supported moderate to poor survival when compared to highly supportive sugars. Sorbose was overall a very poor sugar that did not increase survival over that of the water control. In some cases, survival was less than water, although there was not clear evidence that this sugar was toxic, only very poorly utilized.

Nutritional Reserves and Mating Status

Teneral nutritional reserves are a result of larval diet and larval rearing conditions (Telang and Wells 2004). A crowded larval environment or poor nutritional conditions result in adults that have small body sizes, low nutritional reserves and shorter lifespans (Briegel et al. 2001, McCann 2006). Feeding on carbohydrates as adults can increase nutritional reserves, such as glycogen and lipid. Glycogen is stored in the fat body and flight muscles and can be used for immediate flight energy (Nayar and Sauerman 1971a, 1971b). Lipids are important because they are used for long-term energy, maintenance and survival (Foster 1995, Nayar 1982). Increasing these reserves is critical to the survival and flight potential of a mosquito and its potential to transmit disease.

These studies determined that larval diet played a large role in survival of both male and female *Cx. quinquefasciatus.* Mosquitoes that were fed a larval diet that consisted of lower amounts of food had significantly smaller body sizes, less glycogen and lipid upon adult emergence and lived significantly less time than adults that were fed a high food larval diet. Because mosquitoes that have been collected from the field have been found to be smaller and have lower nutritional reserves than those that were reared under assumedly more optimal laboratory larval conditions (Day and Van Handel 1986), we conclude that these results more closely represent natural conditions.

Mating has also been reported to have an effect on survival. Liles and DeLong (1960) discovered that females lived longer when housed in proximity with males. Males maintained with females lived a shorter amount of time, indicating that females benefitted from their association with males.

Mosquitoes in the longevity assays of our studies were housed in close proximity and due to the potential effect on longevity, it seemed critical to determine whether the assay conditions

supported natural behavior. The effect of larval diet on mating was also examined by determining whether females fed a low or high food diet as larvae contained sperm in their spermathaeca. We found that nearly all of the females in our assay were mated, indicating no negative effect of assay environment or larval diet.

Amino Acids in Nectar Sources

Amino acids are the second most abundant component of nectar and are also present in honeydew (Baker and Baker 1973). Their presence in nectar has elicited much interest in the effect they have on the insects that feed on nectar. Generally, research has focused on butterflies (Mevi-Shutz and Erhardt 2003a, 2003b, 2005, Alm et al. 1990), but because mosquitoes feed on nectar as well, studies have also examined the affect of amino acids on them (Eischen and Foster 1983). Mevi-Shutz and Erhardt (2005) discovered that when fed a poor diet as larvae, the map butterfly lived longer and was more fecund when fed an adult diet containing amino acids. Similarly, *Aedes aegypti* survived longer and fecundity was increased when fed pollen (Eischen and Foster 1983).

We determined that there was an effect of amino acids in the adult diet on the survival of *Culex quinquefasciatus* females. Females that were fed a low food larval diet and had an adult diet with amino acids added lived as long as females that were fed a high food larval diet. While this effect was true if females were fed a poor diet as larvae, the length of survival was only increased by 2 days and further research of this topic to ensure the effect is biologically significant is suggested. Various studies have not found any connection between feeding on a sugar diet with the addition of amino acids and increased longevity (Mevi-Shutz and Erhardt 2003a, 2003b), however, we believe that the benefit of amino acids in the adult diet may be masked by studies using optimally-fed laboratory reared insects.

Future Directions

Studies focused on longevity of mosquitoes will continue to be important because of the association between survival of vectors and disease transmission. We found that differences in survival between species exist depending on adult diet. Because each of the species we examined have such different life-histories (e.g. varying larval habitats, seasonal distribution, sex-differentiated distribution), it may be increasingly important to further understand the effects of different adult diets on individual species. Increased knowledge of the effects of sugars that did not support long life, such as mannose, trehalose and especially sorbose could lead to advances in the development of attracticides. Sorbose has been found to be attractive to and readily imbibed by the blow fly (Fraenkel 1940), but it will be important to examine the phagostimulatory effects this and other poorly supportive sugars have on mosquitoes and how that will direct those used as attracticides.

APPENDIX
PROTOCOL FOR SULPHOSPHOVANILLIN AND HOT ANTHRONE ASSAYS

Mosquito Dry Weights

For dry weight analysis, previously frozen groups of ten males or ten females were frozen further at -80°C for one hour. The caps of the microcentrifuge tubes were opened and the samples were freeze dried for approximately 48 hours to ensure that all moisture was removed. Immediately after freeze drying, the samples were placed in a dessicator to avoid absorption of moisture in the air. The samples were weighed on a microbalance (Sartorius, Germany) and dry weights for the 10 mosquitoes in each sample were recorded for later use in the nutritional analyses. After weighing, the samples were again stored at -20°C for later use.

Preparation for Glycogen and Lipid Analyses

Nutritional reserves at the time of eclosion were determined by measuring the levels of glycogen and triglyceride. The hot anthrone (glycogen) (Van Handel 1965a) and sulphosphovanillin (lipid) (Van Handel 1965a, 1985, as modified by Hahn 2005) assays were conducted. To prepare for these assays, 100 µl of saturated sodium sulfate was added to the microcentrifuge tube while homogenizing the mosquitoes with a motorized homogenizer. Pestles were changed between homogenization of samples. During homogenization, 200 µl of methanol and 100 µl ultrapure water were also added. When the mosquitoes were sufficiently ground into a solution, 500 µl of 1:1 chloroform: methanol was added. This solution was then decanted into a 20 ml glass centrifuge tube labeled with the sample number and the name of the test (glycogen). The remnants of the 1.5 ml microcentrifuge tube were then rinsed with another 500 µl of 1:1 chloroform: methanol, which was added to the same glass centrifuge tube with the first portion of the sample. This process was performed for each sample. The contents of the glass centrifuge tubes were placed in the centrifuge and run at 2500 rpm for 5 minutes. The

crushed insect remnants and the glycogen portion formed a small pellet on the bottom of the tube and the supernatant, or lipid portion, was then transferred to a second 20 ml glass centrifuge tube labeled with the sample number and the type of assay (lipid). The pellet was then washed to ensure removal of all lipids. The extraction was repeated by adding 1000 µl of 1:1 chloroform: methanol. The glycogen portion was resuspended into solution by vortexing. Again, the samples were returned to the centrifuge for 5 minutes and afterward the lipid portion was decanted into the same tube with the previous portion.

The completed lipid extract was placed in the freezer for later use in the sulphosphovanillin assay. The tubes labeled glycogen now contain the precipitated glycogen and some other sugars. These sugars were removed by adding 1000 µl of 66% ethanol saturated with sodium sulfate in water. The pellet was resuspended into solution, centrifuged for 5 minutes and the supernatant discarded. This process was repeated to remove all excess sugars. Glycogen samples were then stored for later use in the hot anthrone assay.

Glycogen Analysis (Hot Anthrone Assay)

After fractionating the glycogen portion from the lipid portion, there were two separate assays to conduct, a glycogen assay and a lipid assay. Prior to beginning the glycogen assay, anthrone reagent and a glucose standard were made. The reagent was 150 mg anthrone added to 100 ml of sulfuric acid/ultrapure water solution (358 ml sulfuric acid and 141.5 ml ultrapure water). The glucose standard was used in place of glycogen and was a 1.0 µg/µl solution (50 mg/ml ultrapure water).

The samples were redissolved in 2.0 ml ultrapure water by stirring, and allowed to partially settle. Aliquots of the redissolved glycogen (100 µl) from each sample were pipetted into two separate tubes (A and B). Duplicating each sample and taking the mean, allowed proper interpretation of the results. The same was done with glucose, using 0, 5, 10, 25, 50, 100 and

125µl as standards. To each tube, 2.4 ml of anthrone reagent was added and the tubes were heated to 90ºC in a water bath for 17 minutes. After addition of the reagent, the tubes were covered to protect from light reactions. After heating, the tubes were placed in an ice bath and kept in the dark for 2 minutes. The samples were vortexed and 125 µl of each sample and its duplicate pipetted into a 96-well microtiter plate. The plate was placed in the spectrophotometer (model, company, city, state)) and absorbance determined at 625 nm.

Lipid Analysis (Sulphosphovanillin Assay)

Prior to the lipid analysis, several steps required completion. The samples were dried to complete dryness with nitrogen to remove all traces of methanol. One milliliter of chloroform was added to each sample to resuspend the lipid. The samples were placed on previously made columns of 0.2 g silicic acid and glass wool. Then, the columns were washed with 4 washes of 1.0 ml of chloroform to separate the non-polar from the polar lipids. This is critical for examining the nectar lipid pool, which is <90% triglycerides, while excluding charged cell membrane lipids. After this separation, the lipid samples contained solely triglycerides and were ready for analysis. The total volume of each sample was measured and recorded. Aliquots of 200 µl from each sample were pipetted into 100 ml glass centrifuge tubes; each was replicated to reduce pipetting error. A 1.0 µg/µl triolein standard (50 mg/ml chloroform) was used as the control, using 0, 5, 10, 25, 50, 75, 100 and 125 µl standards. All samples and standards were dried under nitrogen completely and 200 µl of sulfuric acid added to the tubes and vortexed. The samples were placed for 10 minutes in a 95ºC water bath. They were held at room temperature to cool and develop for 5 minutes. A 2.5 ml aliquot of vanillin reagent (0.6 g vanillin, 100 ml ultrapure water and 400 ml of 85 % phosphoric acid) was added to each sample and standard. The samples were briefly vortexed and placed in a dark cabinet for 10 minutes, again to allow for

a reaction to occur. Samples of 125 µl were transferred into a microtiter plate and absorbance determined, as in the glycogen analysis, in a spectrophotometer, at 525 nm.

Analysis Preparation

Glycogen and lipid contents were measured as µg of glycogen or lipid/ mg of dry weight per group of 10 mosquitoes. The mean glycogen or lipid content was used in statistical analyses.

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BIOGRAPHICAL SKETCH

Erin Vrzal was born in 1980 in Elmira, New York and grew up in Elmira Heights, NY. In 1998, she attended Barton College in Wilson, North Carolina on cross-country and academic scholarships, but transferred to State University of New York, Environmental Science and Forestry (SUNY-ESF) where she graduated in 2002 with a Bachelor of Science in Environmental studies with a focus in biological sciences. During her time at SUNY-ESF, she took an introductory entomology class and completed an internship at the Rosamond-Gifford Zoo at Burnet Park. Her interests in biology and entomology led her to accept a job at the United States Department of Agriculture (USDA), Agricultural Research Service in Gainesville, Florida as a biological science technician working on mosquito behavior. Erin continued to work as a technician there while pursuing her Master of Science degree in Entomology at the University of Florida, and will continue post-graduation at the USDA.