OVIPOSITION STRATEGIES OF *Aedes albopictus* (Skuse) (Diptera: Culicidae): Analyzing Behavioral Patterns for Surveillance Techniques and Control Tactics

By

TIMOTHY JOSEPH DAVIS

A Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

UNIVERSITY OF FLORIDA

2013
To Caryn, Haakon and Sonja
ACKNOWLEDGMENTS

I am very thankful to the Air Force Institute of Technology, Civilian Institution Program for providing me this outstanding opportunity to pursue a PhD in Entomology and a MPH in Environmental and Global Health. Next, I would like to thank the professors, scientists, and staff at the University of Florida, Entomology and Nematology Department and the U.S. Department of Agriculture, Center for Medical, Agricultural and Veterinary Entomology for supplying me with an office, laboratory space, rearing facilities, vehicles, administrative support and camaraderie.

I would to thank my dissertation committee for their invaluable advice and guidance. I thank Dr. Kaufman who was never too busy to listen, provide numerous edits, and keep me progressing towards a goal. These same sentiments are for Dr. Kline who always welcomed me into his office and was a great mentor. A special thanks to Dr. Hogsette who has a great ability to assess situations and find solutions. I thank Dr. Tatem for finding a way to be on my committee and reminding me of the “big picture” questions that need to be answered.

A number of other scientists and staff members were of great help in the completion of this document. I would like to thank Dr. Becnel for allowing me to work in his building; I learned a lot in my short time there. I would also like to thank Joyce Urban who assisted with equipment purchases and provided horticultural expertise. I would like to thank Neil Sanscrainte for his administrative assistance. In addition, I would like to thank Dr. Sivinksi for providing screened cages and Dr. Stuhl for assistance with their set-up. I would like to thank Lois Wood and all of the UF Veterinary Entomology Laboratory members for their assistance with my many projects.
I am especially grateful to Lt Col Burkett who was instrumental in getting me started on my dissertation work.

I thank the staff of the Evergreen Cemetery and private landowners who granted me access to their property in order to conduct research. These people include: Dr. Jerry Hogsette, Ms. Lois Wood, Dr. Kenneth Dodd, Dr. Amy Simonne and Dr. Eric Simonne.

Lastly, I would like to thank my family. I thank my parents who provided unconditional support. I would like to thank my beautiful wife, Caryn for doing it all when I’m not there. Most of all I would like to thank my wonderful children who have taught me more than anyone.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>4</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>9</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>10</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>13</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1 LITERATURE REVIEW OF <em>Aedes Albopictus</em> Biology and Oviposition Ecology</td>
<td>15</td>
</tr>
<tr>
<td>Biology</td>
<td>15</td>
</tr>
<tr>
<td>Distribution and Range Expansion</td>
<td>16</td>
</tr>
<tr>
<td>Taxonomy</td>
<td>17</td>
</tr>
<tr>
<td>Egg Biology</td>
<td>18</td>
</tr>
<tr>
<td>Larval and Pupal Biology</td>
<td>22</td>
</tr>
<tr>
<td>Adult Biology</td>
<td>24</td>
</tr>
<tr>
<td>Oviposition Ecology</td>
<td>27</td>
</tr>
<tr>
<td>Oviposition Site Selection</td>
<td>27</td>
</tr>
<tr>
<td>Density Dependent Larval Mortality</td>
<td>28</td>
</tr>
<tr>
<td>Skip Oviposition</td>
<td>29</td>
</tr>
<tr>
<td>Ovipositing Female’s Requirements</td>
<td>33</td>
</tr>
<tr>
<td>Oviposition Sites as Surveillance and Control Locations</td>
<td>33</td>
</tr>
<tr>
<td>Research Objectives</td>
<td>35</td>
</tr>
<tr>
<td>2 THE EFFECTS OF LARVAL HABITAT QUALITY ON <em>Aedes Albopictus</em> Skip Oviposition</td>
<td>36</td>
</tr>
<tr>
<td>Introduction</td>
<td>36</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>38</td>
</tr>
<tr>
<td>Colony Establishment and Rearing Practices</td>
<td>39</td>
</tr>
<tr>
<td>Larval Habitat Quality</td>
<td>40</td>
</tr>
<tr>
<td>Caged Arenas</td>
<td>41</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>43</td>
</tr>
<tr>
<td>Results</td>
<td>43</td>
</tr>
<tr>
<td>Indoor Caged Arenas</td>
<td>43</td>
</tr>
<tr>
<td>Outdoor Caged Arenas</td>
<td>44</td>
</tr>
<tr>
<td>Discussion</td>
<td>45</td>
</tr>
<tr>
<td>3 AN ATTRACTIVE SELF-MARKING Ovitrap to Measure Dispersal and Determine Skip Oviposition in <em>Aedes Albopictus</em> (SKUSE) Field Populations</td>
<td>60</td>
</tr>
</tbody>
</table>
A  DIGITAL IMAGES OF THE FIELD SITES ASSOCIATED WITH THE 
ASSESSMENT OF AEDES ALBOPICTUS CLUTCH SIZE IN WILD 
POPULATIONS ........................................................................................................... 135

B  DIGITAL IMAGES OF THE FIELD SITES ASSOCIATED WITH THE 
DETERMINATION OF AEDES ALBOPICTUS OVIPosition PREFERENCE 
AS INFLUENCED BY CONTAINER SIZE AND BUDDLEJA DAVIDII PLANTS ... 137

LIST OF REFERENCES ..................................................................................................... 141

BIOGRAPHICAL SKETCH ............................................................................................... 159
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>The mean number of eggs oviposited per <em>Ae. albopictus</em> when exposed to a four ovisite choice assay in indoor or outdoor cages.</td>
<td>51</td>
</tr>
<tr>
<td>3-1</td>
<td>The average proportion of <em>Aedes albopictus</em> (Skuse) gravid females were self-marked by an Attractive Self-Marking Ovitrap and their average daily mortality rates (DMR).</td>
<td>77</td>
</tr>
<tr>
<td>3-2</td>
<td>The average proportion of <em>Aedes albopictus</em> (Skuse) gravid females that were captured using sticky ovitraps following exposure to an Attractive Self-Marking Ovitrap and their mean distance traveled (MDT).</td>
<td>78</td>
</tr>
<tr>
<td>3-3</td>
<td>Number of gravid female <em>Aedes albopictus</em> (Skuse) and <em>Aedes triseriatus</em> (Say) that were captured in sticky ovitraps at a graveyard and on the University of Florida campus in Gainesville, FL.</td>
<td>79</td>
</tr>
<tr>
<td>4-1</td>
<td>The total number of mosquitoes collected using a large aspirator at four residential locations in Gainesville, FL.</td>
<td>101</td>
</tr>
<tr>
<td>4-2</td>
<td>The mean clutch size, number of eggs laid + eggs retained and wing length of <em>Aedes albopictus</em> (Skuse) females either laboratory reared at different larval densities or collected as blood-fed adults.</td>
<td>102</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>2-1</td>
<td>Four ovisite choice arena in a screened cage (61 x 61 x 61cm) employed to evaluate the effect of larval habitat quality on individual <em>Ae. albopictus</em> oviposition patterns.</td>
<td></td>
</tr>
<tr>
<td>2-2</td>
<td>Four ovisite choice arena in a screened cage (2.13 m high x 2.74 m diameter) employed to evaluate the effect of larval habitat quality on individual <em>Ae. albopictus</em> oviposition patterns.</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>Mean number of ovisites containing eggs from indoor caged assays that include an individual <em>Ae. albopictus</em> and four ovisites.</td>
<td></td>
</tr>
<tr>
<td>2-4</td>
<td>Mean proportion of eggs per each container habitat in the low-quality dominant treatment from indoor caged assays.</td>
<td></td>
</tr>
<tr>
<td>2-5</td>
<td>Mean proportion of eggs per each container habitat in the high-quality dominant treatment from indoor caged assays.</td>
<td></td>
</tr>
<tr>
<td>2-6</td>
<td>Mean number of ovisites containing eggs from outdoor caged assays that include an individual <em>Ae. albopictus</em> and four ovisites.</td>
<td></td>
</tr>
<tr>
<td>2-7</td>
<td>Mean proportion of eggs per each container habitat in the low-quality dominant treatment from outdoor caged assays.</td>
<td></td>
</tr>
<tr>
<td>2-8</td>
<td>Mean proportion of eggs per each container habitat in the high-quality dominant treatment from outdoor caged assays.</td>
<td></td>
</tr>
<tr>
<td>3-1</td>
<td>An attractive self-marking ovitrap (ASMO) and sticky ovitrap used to monitor <em>Aedes albopictus</em> (Skuse) skip oviposition.</td>
<td></td>
</tr>
<tr>
<td>3-2</td>
<td><em>Aedes albopictus</em> (Skuse) females exposed to an attractive self-marking ovitrap.</td>
<td></td>
</tr>
<tr>
<td>3-3</td>
<td><em>Aedes albopictus</em> (Skuse) females exposed to an attractive self-marking ovitrap released into a large outdoor enclosure.</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>Aerial images of the two field sites used to evaluate <em>Aedes albopictus</em> (Skuse) skip oviposition located in Gainesville, FL.</td>
<td></td>
</tr>
<tr>
<td>3-5</td>
<td>Mean number of gravid <em>Aedes albopictus</em> (Skuse) adults, <em>Aedes triseriatus</em> (Say) adults and <em>Toxorhynchites rutulus rutulus</em> (Coquillett) eggs per sticky ovitrap at a graveyard in Gainesville, FL.</td>
<td></td>
</tr>
</tbody>
</table>
Mean number of gravid *Aedes albopictus* (Skuse) adults, *Aedes triseriatus* (Say) adults and *Toxorhynchites rutilus rutilus* (Coquillett) eggs per sticky ovitrap at the University of Florida campus, Gainesville, FL. .......................... 85

Linear regression of the captured distance of marked gravid female mosquitoes in relation to five successive four day trapping periods during 2011 and 2012, in Gainesville, FL. .......................................................... 86

Linear regression of the mean distance traveled (MDT) of marked mosquitoes that were captured in the first four days in relation to the mean number captured per sticky ovitrap (ST). .......................................................... 87

Linear regression of the mean distance traveled (MDT) of marked mosquitoes that were captured in captured over five 4-day periods in relation to the mean number captured per sticky ovitrap (ST).................................................. 88

A 30 x 30 x 30 cm cage in which an individual *Aedes albopictus* (Skuse) was placed to complete oviposition................................................................. 103

*Aedes albopictus* (Skuse) wing lengths were measured from the base of the costa to the wing margin, exclusive of fringes, using the Keyence VHX-600E digital microscope (Keyence Corporation, Osaka, Japan)............................................ 104

Linear regression analysis of emergent adult *Aedes albopictus* (Skuse) adult clutch size, number of eggs laid + eggs retained, and wing length in relation to larval rearing density. ...................................................... 105

Linear regression analysis of clutch size and number of eggs laid + eggs retained on the wing-length of laboratory reared *Aedes albopictus* (Skuse)..... 106

Linear regression analysis of clutch size and number of eggs laid + eggs retained on the wing-length of wild-caught *Aedes albopictus* (Skuse)............ 107

Gravid *Aedes albopictus* (Skuse) females exposed to a four choice arena inside an outdoor screened enclosure......................................................... 122

Suburban yard in which *Aedes albopictus* (Skuse) oviposition preference was examined for three different sized black containers (473, 946, 1892 mL). .... 123

Gravid *Aedes albopictus* (Skuse) females attempting to feed from flowering butterfly bush (*Buddleja davidii* Franchett cultivar ‘Guinevere’). .................. 124

Mean proportion of *Aedes albopictus* (Skuse) eggs recovered from different sized containers, 473 mL and 1,892 mL, that occur with and without flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’). .................. 125

Mean proportion of *Aedes albopictus* (Skuse) eggs recovered from different sized containers, 473 mL (S) and 1892 mL (L), that occur with (BD +) and
without (BD-) flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’). .......................................................... 126

5-6 Mean proportion of *Aedes albopictus* (Skuse) eggs recovered from different sized containers, 473 mL and 1892 mL, which occur with butterfly bushes that retain flowers or with the flowers removed.......................................................... 127

5-7 Mean number of eggs recovered from containers with and without flowering butterfly bushes and different sized containers: 473 mL, 946 mL, 1892 mL..... 128

5-8 Mean number of eggs per ovitrap at each field site................................. 129

A-1 Digital images of the field sites in Gainesville, FL, associated with the assessment of *Aedes albopictus* (Skuse) clutch size. ........................................... 135

B-1 Digital images of field site 5-1. ................................................................. 137

B-2 Digital images of field site 5-2.................................................................. 138

B-3 Digital images of field site 5-3.................................................................. 139

B-4 Digital images of field site 5-4.................................................................. 140
Aedes albopictus (Skuse) is an invasive mosquito species that vectors human pathogens. Being a day-biting mosquito that is not attracted to standard surveillance approaches, surveillance and control measures for Ae. albopictus often are based on its oviposition behavior and this behavior warrants further examination. Aedes albopictus is a container-inhabiting mosquito that oviposits in resource-limited habitats that and encounters density dependent conditions that reduce fitness. To mitigate larval competition, Ae. albopictus females may choose to distribute eggs from a single gonotrophic cycle among multiple containers through skip oviposition.

A series of laboratory and field studies were conducted to determine if Ae. albopictus performs skip oviposition. In field studies, 37 wild Ae. albopictus that visited an attractive self-marking ovisite were subsequently captured at a sticky ovitrap within a four day period. Since the average Ae. albopictus gonotrophic period is 4.5-6 days, the wild-caught Ae. albopictus must have visited two oviposition sites within a single gonotrophic period, thereby providing field-based indirect evidence of skip oviposition.
Density dependent effects on oviposition outcomes were examined. In a four-choice arena, individual *Ae. albopictus* oviposited in significantly fewer containers (1.4-1.5 containers) when presented with ovisites of high quality larval habitat (uncrowded conditions) compared with oviposition in low quality (crowded conditions) larval habitats (2.5-2.9 containers). *Aedes albopictus* clutch size was evaluated in field populations and compared with the clutch size of mosquitoes reared in the laboratory at different levels of larval crowding. Field populations varied significantly in mean clutch size (23 to 46), which was comparable to the mean clutch sizes of females reared at the larval densities of 9 (20 eggs) and 3 (53 eggs) larvae/3 mL of water.

The oviposition preference of *Ae. albopictus* for containers of selected sizes and the influence of flowering butterfly bush (*Buddleja davidii* Franchett cultivar ‘Guinevere’) were examined. Significantly more eggs were oviposited in large containers than in medium and small containers. Significantly more eggs were oviposited in containers adjacent to flowering butterfly bushes than in those without a flowering butterfly bush. This was the first study to demonstrate an oviposition preference for ovisites associated with a flowering landscape plant.
Biology

*Aedes albopictus* (Skuse), also known as the Asian tiger mosquito, is an invasive and pestiferous species with both medical and veterinary significance (Hawley 1988). This mosquito will readily feed on humans and domestic animals (Colless 1959) and has been shown to be a competent vector of pathogens (Gratz 2004). Besides being a threat in its historic geographic distribution, the Asian tiger mosquito has proven adept at invasion and establishment in new countries often in association with modern human movements (Benedict et al. 2007). In the last 30 years, *Ae. albopictus* has successfully become established in 29 countries (Enserink 2008). Many aspects of this mosquito make it a very efficient invasive species; these include feeding plasticity, capacity to reproduce in close association with humans, and utilization of both natural and artificial container habitats. *Aedes albopictus* is an ecological generalist that continues to provide a challenge to the mosquito control community.

Where it occurs, the Asian tiger mosquito regularly bites humans with daily biting rates of 314 per person having been recorded (Almeida et al. 2005). *Aedes albopictus* females are most often diurnal feeders and target the area around the lower legs, but will bite any exposed skin (Robertson and Hu 1935). This mosquito not only feeds on humans, but also is known to be a catholic feeder with blood meal analyses having shown human, cat, dog, and avian sources (Richards et al. 2006). In Thailand, host preference studies have resulted in the following rank order human > pig > dog > chicken (Sullivan et al. 1971). The ability of this mosquito to utilize a broad range of
hosts allows it to capitalize on what is available for survivorship and preferentially target humans when available, thus contributing to its pest status.

Besides being an annoyance biter, the Asian tiger mosquito is also a vector of pathogens. Recent outbreaks of chikungunya in Asia, the Indian Ocean region, and in parts of Europe, have resulted in over 265,000 reported cases and 237 deaths (Charrel et al. 2007) and were attributed to *Aedes albopictus* (Reiter et al. 2006, Pages et al. 2009). This mosquito transmits dengue virus and has been implicated in several dengue epidemics (Russell et al. 1969, Chan et al. 1971). In addition to the aforementioned pathogens, *Ae. albopictus*, transmits dog heartworm (*Dirofilaria immitis*) (Chellapah and Chellapah 1968) and is an efficient laboratory vector of a variety of other viruses (Shroyer 1986).

**Distribution and Range Expansion**

The Asian tiger mosquito is believed to have originated from the forested regions of Southeast Asia (Hawley 1988). It has undergone range expansion throughout much of Asia, from the Islands of Indonesia westward to Madagascar (Surtees 1966). The northerly distribution in Asia includes Beijing China, Seoul Republic of Korea, and Honshu Island, Japan (Huang 1972, Tanaka et al. 1979). Recent introductions of *Ae. albopictus* have led to its establishment in many additional regions of the world. Benedict et al. (2007) summarized the expanding distribution of this mosquito to include much of North America, Central America, and South America; central, eastern, and southern Europe; and into portions of western Africa.

Once established, populations of this species have demonstrated continued expansion. In the United States, *Ae. albopictus*, has been present in Hawaii since the mid-1900’s (Bonnet 1948), but a substantial breeding population was not found on the
continental U.S. until 1985 in Houston, Texas (Sprenger and Wuithiranyagool 1986). Within 10 years of this introduction, the Asian tiger mosquito spread to many states east of the Mississippi river to include those as far north as Minnesota, Indiana, Ohio, and New Jersey (Moore and Mitchell 1997).

The used tire trade has been implicated as the most likely source of this mosquito’s assisted movement (Reiter and Sprenger 1987). Aedes albopictus lay their eggs on the interior surface of these tires and the larvae develop in the water that is trapped inside. Infested tires are then moved throughout the world to be used in a variety of industries (Reiter and Darsie 1984), often being stored for long periods near mosquito-free tires thus facilitating new infestations. In addition to the used tire industry, a newly discovered route of Asian tiger mosquito movement is the trade in live plants. Introductions of Ae. albopictus into southern California and the Netherlands have been associated with the importation of Dracaena sanderiana, known as lucky bamboo, from China (Madon et al. 2002, Scholte et al. 2008).

**Taxonomy**

*Aedes albopictus* was first described as “the banded mosquito of Bengal” by Skuse (1894) from specimens collected in Calcutta, India. This mosquito is a member of the *Stegomyia* subgenus, the Scutellaris group, and the Albopictus subgroup. Hawley (1988) indicates there are 12 species within the Albopictus subgroup.

The separation of *Ae. albopictus* from other members of the Scutellaris group can be difficult, especially in areas of overlapping distribution (Ho et al. 1972). In Southeast Asia, the adults of *Ae. albopictus* are differentiated from those of *Ae. pseudalbopictus* (Borel), *Ae. malayensis* Colless, and *Ae. seatoi* Huang using the dichotomous keys of Huang (1971, 1972, 1979). In Japan and the Republic of Korea,
the keys of Tanaka et al. (1979) and Toma and Miyagi (1986) can be used to separate *Ae. albopictus* adults from those of *Ae. flavopictus* Yamada and *Ae. riversi* Bohart and Ingram.

Identifying the larval stage of *Ae. albopictus* in much of Asia poses a greater challenge as the larvae share similar habitat and almost identical morphology with those of *Ae. scutellaris* (Walker), *Ae. malayensis*, *Ae. riversi*, *Ae. flavopictus* and *Ae. alcasidi* Huang (Ho et al. 1972, Hawley 1988). The keys of Tanaka et al. (1979) and Huang (1971) are used to separate the larval stage. In addition to traditional morphological identification, molecular assays have been developed to distinguish among *Ae. scutellaris*, *Ae. albopictus*, and *Ae. aegypti* (Linnaeus) (Beebe et al. 2007, Hill et al. 2008).

In North America, no other members of the Scutellaris group are present and the adult Asian tiger mosquito often can be distinguished without the aid of magnification (Darsie 1986). The *Ae. albopictus* larval stage can share its habitat with *Ae. aegypti*, *Ae. triseriatus* (Say), and *Ae. japonicus japonicus* (Theobald) and the keys of Darsie and Ward (2005) should be used to separate them.

**Egg Biology**

Studies have been performed to determine factors that affect survivorship, development time, and hatching stimuli of the Asian tiger mosquito egg stage (Surtees 1966, Ho et al. 1972, Hawley 1988). These factors are important in planning surveillance activities and developing control plans. Recruitment into the next generation through the survivorship of eggs assists in predicting population growth rates and can translate into the development of action thresholds. Determining the time needed for eggs to reach maturation provides useful information in developing
generation times. Lastly, the abiotic and biotic factors that influence egg hatching supply some of the inputs required for modeling population dynamics and predicting peak abundance.

According to Hawley (1988) there are three factors that most affect *Ae. albopictus* egg survival: desiccation, predation, and climate. The importance of each factor on egg survival depends upon the time of year and the geographic location. These factors are often difficult to assess in the natural habitat and most studies have been performed in the laboratory.

One life table study was completed in a tropical field environment and the egg mortality was assessed at 9.9% (Chan 1971). This study followed eggs that had been deposited in tin cans within 24 hours, after which the cans were covered with a fine mesh. Thus, the effects of desiccation and tropical climate were assessed, but predation was not fully examined. Studies of other *Aedes* mosquito eggs also show similar low mortality rates (Southwood et al. 1972, Hawley 1985).

Laboratory studies have demonstrated that *Ae. albopictus* eggs are most susceptible to desiccation within 24 hours after oviposition (Hien 1975, Imai and Maeda 1976). After this initial vulnerability period, the eggs are able to withstand extended periods of drying (Gubler 1970). Hien (1975) found 100% mortality in the eggs of an *Ae. albopictus* population from Vietnam if dried immediately after oviposition. In a Japanese population, mortality was near 100% if drying occurred at less than 12 hours, decreased to 40% mortality when eggs were dried at 16 hours old and no significant mortality occurred if eggs were dried after 24 hours (Imai and Maeda 1976).
Dried *Ae. albopictus* eggs have remained viable for many months in both field (Chow 1949, Wang 1962) and laboratory studies (Ishii et al. 1954, del Rosario 1963, Gubler 1970, Hein 1975). del Rosario (1963) found that a portion of dried eggs recovered from tree-holes, bamboo stumps, and small containers hatched after three months when held at room temperature. In the laboratory Gubler (1970) found nearly 50% survivorship after three months in eggs maintained at a relative humidity of 70-75% and a temperature of 25°C. At the same temperature and a slightly lower relative humidity (60-70%) Hein (1975) observed a 95% hatch rate after two months and 24% eclosion after four months.

*Aedes albopictus* egg survivorship has not only been measured in time but also over seasonal temperature fluctuations, particularly in winter (Chow 1949, Ishii et al. 1954, Wang 1962, Toma et al. 1982, Hawley et al. 1989). A difference in winter survivorship exists between populations originating in temperate and tropical regions. Hawley et al. (1989) exposed eggs from tropical Asia to an Indiana winter and recorded near 100% mortality. In contrast, eggs from a temperate region of Asia experienced considerably lower mortality.

In addition to egg survival, the time required for eggs to develop and eclose (embryonation period) has been measured for *Ae. albopictus*. Eggs originating from different regions and held at selected constant temperatures were found to have development times ranging from two to six days (Tseng and Wu 1951, del Rosario 1963, Hien 1975, Livingstone and Krishnamoorthy 1985).

The embryonation period can be influenced by diapause in populations of *Ae. albopictus* that exhibit overwintering behavior. Egg diapause completion requires two to
six months and is influenced by the photoperiodicity experienced by the adult females (Ho et al. 1972). When a population of *Ae. albopictus* females from Shanghai, China, was exposed to light for 24 hours, 85% of the eggs hatched within seven days (Wang 1966). This same population had only 30% of their eggs hatch within seven days when exposed to eight hours of light and 16 hours darkness (Wang 1966). Photoperiodicity is seen only in populations occurring in or originating from temperate climates (Hawley et al. 1987).

*Aedes albopictus* egg survivorship and development concludes when the egg either hatches or dies. Eclosion occurs after the egg is covered by water and is influenced by the interaction of several complex components (Hawley 1988). Water oxygen content has been found to be an important stimulus in egg hatching (Hien 1975, Imai and Maeda 1976). However, egg hatching is influenced not only by the oxygen content, but also by the interaction of egg dessication (Imai and Maeda 1976). Imai and Maeda (1976) used 3-d-old moist eggs from a recently colonized *Ae. albopictus* population from Kyoto, Japan, and observed a 94% hatch rate when eggs were exposed to water containing 1.5 ppm oxygen, 79% at 6.0 ppm, and 62% at 7.6 ppm. In contrast, eggs that were allowed to dry for 24 hours had a hatch rate of 20% when exposed to water with high oxygen content (7.2 ppm) and a near 100% hatch rate when exposed to water with low oxygen content (1.7 ppm) (Imai and Maeda 1976). Therefore, in regards to water oxygen content as a hatching stimulus, eggs that have not dried are not as sensitive as eggs that are dried for 24 hours.

In measuring the effects of hatching stimuli it is important to recognize the influence of laboratory colonization (Halcrow 1955, Mogi 1982). In a laboratory colony
from Mauritius, four to seven day old eggs hatched immediately and synchronously when flooded with water (Halcrow 1955), most likely as a result of artificial selection pressure on the colony from laboratory rearing practices. Mogi (1982) compared two strains from Japan, one recently established and one older laboratory strain. It was documented that upon flooding there was a 95% hatch in the older laboratory strain, while only a small portion hatched in the recently established strain. However, after a month of laboratory rearing and the addition of yeast and mouse feces to the water, a greater hatch occurred in the recently colonized strain (Mogi 1982).

**Larval and Pupal Biology**

*Aedes albopictus* is believed to have originated in the sylvatic habitat of Southeast Asia, based on the evidence that most members of the Albopictus Subgroup inhabit tree holes in the larval stage (Hawley 1988). Indeed, in the tea plantations and bamboo forests of northern Thailand, *Ae. albopictus* larvae are frequently found in tree stumps and the adults are one of the most common pests (Birks 1952, Scanlon and Esah 1965). In addition to forested regions, *Ae. albopictus* is often found in close association with humans, inhabiting human-made water holding containers (Surtees 1966). Surtees (1966) and Hawley (1988) have provided summaries of the numerous surveys conducted to examine the differing habitats of *Ae. albopictus* larvae. Everything from ant traps and coconut shells to rock pools and discarded tires have been found to be suitable for *Ae. albopictus* larval development (Surtees 1966, Hawley 1988).

After eclosion, Asian tiger mosquito larvae feed, grow, and develop through four molts before reaching the pupal stage. Asian tiger mosquito larvae consume microorganisms associated with the decay of plant and animal material (Merritt et al.)
The development time of the larval and pupal stage can vary; however most studies have found the development time of the larval and pupal stages to be 5-10 s and 1-2 days, respectively (del Rosario 1963, Hien 1975, Hawley 1988). These experiments were conducted under laboratory conditions with temperatures near 25 °C and provision of optimal amounts of food. When larvae were fed suboptimal amounts of food, their development period was greatly increased to 24-58 days (Mori 1979). In tropical field conditions, Chan (1971) found the combined larval and pupal development time for *Ae. albopictus* to be approximately 19 days.

*Aedes albopictus* larvae occur in container habitats that vary in biotic and abiotic characteristics. The four abiotic larval habitat characteristics found to have the greatest impact on habitat productivity include nutrient levels (Mori 1979), water temperature (Teng and Apperson 2000), water level (Akram and Lee 2004), and dissolved oxygen (Su et al. 2012). Biotic factors that influence *Ae. albopictus* larval habitat include predation, parasitism and competition (Gratz 2004).

Several aspects and outcomes of intraspecific and interspecific competition have been examined for the larvae of this species (Hawley 1988). Multiple life history characteristics were shown to be affected as a result of intraspecific competition for larval food resources (Chan 1971, Mori 1979, Blackmore and Lord 2000, Reiskind and Lounibos 2009). Mortality rates increase when food is limited in the larval environment (Chan 1971). Mori (1979) demonstrated that *Ae. albopictus* larval development time is extended when larval nutrition is reduced and larval rearing density is increased. This author also found that adult size decreased as the number of larvae within a container habit increased and larval food was restricted. In similar experiments, Blackmore and
Lord (2000) reared *Ae. albopictus* at a range of densities to produce adults of various sizes and found that smaller adults laid fewer eggs. However, intraspecific competition was found not to influence adult longevity (Reiskin and Lounibos 2009).

Most studies involving *Ae. albopictus* interspecific competition have involved studies with *Ae. aegypti*. *Aedes albopictus* and *Ae. aegypti* populations have invaded many regions and their distributions have overlapped and changed both temporally and spatially. In Southeast Asia, many *Ae. albopictus* populations were replaced by *Ae. aegypti* (Hawley 1988). Conversely, in Florida many *Ae. aegypti* populations have been displaced by *Ae. albopictus* (O’Meara et al. 1995). These two species occupy container habitats in the larval stage. Therefore, interspecific larval competition of these two species has been examined and under most conditions *Ae. albopictus* is the dominant competitor (Barrera 1996, Juliano 1998, Daugherty et al. 2000, Braks et al. 2004, Yee et al. 2004, Murrel and Juliano 2008). However, factors other than interspecific larval competition are theorized to impact *Ae. albopictus* and *Ae. aegypti* population distributions (Chan 1985, Juliano et al. 2004, Tripet et al. 2011).

**Adult Biology**

*Aedes albopictus* adult males generally emerge from the pupal stage before the females. The average lifespan for females and males ranged from 24-60 days and 7-21 days, respectively, when the mosquitoes were held at temperatures near 25°C and a humidity level of at least 30% (del Rosario 1963, Gubler 1970, Gubler and Bhattacharya 1971, Hien 1976a, Hawley 1988). However, longevity is greatly reduced if the Asian tiger mosquito is not provided either blood or sugar (Hawley 1988). *Aedes albopictus* utilize vertebrate blood to generate eggs and sugar meals for production of metabolic energy.
Adult *Ae. albopictus* reside in terrestrial habitats, acquiring sugar from plant nectar (Oda 1964, Burkett et al. 1999) and possibly other carbohydrate sources such as honeydew. Both sexes require nectar meals throughout their adult lifespan and the availability of these carbohydrates may be limited (Foster 1995). Sugar meals provide metabolic energy for flight, mating, and survival. Harada et al. (1975) found 65.1% of males and 53.6% of females positive for fructose in wild *Ae. albopictus* collected from Japan. Laboratory feeding studies of nectar from flowering plants demonstrated that *Ae. albopictus* has a preference among flowers from different plant species (Harada et al. 1975). These authors also recorded a decrease from 59.1% to 18.3% in the number of *Ae. albopictus* positive for fructose following a typhoon that damaged most flowering plants in the area (Harada et al. 1976).

Adult female *Ae. albopictus* require a blood meal to produce viable eggs. Proteins acquired from vertebrate blood provide the amino acids that are needed to synthesize vitellogenin, the egg yolk protein (Hagedorn 1974). Autogeny has been reported in this species, but egg production was slight with a range of 2-4 eggs per female (Bat-Miriam and Craig 1966). *Ae. albopictus* does not require multiple blood meals to develop a batch of eggs. However, 90% of a laboratory population was observed to take a second blood meal on the day following an initial blood meal during a single gonotrophic cycle (Hien 1976a). A much lower proportion of 8%-19% per gonotrophic cycle acquiring multiple blood meals was reported in field studies (Gould et al. 1970, Chan 1971). The amount of blood taken per blood meal is related to the size of the mosquito and ranges from 0.2-4.2 mg (Hawley 1988).
The number of eggs that *Ae. albopictus* produce after a blood meal averages between 42 to 88 for the first gonotrophic cycle (del Rosario 1963, Gubler 1970, Chan 1971, Gubler and Bhattacharya 1971, Hien 1976b, Mori 1979, Hawley 1988, Blackmore and Lord 2000). Fewer eggs tend to be produced in subsequent gonotrophic cycles (Hien 1976b, Chan 1971). Asian tiger mosquitoes have an average fecundity of 300-345 eggs (Gubler 1970, Gubler and Bhattacharya 1971, Hien 1976b, Hawley 1988). The length of the gonotrophic cycle is dependent on temperature with cooler temperatures extending the cycle length and warmer temperatures shortening it (Hawley 1988). In a strain of *Ae. albopictus* from Calcutta, India, the average gonotrophic cycle was 4.6 days when maintained at 26°C (Gubler and Bhattacharya 1971). In a field study in Nagasaki, Japan, the average gonotrophic cycle was found to be 5 days during a period where the average temperature was 25°C (Mori and Wada 1977).

*Aedes albopictus* disperse throughout their adult environment to locate resting sites, nectar meal sources, blood meal sources, mates and oviposition sites. The average flight range of this mosquito is thought to be less than 120 m (Bonnet and Worcester 1946, Niebylski and Craig 1994, Lacroix et al. 2009, Marini et al. 2010). However, this measure varies depending on ecological conditions (Hawley 1988). In a mark-release-recapture study, Mori (1979) observed greater dispersal distances when the release environment contained poor resting habitat. In addition, Mori (1979) also observed greater dispersal in females that were reared in high larval density conditions when compared to females reared in a low larval density environment. Indeed, dispersal distances of 600-1000 m have been observed in *Ae. albopictus* studies where
the mosquitoes have been released (Rosen et al. 1976, Honorio et al. 2003, Maciel-de-Freitas et al. 2006). Therefore, \textit{Ae. albopictus} dispersal distance is determined by many variables that influence flight behavior and will vary based on the conditions experienced.

**Oviposition Ecology**

Many factors influence \textit{Ae. albopictus} oviposition. Environmental conditions and the female’s physiological state are prerequisites for oviposition to occur. Some of these conditions in many mosquito species were discussed in a review by Bentley and Day (1989) and the review of Hawley (1988) provided some specific characteristics of \textit{Ae. albopictus} oviposition. Nonetheless, Asian tiger mosquito egg-laying behavior has not been discussed in any review with regards to both abiotic and biotic factors and how these conditions influence the oviposition outcome. The following review will synthesize some aspects of \textit{Ae. albopictus} oviposition ecology.

**Oviposition Site Selection**

\textit{Aedes albopictus} eggs are laid in both artificial and natural water holding containers. The female deposits the eggs singly on the sides of the container slightly above the water line. Several characteristics of container habitats have been examined in preference studies. Field studies have shown that \textit{Ae. albopictus} oviposit more eggs in black colored containers than in white colored containers (Yap 1975, Hoel et al. 2011). The texture of the container also influences oviposition, with rough surfaces being preferred to smooth surfaces (del Rosario 1963, Hien 1976b). Lambrecht (1971) found more eggs in oviposition sites located in shaded areas as opposed to ovisites in open areas. \textit{Aedes albopictus} also has demonstrated a preference to oviposit near ground level (Amerasinghe and Alagoda 1984, Obenauer et al. 2009b).
Ovisite contents have been found to impact the oviposition preference of *Ae. albopictus*. The larvae of this mosquito feed on microorganisms associated with decaying vegetation and animal matter. Thus, studies have shown preferences for waters that have been conditioned by different types of decaying vegetation. Gubler (1971) found that containers that held oak leaf and grass infused water were preferred oviposition sites in comparison to water containing no organic matter. Further studies confirmed these results (Trexler et al. 1998, Obenauer et al. 2009b). In addition, the previous and current presence of conspecific larvae has been shown to influence mosquito production. Allan and Kline (1998) documented that water conditioned by larvae was attractive. However, containers with high densities of *Ae. albopictus* larvae were repellent to ovipositing females (Yoshioka et al. 2012).

**Density Dependent Larval Mortality**

A primary factor in larval mortality among container inhabiting mosquitoes is density dependence (Service 1985, Washburn 1995). Indirect and direct competition for access to larval nutritional resources has been shown to increase *Ae. albopictus* larval mortality, reduce resultant adult size, reduce female fecundity and trigger adult behavioral responses (Chan 1971, Mori 1979, Blackmore and Lord 2000, Yoshioka et al. 2012). Consequently, density dependent factors influence how container-inhabiting mosquitoes utilize this habitat.

Container-inhabiting mosquitoes have evolved differing ways of interacting with the resource-limited container habitat. The ability to outcompete other container inhabitants for resources was found in *Ae. albopictus* larvae when in the presence of *Aedes polynesiensis* Marks larvae (Lowrie 1973). Lounibos (1981) reported habitat segregation among African treehole mosquitoes by species preferences for sizes of
treeholes. One feature of mosquito ecology that directly affects the next generation, and thus is clearly acted on by evolutionary selection pressures, is mosquito oviposition (Bentley and Day 1989). In some species, the eggs from a single gonotrophic cycle are deposited singly in several clutches over multiple containers. This behavior has been referred to as interval or installment egg laying (Christophers 1960) and skip oviposition (Mogi and Mokry 1980). In container habitats where resources may be low and competition may be high, distributing eggs over several containers could provide the offspring with better developmental conditions and ensure that most or all of a female’s batch are not lost.

**Skip Oviposition**

Skip oviposition behavior may occur in *Ae. albopictus* and other container-inhabiting mosquitoes. Currently, little work has been done to examine this behavior in *Ae. albopictus*. Therefore, in the following section, skip oviposition behavior will be examined in several species of container-inhabiting mosquitoes to provide a foundation within which to examine *Ae. albopictus* skip oviposition.

Clements (1999) defines a batch of eggs as the full compliment of eggs matured and laid in a single gonotrophic cycle and an egg clutch is defined as the number of eggs laid by a female at one site. Eggs from a batch laid singly over a period of time, provides the prerequisite conditions in which selection pressures could act to favor individuals that deposit their eggs spatially among several containers instead of putting them all in one. If females that deposited their eggs among multiple containers had increased fitness in comparison to those that deposited all eggs in one container then the former would have an evolutionary advantage and thus this behavior would become widespread in the species (Harrington and Edman 2001). In several species of
container-inhabiting mosquitoes, oviposition of a full batch of eggs has been observed to occur over several days. Christophers (1960) observed that in many populations of Ae. aegypti, females tended to deposit their egg batch over several days in several clutches, but that in most laboratory colonies of this species all eggs were deposited within several hours. Gillett (1962) made similar observations of Ae. aegypti and noted eggs from a single batch being laid in installments over several 24 hour intervals. In Ae. albopictus it has been observed that oviposition of a batch of eggs generally occurs over several days (Hawley 1988).

Studies to determine the presence of skip oviposition have been conducted in both laboratory and field settings. Only a few species have been examined for this behavior and much of the work has been conducted in a laboratory setting. Concerns with artificial selection pressures of laboratory colonies and the interference with behavior in a caged laboratory setting should be noted when reviewing these studies. Field studies in which natural populations are examined for this behavior would be optimal, but these are difficult to perform when evaluating how a lone female mosquito oviposits in both space and time. A summary of both laboratory and field studies together does provide a solid foundation for this behavior existing in multiple species of container-inhabiting mosquitoes, including Ae. albopictus.

Laboratory studies that have described this behavior often were not performed to directly examine installment egg laying over multiple ovipositions. Rather, many of the studies in which this behavior was noted were conducted to investigate the effects of various potential ovipositional attractants. Fay and Perry (1965) evaluated Ae. aegypti ovipositional preference for the texture, color and shape of the container, as well as the
contents. A similar study had been performed previously (O’Gower 1957) but Fay and Perry (1965) evaluated *Ae. aegypti* females individually in a multiple choice arena and noted that the majority oviposited in multiple ovisites. Later, Corbet and Chadee (1993) standardized a multiple choice arena for testing ovisite preference by individual females and found many of the *Ae. aegypti* oviposited in multiple containers. Trexler et al. (1998) used the standardized multiple choice arena of Corbet and Chadee (1993) to examine ovisite preferences of *Ae. albopictus* and *Ae. triseriatus*. Trexler et al. (1998) found that these two container-inhabiting mosquitoes oviposited the majority of the time in multiple containers. While not extensive, these studies have demonstrated that in a laboratory setting, several species of container inhabiting mosquitoes perform skip oviposition behavior.

In contrast to the laboratory studies previously described, field studies have been performed to examine the occurrence of this behavior. Initial observations were made utilizing patterns of eggs per ovisite from several different species (Buxton 1927, Rozeboom et al. 1973, Makiya 1976, Chadee and Corbet 1987, Kitron et al. 1989). Buxton (1927) found that the average number of eggs deposited by *Ae. aegypti* females to be 20 in field observations. Christophers (1960) noted that because 20 eggs is less than the average batch size recorded in laboratory reared specimens then these results provide evidence of skip oviposition behavior. Later, Chadee and Corbet (1987) made the same conclusion following observations on *Ae. aegypti* egg distributions from Trinidad. Rozeboom et al. (1973) found a similar pattern of oviposition in both *Ae. albopictus* and *Ae. polynesiensis* with many oviposition sites containing fewer eggs than the average batch size. Makiya (1976) analyzed distributions of *Ae. albopictus* eggs
and made an analogous conclusion. Mogi and Mokry (1980) examined *Wyeomyia smithii* (Coquillett) egg distributions in pitcher plants and found very few eggs (often one) per pitcher and described this behavior as “skip oviposition” from plant to plant. Similar observations were made with *Ae. triseriatus* egg distributions with clutches of eggs being less than the average egg batch size (Kitron et al. 1989).

Further field studies of skip oviposition behavior were conducted with *Ae. aegypti* populations using molecular techniques (Apostol et al. 1994, Colton et al. 2003) and by marking with rubidium (Reiter et al. 1995, Liew and Curtis 2004). Molecular techniques were used to determine relatedness among individuals within and between containers to establish family relationships. Apostol et al. (1994) using random amplified polymorphic DNA amplified by polymerase chain reaction (RAPD-PCR) found that an average of approximately 11 eggs per ovisite were laid by a given female *Ae. aegypti*. Colton et al. (2003) analyzed family distributions using restricted fragment polymorphism (RFLP) markers and found individuals from the same families dispersed among several containers. Using marking techniques researchers reported similar results in the distribution of small egg clutches over multiple ovisites (Reiter et al. 1995, Liew and Curtis 2004). Blood meals containing rubidium were fed to females and the gravid mosquitoes were released to oviposit. Ovitraps were placed at given distances and used to examine adult dispersal as documented by oviposition events. Many ovisites contained very small numbers of rubidium marked eggs, implying that a marked female was depositing her eggs among multiple ovisites.

Combining overall conclusions from all the studies conducted in reference to this subject, only populations from five species have demonstrated some capacity for
installment egg laying over multiple ovisites. Further studies of this behavior in container-inhabiting mosquitoes are warranted to determine the commonality of skip oviposition.

**Ovipositing Female’s Requirements**

In anautogenous mosquito species, egg development occurs after a female has acquired a blood meal. *Aedes albopictus* are capable of ovipositing an average of two to five days after taking a blood meal (Hawley 1988). During this time period *Ae. albopictus* rests in vegetative sites (Bohart 1956) that are presumably of a preferred microclimate. Once eggs have reached maturation, preovipositional behavior is initiated which includes ovisite location and selection (Bentley and Day 1989). Searching flights are required to find suitable oviposition sites. The metabolic state of the mosquito is an important aspect during ovisite location. In a survey of Connecticut mosquitoes it was found that mosquitoes in all stages of follicular development were positive for fructose (Magnarelli 1978b). Depletion of energy reserves during oviposition may require the mosquito to seek nectar sources. Therefore, sugar availability in the field may influence *Ae. albopictus* oviposition (Bentley and Day 1989).

**Oviposition Sites as Surveillance and Control Locations**

The ability to predict the location of medically important mosquitoes at specific times within their lifecycle allows targeted surveillance and control applications. Knowing that a gravid, container-inhabiting mosquito performing skip oviposition will visit multiple ovisites provides prediction potential. Utilizing the probability that a container-inhabiting mosquito will visit multiple ovisites due to its behavior has been both proposed for and used as the basis for several control techniques.
Reiter (2007) has proposed that skip oviposition behavior has been historically important in *Ae. aegypti* control programs. Residual pesticide treatments were routinely applied to *Ae. aegypti* larval habitats during the control programs of the 1960s (Reiter 2007). Reiter (2007) theorized that *Ae. aegypti* adults performed skip oviposition and that the ovipositing female had an increased probability of encountering pesticide treated container habitats if it visited multiple habitats. Indeed, during these campaigns *Ae. aegypti* was successfully controlled in 22 countries (Schliessman and Calheiros 1974).

Lethal ovitraps have been proposed as a method of controlling container-inhabiting mosquitoes (Zeichner and Perich 1999, Perich et al. 2003, Sithiprasasna et al. 2003, Williams et al. 2006, Ritchie et al. 2008). Zeichner and Perich (1999) developed a potential control technique that utilized a pesticide treated velour strip as an oviposition substrate placed in a cup for a gravid female to encounter. If the target population performed multiple ovipositions in one gonotrophic cycle then the females would have a higher probability of encountering this pesticide than if oviposition was restricted to one container. Utilization of the lethal ovitrap in field studies has resulted in *Ae. aegypti* population reduction in Brazil (Perich et al. 2003) and varied results in Thailand (Sithiprasasna et al. 2003).

Similar to the lethal ovitrap, it has been proposed to utilize ovisites to treat ovipositing females with a control measure which thereafter is widely dispersed into the mosquito populations’ developmental sites (Gaugler et al. 2012). In a laboratory setting, gravid *Ae. albopictus* have been shown to visit ovisites containing an insect growth regulator (IGR), pyriproxifen, and carry it to other ovisites (Gaugler et al. 2012).
Pyriproxifen works more efficiently as a larvicide than an adulticide and affects container-inhabiting mosquito larvae at very small doses (Devine et al. 2009). Therefore, skip ovipositing mosquitoes that encounter an IGR-treated ovisite have the potential to move the toxicant to multiple locations.

The behavior of ovipositing an egg batch over multiple ovisites is an important aspect of container-inhabiting-mosquito ecology. This behavior could be wide spread among container inhabiting mosquitoes as evolutionary pressures may have selected for it. Skip oviposition behavior also provides a predictive tool to be utilized in control measures. Future work to explore the extent and the details of this behavior will be critical to integrating it into container-inhabiting mosquito management.

**Research Objectives**

To date, only one laboratory studies have been completed to examine skip oviposition behavior in *Ae. albopictus* (Trexler et al. 1998). In the following four studies aspects of *Ae. albopictus* oviposition behavior were examined to elicit improvements for both surveillance techniques and control tactics:

1. Determine if *Aedes albopictus* will exhibit skip oviposition and examine the effects of larval habitat quality on oviposition, in small indoor and large outdoor caged arenas.

2. Develop and deploy an attractant self-marking ovitrap (ASMO) to directly examine *Ae. albopictus* dispersal and indirectly determine skip oviposition.

3. Assess *Ae. albopictus* clutch size in wild populations and in a colony reared at differing larval densities.

4. Examine *Ae. albopictus* oviposition preference for different size containers and the influence of adjacent flowering plants.
CHAPTER 2
THE EFFECTS OF LARVAL HABITAT QUALITY ON Aedes albopictus SKIP OVIPosition

Introduction

Container-inhabiting mosquito larvae reside in environments that vary in resource availability (Walker et al. 1991). Competition for limited food among mosquito larvae can result in mortality (Mori 1979). Accordingly, density dependent larval mortality has been proposed as a major population regulating factor in container-inhabiting mosquito species (Service 1985). To mitigate larval competition for nutritional resources, female mosquitoes may choose to disperse their eggs over multiple ovipositions. This behavior has been observed in several container-inhabiting mosquito species. Aedes aegypti (Linneaus) females have been found to distribute a batch of eggs in installments by laying clutches of eggs over multiple ovipositions (Christophers 1960, Fay and Perry 1965). Mogi and Mokry (1980) observed Wyeomyia smithii (Coquillett) females depositing eggs at multiple sites during a single gonotrophic cycle and described this behavior as skip oviposition. It has been theorized that skip oviposition behavior is selected for because the female distributes larval survival risk through placement of offspring in multiple locations and reduces sibling competition for resources (Harrington and Edman 2001).

Alternatively, if the female finds a “high-quality” larval habitat all eggs may be laid to conserve energy and limit the probability of adult mortality before complete egg deposition, resulting in selection against skip oviposition behavior (Harrington and Edman 2001).

Evidence for skip oviposition by Aedes albopictus (Skuse) is limited. It has been reported in several studies that when surveying for Ae. albopictus eggs, the number of eggs collected per oviposition habitat is often much less than the average female egg
batch per gonotrophic cycle (Rozeboom et al. 1973, Makiya 1976, Takagi et al. 1995, Rozilawati et al. 2007). These results have been interpreted as evidence of skip oviposition (Hawley 1988). There are several concerns with the conclusion that skip oviposition has been observed based on the number of eggs found in surveillance studies. First, in the surveillance studies of Rozeboom et al. (1973), Takagi et al. (1995), and Rozilawati et al. (2007) only eggs deposited on the oviposition substrate located in each ovitrap were counted. However, it has been found that *Ae. albopictus* will lay a portion of their eggs on the water surface (Chan 1971), perhaps resulting in an under estimate of eggs per trap. In addition, the size of the ovitraps used in the surveillance studies (Rozeboom et al. 1973, Makiya 1976, Takagi et al. 1995, Rozilawati et al. 2007) was uniform and relatively small. Second, the average egg batch size of *Ae. albopictus* has been measured only under optimal laboratory conditions and the field environment may greatly influence the number of eggs produced (Hawley 1988). Third, only one laboratory study provided evidence that *Ae. albopictus* performed skip oviposition and this study focused on the effect of organic infusions as an attractant (Trexlar et al. 1988). The study was conducted in small (30 cm x 30 cm x 30 cm) cages, and the influence of the larval habitat quality on oviposition was not fully examined (Trexlar et al. 1998).

Increased larval density in relation to food supply causes an increase in larval mortality and a decrease in adult concomitant size (Hien 1975, Mori 1979, Dutton and Sinkins 2004). A reduction in adult *Ae. albopictus* size has been correlated to a reduction in fecundity (Blackmore and Lord 2000). Thus, larvae developing in “low-quality” habitat will encounter inadequate nutrition resulting in high mortality and
production of smaller females with reduced fitness as compared to those developing in a habitat of higher quality.

Larvae reared at reduced density with adequate larval nutrition produce larger adults and larger adults produce more offspring when compared to larvae reared at high density with inadequate nutrition (Blackmore and Lord 2000). Larvae reared under normal rearing practices encounter minimal competition for nutritional resources and represent “high-quality” habitat. These larvae will produce more eggs and subsequently have an evolutionary advantage as opposed to larvae that develop in low-quality habitat conditions. Allan and Kline (1995, 1998) found that *Ae. albopictus* show an ovipositional preference for water taken from standard larval rearing practices when compared to well water. In addition, Yoshioka et al. (2012) observed more oviposition in containers with low larval density compared with those having highed larval density.

The objectives of this study were to determine if *Ae. albopictus* exhibited skip oviposition in caged indoor and outdoor environments and elucidate the influence of larval habitat quality on egg deposition. Two developmental conditions were designed to simulate quality differences in larval habitat: crowded larval habitat represented low-quality habitat and uncrowded larval habitat represented high-quality habitat.

**Materials and Methods**

A series of multiple choice studies were conducted to test the hypothesis that a single female *Ae. albopictus* will deposit her eggs in one container during a single gonotrophic cycle or alternatively, she will deposit them in more than one container (H₀: μ = 1, Hₐ: μ > 1). Additionally, the effect of larval habitat quality was examined on the oviposition pattern of individual gravid females over multiple ovisites. Each female
tested was introduced into a four-ovisite arena in which to deposit eggs (Figs. 2-1 and 2-2).

**Colony Establishment and Rearing Practices**

*Aedes albopictus* larvae were collected from four locations in Alachua County, Florida. All collections were made near human habitations, with two sites in rural areas and two sites in suburban areas. Approximately 1000 larvae were collected between September and October, 2010. Larvae were collected using aquarium fish nets (7.6 x 7.6 cm, Marina Pet Products, Waverly, NY) to strain out the water from the habitat. Larvae were placed in 355 mL clear plastic cups (Solo Cup Company, Chicago, IL) and held in screened cages (46 x 46 x 46 cm, Bioquip, Rancho Dominguez, CA) at the University of Florida Veterinary Entomology Laboratory. Larvae were fed ground TetraFin™ goldfish flakes daily, until pupation. *Aedes albopictus* adults (F0) were separated from other species of emergent adult mosquitoes and used to establish the colony.

The rearing of *Ae. albopictus* was adapted from the United States Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology (USDA-CMAVE) protocol. *Aedes albopictus* adults were held in screened cages (46 x 46 x 46 cm) maintained in a rearing room at 25 ± 1°C, 75 ± 5% RH, and a 12:12 (L:D) photoperiod at the University of Florida Veterinary Entomology Laboratory. Adults were provided a 5% sucrose solution that was wicked through rolled white blotting paper (7 x 7 cm). Blood meals were offered to the caged adults twice weekly starting one week post emergence. Approximately 50 mL of citrated bovine blood was placed in a sausage casing (Sausage Maker, Buffalo, NY) warmed to around 37°C, and suspended inside the adult cages.
Plastic cups lined with number 76 seed germination paper (15 cm x 4 cm high, Anchor Paper, St. Paul, MN) and filled with 300 mL of deionized water were placed in adult cages to collect eggs. Seed germination paper lined with eggs was removed weekly and dried for 24 hr prior to storage in re-sealable plastic bags. A damp paper towel was placed in the bags to maintain high humidity until the eggs were required for hatching.

To initiate egg hatch, seed germination paper lined with eggs was placed in a plastic cup (355 mL) with 300 mL of deionized water and 0.1 g of ground TetraFin™ fish food for 24 hr. The newly eclosed larvae were distributed at approximately 500 per enamel pan (30 cm x 23 cm x 5 cm high). Pans contained approximately 2 liters of deionized water and were held in environmental growth chambers 29 ± 1°C. The larvae in each pan were fed 0.5 g of ground fish flakes initially, and every 48 hr thereafter. The time from eclosion until pupation was approximately seven days. Pupae were removed from the enamel pans using a fish net and approximately 500 were placed in 355 mL plastic cups with 300 mL of deionized water. Cups were then placed in mesh cages (46 x 46 x 46 cm) to allow adult emergence.

The *Ae. albopictus* colony was established to conduct oviposition evaluations. Both larvae and adults were used in this study. To minimize the effects of colony selection pressures only generations F3-F12 were utilized.

**Larval Habitat Quality**

An evaluation of larval habitat quality on *Ae. albopictus* oviposition over multiple habitats during a single gonotrophic cycle was conducted. Multiple choice oviposition assays were conducted with gravid females being presented containers with water and larvae collected from rearing pans that had held differing densities of larvae. High and
low-quality larval habitat was based on larval rearing densities established in previous studies where maximum and minimum adult size and egg production, respectively were determined (Mori 1979, Blackmore and Lord 2000, Dutton and Sinkins 2004). Thus, a habitat was considered high-quality when larvae were reared at a density of 1 larva per 3 mL and provided approximately 0.5 g of ground fish food per 1,000 mL initially and every 48 hours thereafter. Habitat was considered of low-quality when larvae reared at a density of 10 larvae per 3 mL with the same amount of food as in the high-quality conditions. Specified larval habitat conditions were provided for adult oviposition in the multiple choice assays when the larvae were in the second and third instars. The specified habitat was acquired by agitating individual rearing density trays to uniformly distribute larvae, food, and waste in the water column and then adding 120 mL of the habitat water to a 473 mL black cup. For each replication a new larval rearing tray of the appropriate rearing density was used.

**Caged Arenas**

In the indoor cage study, 15 cages (Bioquip, Rancho Dominguez, CA) (61 x 61 x 61 cm), were used in the mosquito rearing building at the USDA-CMAVE. The cages were held at 25 ± 1°C, 75 ± 5% relative humidity, and 12:12 (L:D) photoperiod. Within each cage, four 473 mL black cups were placed equidistant from each other representing the multiple choice arena (Fig. 2-1). Each cup contained 120 mL of the treatment habitat and was lined with seed germination paper. Females seven days old were allowed to mate and feed from the bovine blood-filled sausages and were held for five days without additional blood. Individual mosquitoes were transferred using a mouth aspirator (John W. Hock, Gainesville, FL) into center of a test cage and allowed
to oviposit for three days. Both, the number of eggs per container per cage and the number of containers with eggs per cage were recorded.

Each treatment consisted of a four-cup choice arena presented to an individual gravid female. There were five treatments and each treatment was comprised of differing assemblages of larval habitat quality to enable an evaluation of substrate options on the adult female’s oviposition choice. Treatments consisted of cages containing either: well-water (4 cups of well-water), high-quality habitat (4 cups of high-quality larval habitat), low-quality habitat (4 cups of low-quality larval habitat), low-quality dominant (3 cups of low-quality habitat and 1 cup of high-quality habitat), and high-quality dominant (3 cups of high-quality habitat and 1 cup of low-quality habitat). The five treatments were repeated three times in a randomized complete block design resulting in three blocks of five cages each for a total of 15 cages per replicate. Blocking was utilized to account for variability in the rearing facility. This study was replicated 10 times with each treatment being tested 30 times for a total of 150 experimental units.

The previously described experiment was conducted in large cages in an outdoor environment to simulate field conditions. This experiment provided several enhancements over the laboratory, including an enlarged search arena for adult females to operate within during the experiment, and the introduction of typical outdoor parameters, both of which may influence the detection of oviposition substrates. Five cages (2.13 m high x 2.74 m diameter) constructed of a polyvinyl chloride (PVC) pipe frame (2.54 cm diameter) and screening (18 x 14 mesh) were positioned at the USDA-CMAVE (Fig. 2-2). Cups and treatments used in the indoor small cage study were used
for the outdoor large cage study. The cups were placed equidistant from each other and inside cinder blocks in order to provide a shaded habitat. Additionally, these cinder blocks were used to prevent screened enclosures from being moved by wind gusts. One blooming *Buddleja davidii* plant was placed in the center of each cage to provide a resting location and nectar source. Treatments were assigned to cages in a randomized complete block design with individual blocks being tested sequentially. Both, the number of eggs per container per cage and the number of containers with eggs per cage were recorded. Each experiment consisted of five cages that contained a treatment assay and this was replicated 22 times for a total 110 experimental units.

**Statistical Analysis**

The hypothesis that the mean number of eggs laid per female for each treatment and for each block was equal was examined using a two-way analysis of variance (ANOVA) for both the indoor and outdoor cages. To evaluate treatment and block effects, the mean number of cups with eggs for each treatment was examined using a two-way ANOVA for both the indoor and outdoor cage studies. The Tukey-Kramer Honestly Significant Difference (HSD) multiple means comparison was used to examine differences among the treatments for both the indoor and outdoor cage studies. Means were considered significantly different if the p-values were below 0.05 for each pairwise comparison. JMP® Version 8 was used for all statistical analyses (SAS Institute 2009).

**Results**

**Indoor Caged Arenas**

The average number of eggs produced per *Ae. albopictus* was not significantly different when presented different treatment assays in indoor cages (Table 2-1). Individual females demonstrated skip oviposition by depositing eggs in more than one
container in caged arenas as confirmed by distributions among the varying larval habitat quality treatments \( (F_{4,135} = 7.68, \: P < 0.01) \) (Fig. 2-3). *Aedes albopictus* oviposited in significantly more containers when presented only low-quality larval habitat ovisites as compared to oviposition patterns from arenas that contained only high-quality and high-quality dominant habitats. When presented ovisites that contained only well water, *Ae.* *albopictus* oviposited in a similar number of containers as when arenas contained ovisites that had all low-quality larval habitat.

The egg deposition pattern within the low-quality dominant arena presented a differential pattern in the ovisites that contained eggs, with significantly more eggs being recovered in the single high-quality ovisite \( (F_{3,108} = 6.30, \: P < 0.01) \) (Fig. 2-4). No differences in the proportion of eggs recovered per ovisite were observed in the high-quality dominant arena, including selection against the low-quality ovisite (Fig. 2-5).

**Outdoor Caged Arenas**

*Aedes albopictus* demonstrated skip oviposition in the large outdoor cages by laying eggs in more than one ovisite. The mean number of eggs produced by each female when exposed to the different larval habitat treatments was not significantly different (Table 2-1). Similar to the small indoor cage study, the skip oviposition behavior was affected by the larval habitat quality treatments \( (F_{4,105} = 33.35, \: P < 0.01) \) (Fig. 2-6). Significantly more within-treatment ovisites contained eggs in the well-water, low-quality habitat, and low-quality dominant treated arenas as compared to the number of ovisites containing eggs in the high-quality habitat treatment. The low-quality dominant arena had a greater proportion of eggs in the high-quality habitat \( (F_{3,84} = 4.03, \: P < 0.01) \), although, one of the low-quality habitats was not significantly different (Fig. 2-
The high-quality dominant arena had no differences in the proportion of eggs per ovisite (Fig. 2-8).

**Discussion**

Skip oviposition is an important aspect in understanding population dynamics and dispersal in container-inhabiting mosquitoes (Mogi and Mokry 1980, Corbet and Chadee 1993, Reiter 2007, Wong et al. 2011, Oliva et al. 2013). Mosquitoes that perform skip oviposition may travel greater distances during oviposition than mosquitoes that oviposit all of an egg batch in a single container (Oliva et al. 2013). Increases in dispersal by ovipositing mosquitoes can have implications in vector-borne disease epidemiology (Reiter 2007). *Aedes albopictus* that are gravid will have previously acquired a blood-meal and potentially a pathogen. Dispersal in search of multiple oviposition sites can result in vectors carrying the pathogen into areas away from the site of initial acquisition and facilitate disease spread. In the present study, *Ae. albopictus* performed skip oviposition when arenas were within small indoor cages (61 x 61 x 61 cm) and when the cups were four times further apart in large outdoor cages (2.13 m high x 2.74 m diameter) (Figs. 2-1 and 2-2). Additional support for skip oviposition behavior was demonstrated with the poorest habitat examined. In these comparisons, individual females distributed their respective egg batch among greater than 75% if the available poor-quality, well-water only ovisites were provided (Fig. 2-3, 2-6). These results are especially important when considering the flight capabilities of this mosquito. The maximum distance dispersed during skip oviposition could be much greater than our current findings, as evidenced by a published record of *Ae. albopictus* ovipositing 320 m away from a release point (Liew and Curtis 2004).
Other studies have found evidence for and against skip oviposition behavior occurring in container-inhabiting mosquitoes (Buxton 1927, Rozeboom et al. 1973, Makiya 1976, Chadee and Corbet 1987, Kitron et al. 1989, Trexler et al. 1998, Apostol et al. 1994, Reiter et al. 1995, Harrington and Edman 2001, Colton et al. 2003, Liew and Curtis 2004, Oliva et al. 2013). However, many of these studies were focused over short time periods and with little-to-no reference to larval habitat conditions. Trexler et al. (1998) is the only study we have found in the literature to report evidence of *Ae. albopictus* skip oviposition from individual females. The study by Trexler et al. (1998) did not examine the influence of larval habitat quality on skip oviposition but did report that more ovisite cups were utilized by females when presented only water and fewer cups were used when they contained oak leaf infusion water. Water that contains decaying vegetation has microorganisms present that container-inhabiting mosquitoes feed upon as larvae (Walker et al. 1991). The microorganisms within oak leaf infusion water have been found to produce oviposition-stimulating kairomones for *Ae. aegypti* (Ponnusamy et al. 2008). Therefore, the volatiles associated with container habitats may serve as cues for skip ovipositing females in assessing the oviposition site.

This is the first study to demonstrate that larval habitat quality impacts *Ae. albopictus* skip oviposition behavior. *Aedes albopictus* dispersed an egg batch, on average, among 2-3 cups when presented an arena containing low-quality habitats and among 1-2 cups when provided high-quality habitats. The larval habitat quality was found not to affect the number of eggs laid by *Ae. albopictus*, with females having oviposited an average of between 54.2-68.7 eggs. Therefore, larval habitat quality does not impact the number of eggs produced, but does affect the distribution of the eggs.
Previous studies have determined that ovipositing mosquitoes are able to assess the larval habitat (Reisen and Siddiqui 1978) including *Ae. albopictus* (Allan and Kline 1995, Yoshioka et al. 2012). Allan and Kline (1995) reported that *Ae. albopictus* displayed an oviposition preference for cups that contained water from colony larval rearing trays that were maintained with uncrowded conditions when compared to cups containing water. Yoshioka et al. (2012) found that *Ae. albopictus* oviposition was affected by the density of conspecifics within the oviposition site. Our study establishes that these mosquitoes can assess larval habitat quality and choose to distribute their egg batch based on larval habitat assessments. Consequently, skip oviposition in *Ae. albopictus* is expressed as a behavioral decision that is regulated by the quality of the larval habitat with high-quality habitat inhibiting this behavior and low-quality habitat promoting skip oviposition.

The results of the present study indicate that *Ae. albopictus* may have evolved the ability to distinguish among environmental conditions in order to limit density dependent larval mortality and maximize recruitment. Biotic conditions, such as competition among larvae for limited resources can alter adult mosquito oviposition outcomes (Mori 1979). In addition to competition, parasitism may impact density dependent larval mortality and in turn influence skip oviposition in container-inhabiting mosquitoes. Zahiri and Rau (1998) reported that *Ae. aegypti* larvae occurring in high densities were infected with the digenean *Plagiorchis elegans* (Rudolphi) and that water titrated from this larval habitat was an ovipositional repellent. Therefore, stress associated with crowded larval conditions may allow for an increase in parasitic infection and contribute to mosquito assessment of low-quality habitats. Similarly, Nalen et al. (2013) reported that *Ae. aegypti* laid fewer eggs in cups containing larvae
infected with the microsporidium *Edhazardia aedis* (Kudo) and attributed the repellency to chemicals produced by the parasitized larvae. The gregarine parasite *Ascogregarina taiwanensis* (Lien and Levine) has been commonly found in *Ae. albopictus* in Florida (Blackmore et al. 1995) and this parasite-host interaction may affect *Ae. albopictus* skip oviposition behavior. Furthermore, biotic factors impacting larval habitat quality, along with abiotic factors are theorized to influence density dependent population effects (Dieng et al. 2003). The physical features of oviposition sites may influence *Ae. albopictus* habitat quality and potentially affect skip oviposition performance. In *Ae. aegypti*, Oliva et al. (2013) reported that the percentage of females performing skip oviposition increased from 32.7% to 94% when containers were painted black. Our present study focused on the biotic factors associated with larval habitat quality, however, additional studies are required to examine biotic factors, abiotic factors and their interactions to better understand how container-inhabiting mosquitoes assess their environment in order to limit density dependent larval effects.

Bentley and Day’s (1989) review of mosquito oviposition collated information from several studies that examined oviposition preference among both containers that had conspecifics and water that had previously contained conspecifics. The authors concluded that adult mosquitoes were most likely detecting differences in the water by olfactory cues that may be related to larval waste or a pheromone produced by the larvae. Therefore, the *Ae. albopictus* in the present study may have assessed the larval habitat quality by volatiles in concentrations and combinations associated with the differing habitats. In our study when choice arenas contained both high and low-quality habitats, the proportion of eggs per container within an arena was dependent upon the
number of each larval habitat present (Figs. 2-4, 2-5, 2-7, and 2-8). The observed behavior may have resulted from the different concentrations or combinations of volatiles present in the low-quality dominant and high-quality dominant arenas. In the arenas that were low-quality dominant, the largest proportion of eggs were oviposited in the high-quality habitat, but, in the high-quality dominant arena the habitats were found not to differ in the average proportion of eggs received. The results of the high-quality dominant assays indicate that the repellency of the lone low-quality habitat may have been negated by the presence of the three high-quality habitats. Likewise, in an examination of *Ae. aegypti* responses to volatiles, Kline et al. (2003) found that the compound linalool negated a behavioral response by causing a reduction in the number of mosquitoes that initiated a host-searching flight in response to human volatiles. Further research is needed to identify the volatiles and compounds acting as skip oviposition stimulants or repressors.

Understanding when and how skip oviposition behavior is expressed will be an important aspect in successfully employing mosquito control techniques utilizing container habitats. As such, mosquitoes that perform a greater degree of skip oviposition will have a higher probability of encountering these mosquito control ovisites. Therefore, these control techniques will be more successful when larval habitat quality is poor and more containers are being visited by skip ovipositing females. In contrast, during times where high-quality habitats are abundant, these control techniques may have limited success due to the reduced number of containers visited by skip ovipositing mosquitoes and the availability of competing ovisites. This choice shifting
oviposition behavior may explain the mixed success of lethal ovitraps in different environments (Perich et al. 2003, Sithiprasasna et al. 2003).

A limitation of our present studies is that they were conducted in caged environments. Further studies that occur in a field environment are needed to assist in understanding *Ae. albopictus* skip oviposition behavior and how this mosquito is able to distinguish among larval habitats. Nonetheless, this research has provided insight into the factors that may affect skip oviposition. Specifically, the understanding that skip oviposition may occur at varying degrees within a population and that factors such as larval habitat quality will determine oviposition outcomes.
Table 2-1. The mean number of eggs oviposited per *Ae. albopictus* when exposed to a four ovisite choice assay in indoor or outdoor cages.

<table>
<thead>
<tr>
<th>Habitat treatment</th>
<th>Indoor cages mean (± SEM)</th>
<th>Outdoor cages mean (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Well-water</td>
<td>30</td>
<td>54.2 (3.0)</td>
</tr>
<tr>
<td>Low-quality</td>
<td>30</td>
<td>65.1 (3.0)</td>
</tr>
<tr>
<td>Low-quality dominant</td>
<td>30</td>
<td>54.2 (5.3)</td>
</tr>
<tr>
<td>High-quality dominant</td>
<td>30</td>
<td>59.9 (5.5)</td>
</tr>
<tr>
<td>High-quality</td>
<td>30</td>
<td>63.7 (5.6)</td>
</tr>
</tbody>
</table>

ANOVA: $F_{4,135} = 0.83, P > 0.51$ $F_{4,105} = 0.96, P > 0.43$

$^1$ Treatments consisted of cages containing either: well-water (4 cups of well water), low-quality habitat (4 cups of low-quality larval habitat), low-quality dominant (3 cups of low-quality habitat and 1 cup of high-quality habitat), high-quality dominant (3 cups of high-quality habitat and 1 cup of low-quality habitat), and high-quality habitat (4 cups of high-quality larval habitat).
Figure 2-1. Four ovosite choice arena in a screened cage (61 x 61 x 61cm) employed to evaluate the effect of larval habitat quality on individual *Ae. albopictus* oviposition patterns. Photo courtesy of Timothy J. Davis.
Figure 2-2. Four ovisite choice arena in a screened cage (2.13 m high x 2.74 m diameter) employed to evaluate the effect of larval habitat quality on individual *Ae. albopictus* oviposition patterns. Photo courtesy of Timothy J. Davis.
Figure 2-3. Mean number of ovisites containing eggs from indoor caged assays that include an individual *Ae. albopictus* and four ovisites. Columns with the same letter are not significantly different based on Tukey-Kramer HSD multiple means comparison ($F_{4,135} = 7.68, \ P < 0.01$).
Figure 2-4. Mean proportion of eggs per each container habitat in the low-quality dominant treatment from indoor caged assays. Columns with the same letter are not significantly different based on Tukey-Kramer HSD multiple means comparison ($F_{3,108} = 6.30$, $P < 0.01$).
Figure 2-5. Mean proportion of eggs per each container habitat in the high-quality dominant treatment from indoor caged assays. Columns with the same letter are not significantly different based on Tukey-Kramer HSD multiple means comparison.
Figure 2-6. Mean number of ovisites containing eggs from outdoor caged assays that include an individual *Ae. albopictus* and four ovisites. Columns with the same letter are not significantly different based on Tukey-Kramer HSD multiple means comparison ($F_{4,105} = 33.35$, $P < 0.01$).
Figure 2-7. Mean proportion of eggs per each container habitat in the low-quality dominant treatment from outdoor caged assays. Columns with the same letter are not significantly different based on Tukey-Kramer HSD multiple means comparison ($F_{3,84} = 4.03, P < 0.01$).
Figure 2-8. Mean proportion of eggs per each container habitat in the high-quality dominant treatment from outdoor caged assays. Columns with the same letter are not significantly different based on Tukey-Kramer HSD multiple means comparison.
CHAPTER 3
AN ATTRACTIVE SELF-MARKING OVITRAP TO MEASURE DISPERSAL AND DETERMINE SKIP OVIPOSITION IN Aedes albopictus (Skuse) FIELD POPULATIONS

Introduction

Mosquito dispersal is an area of study that is important for understanding disease epidemiology and developing effective control strategies (Service 1997). Mosquitoes disperse to locate sugar sources, mates, resting sites, blood meals and oviposition sites. In mosquitoes that display skip oviposition, the female will disperse over an area in search of multiple oviposition sites within a single gonotrophic cycle (Mogi and Mokry 1980). *Aedes albopictus* (Skuse), the Asian tiger mosquito, may exhibit skip oviposition behavior (Rozeboom et al. 1973).

Several studies have been completed to examine *Ae. albopictus* dispersal (Bonnet and Worcester 1946, Mori 1979, Niebylski and Craig 1994, Honorio et al. 2003, Liew and Curtis 2004, Maciel-de-Freitas et al. 2006, Lacroix et al. 2009, Marini et al. 2010). While most studies have found Asian tiger mosquitoes remain in an area of less than 120 m (Bonnet and Worcester 1946, Niebylski and Craig 1994, Lacroix et al. 2009, Marini et al. 2010), other studies have found this mosquito can travel distances of over 800 m (Honorio et al. 2003, Maciel-de-Freitas et al. 2006). In mosquitoes that exhibit skip oviposition, little information has been reported in regards to the distance traveled between oviposition sites (Honorio et al. 2003, Liew and Curtis 2004). This knowledge is an important regulator as new approaches in *Ae. albopictus* control utilize skip ovipositing mosquitoes to distribute an insect growth regulator (IGR) to multiple larval habitats (Caputo et al. 2012, Gaugler et al. 2012). The distance traveled during skip
oviposition will determine the extent to which a control measure can be dispersed utilizing auto-dissemination techniques.

An attractive self-marking ovitrap (ASMO) using fluorescent marking powders was developed and used to directly examine the distance traveled from one oviposition site to another and indirectly to determine the occurrence of skip oviposition. Laboratory studies were completed to examine the ASMO’s marking potential, the influence of marking dusts on survivorship, and the ability of sticky ovitraps to recapture marked mosquitoes. The ASMO was deployed in the field to mark naturally occurring populations of *Ae. albopictus* and sticky ovitraps were placed to capture the marked mosquitoes at secondary oviposition sites. Establishing if field populations of *Ae. albopictus* display skip oviposition and determining the distance traveled between oviposition sites would benefit mosquito control programs that seek to exploit skip oviposition behaviors.

**Materials and Methods**

**ASMO Evaluation Studies**

Two preliminary studies were conducted to test the effectiveness of the ASMO. An indoor cage study examined the ability of an ASMO to mark *Ae. albopictus* females and to determine the influence of fluorescent dusts (Day-Glo Color Corp., Cleveland, OH and United States Radium Corp., Hackettstown, NJ) on survivorship after marking. A follow-up outdoor screened enclosure study was conducted to examine the potential of *Ae. albopictus* females to be self-marked by an ASMO, for sticky ovitraps to collect the marked mosquitoes in large outdoor cages, and to determine the influence of fluorescent dusts on dispersal distance.

The ASMO consisted of a 1.8 L plastic container (Plastic Packaging Corp., West Springfield, MA) painted black with a 6 cm hole cut in the lid (Fig. 3-1a). A 150 x 15 mm
plastic Petri dish lid (Corning Inc., Corning, NY) was placed at a height of 4 cm within the container (Fig. 3-1b). The Petri dish had an 8 cm hole cut in center. Fluorescent dusts were applied using a 113 g bulb duster (The Centro Company, North Liberty, IA) to coat the inside of the ovitrap. An oak leaf infusion (600 mL) was added as an attractant. The oak leaf infusion was formulated by adding 150 g of fallen live oak (Quercus virginiana Miller) leaves to 15 liters of well water in a container that is sealed, placed outside and allowed to ferment for seven days, as modified from Allan and Kline (1995).

The indoor ASMO marking evaluation study consisted of five treatments represented by infusion-baited ASMOs treated individually with either one of the powders that consisted of two Day-Glo® (Cleveland, OH) fluorescent powders (Horizon Blue®, Rocket Red®) or, two US Radium (Hackettstown, NJ) fluorescent powders (Yellow 2267 and Green 1953), or an undusted control. The study was conducted in a randomized complete block design with three blocks of five cages for a total of 15 cages per replication. Blocking was utilized to account for variability in the rearing facility. This test was replicated five times, providing an overall n = 15 for each treatment. Each treatment ASMO was placed individually inside a cage (Bioquip, Rancho Dominguez, CA) (61 x 61 x 61 cm). The cages were kept in the mosquito rearing building at the United States Department of Agriculture’s Center for Medical, Agricultural, and Veterinary Entomology (USDA-CMAVE) in Gainesville, FL, and held at 25 ± 1°C, 75 ± 5% relative humidity, and 12:12 (L:D) photoperiod. Mosquitoes were provided cotton balls that were soaked in a 5% sucrose solution for the duration of the study.
The mosquitoes used in the indoor ASMO evaluations were blood fed 7 d after emergence using bovine blood-filled sausages, and then held for an additional 5. Twenty gravid *Ae. albopictus* (laboratory colony generations F2-F7) were released into each cage containing a treatment ASMO. Following 48 h of access to the ASMO, mosquitoes were removed by aspiration and examined on a chill table (Bioquip, Rancho Dominguez, CA) with a long-range ultra violet (UV) light (Scorpion Master, Chandler, AZ). Long-range UV light results in the fluorescent powders glowing, thus aiding in the ability of the researcher to record the number marked (Fig. 3-2). All marked mosquitoes were returned to their respective cages from which the ASMO had been removed and the unmarked mosquitoes were discarded. Thereafter, every 24 h dead mosquitoes were removed from the cages and the daily mortality rate (DMR) was recorded until all mosquitoes were dead. The DMR was calculated as number of deaths during a 24 h interval divided by the number of individuals alive during that start of that interval times 100 (Smiley 1985). The mean DMR was determined and compared for each treatment.

An outdoor screened enclosure ASMO evaluation study examined the potential for *Ae. albopictus* females to self-mark at an ASMO and subsequently be captured at a sticky ovitrap, as well as to determine the influence of fluorescent dusts on dispersal distances. *Aedes albopictus* (generations F4-F9) and were fed using bovine blood-filled sausages and held for five days prior to testing. The five treatments, four powders and undusted control used in the indoor cage study were used in this study. A 61 x 61 x 61 cm cage was placed in the center of an outdoor screened 9.2 m wide x 18.3 m long x 4.9 m high, peaking at 6.1 m along the length of the enclosure ceiling (Kline 1999). The large outdoor enclosures were located at USDA-CMAVE. Each treatment consisted of
20 gravid *Ae. albopictus* initially released into the smaller cage containing an infusion-baited ASMO. After a 24 h exposure to the ASMO, the top of the smaller cage was opened inside the screened enclosure releasing the marked mosquitoes into the larger cage (Fig. 3-3) into which 16 infusion-baited sticky ovitraps were placed, prior to opening of the cage containing the mosquitoes and ASMO. The sticky ovitraps were distributed evenly by dividing the area within the large screened enclosure into four concentric rings and placing a trap every 3.14 m2. This resulted in the following number of traps placed at each concentric ring: one trap at 0-1 m, three traps at 1-2 m, five traps at 2-3 m, and seven traps at 3-4 m, yielding 16 traps per enclosure. Sticky ovitraps consisted of the aforementioned 1.8 L black plastic containers outfitted with two sticky panels (Fig. 3-1c). The sticky panels consisted of 7 cm x 21.6 cm strips of transparency film (3M, St. Paul, MN), coated with Catchmaster bulk glue (The Catchmaster Co., Brooklyn, NY) that were attached to the container’s interior with paper clips (Ritchie et al. 2003). The sticky panels were collected 24 hours after opening the initial ASMO-containing treatment cage. The number of marked and unmarked mosquitoes on the sticky panels was recorded. The mean distance traveled (MDT) was calculated and compared for each treatment. This study was conducted as a complete randomized design with 5 replications per treatment.

**Field Studies**

A field experiment was conducted to determine if *Ae. albopictus* females will visit two oviposition sites within a gonotrophic cycle and to assess the distance traveled between oviposition sites. The field experiments were carried out at two locations (Site 2-1 a graveyard in Gainesville, FL and Site 2-2 on the University of Florida campus in
Gainesville, FL) and replicated six times at each location over the summers of 2011 and 2012.

An infusion baited ASMO was placed at a known Ae. albopictus developmental site at each location. A radius of 150 m around the ASMO was marked and 50 sticky ovitraps were distributed over this area (Fig. 3-4). To facilitate distribution of the sticky ovitraps, the trapping area was divided into five concentric rings and one sticky ovitrap was placed approximately every 1,413 m$^2$ to equalize trapping density. Thus, the trapping density is represented by the following number of traps placed within each concentric ring: two traps at 0-30 m, six traps at 30-60 m, 10 traps at 60-90 m, 14 traps at 90-120 m, and 18 traps at 120-150 m. Exact trap placement was dependent on the appropriate available habitat and site restrictions and thus, traps were more clustered in some areas within a ring. Regardless of trap-to-trap placement, all traps were placed in habitats that were shaded and contained vegetation (Lambrecht 1971). All sticky ovitraps were georeferenced with a hand-held global positioning system (GPS) device (MiTAC International Corp., San Dimas, CA).

ASMOs were treated with one of the previously described four fluorescent colors and every four days the ASMO was replaced with a new ASMO containing a color not previously used. Thus, all four colors were placed in the field individually and consecutively resulting in the following pattern of placement: red on days 0-4, blue on days 4-8, yellow on days 8-12, and green on days 12-16. Following the 12-16 day exposure, no ASMO was present for an additional 16 days. This resulted in each trial consisting of a 32-day period with self-marked mosquitoes of each color being available.
to be trapped at gravid sites for a minimum 20 days. Each 32-day trial was replicated six times between June 5 - September 5, 2011 and June 5 - September 5, 2012.

All sticky ovitraps were serviced every four days wherein sticky panels were changed, the oak infusion was replaced, and any missing or damaged traps were substituted. Removed sticky panels were examined under UV light and the number of marked and unmarked *Ae. albopictus* and *Aedes triseriatus* (Say) were recorded. *Aedes albopictus* and *Ae. triseriatus* were identified using the keys of Darsie and Morris (2003). Thereafter, the collected mosquitoes were dissected to determine if developed eggs were present using the method of Lounibos et al. (1990). During the servicing of the sticky traps the number of *Toxorhynchites rutilus rutilus* (Coquillett) eggs was recorded. *Toxorhynchites r. rutilus* identification was confirmed by rearing approximately 75% of the eggs to the adult stage and determining species using the keys of Carpenter and LaCasse (1955). During each replicate, captured self-marked mosquitoes were recorded by color for each trapping period. Those marked mosquitoes that were captured within four days of the same color being placed were considered as visiting two ovisites within a gonotrophic cycle, based on the average time per gonotrophic cycle of 4.5 to 6 days for *Ae. albopictus* (Gubler and Bhattacharya 1971, Mori and Wada 1977).

**Statistical Analysis**

Data from the indoor ASMO evaluation were analyzed to determine the effectiveness of the ASMO to mark mosquitoes and the influence of marking dusts on the DMR. The hypothesis that the mean number of mosquitoes marked for each treatment was equal was tested using a two-way Analysis of Variance (ANOVA) in JMP® Version 8 (SAS Institute 2009). In addition, the DMR for each treatment was
examined using the two-way ANOVA. The Tukey-Kramer Honestly Significant Difference (HSD) multiple means comparison was completed to examine differences among the treatment means. Means were considered significantly different if the p-values were below 0.05 for each pairwise comparison.

The outdoor screened enclosure ASMO evaluation data were analyzed to determine the influence of marking dusts on dispersal distances. Treatment MDTs were compared using a one-way ANOVA statistical test and the Tukey-Kramer HSD multiple means comparison in JMP® Version 8 (SAS Institute 2009).

The field study examined the distance traveled by naturally occurring female *Ae. albopictus* and *Ae. triseriatus* following visitation to an ASMO. The MDT was calculated for those mosquitoes captured in the first four-day period following an initial color placement during each of the six replicates (n = 12). In addition, the MDT during a 20-day period was calculated for each of the six replicates (n = 12). In order to account for unequal sticky trap distribution and sticky trap loss, the MDT was calculated according to Lillie et al. (1981): 

\[ MDT = \frac{\sum (EC \times median \text{ distance of annulus}) \text{ for all annuli}}{total \ EC} \]  

where the estimated captures (EC) are the number of captures that would be expected if the trap density was equal in each annulus:

\[ EC = \frac{Number \ of \ observed \ marked \ captures \ per \ annulus}{Number \ of \ sticky \ ovitraps \ per \ annulus} \times CF \]  

where the correction factor (CF) was used to account for differences in trap densities among annuli:

\[ CF = \frac{area \ of \ annulus}{total \ trapping \ area} \times total \ number \ of \ sticky \ ovitraps \ in \ trapping \ area \]
A two-way ANOVA was completed in JMP® Version 8 (SAS Institute 2009) to determine whether or not the site and year affected the MDT. This comparison was conducted for the MDT within the first four days of each color placement and the overall 20-day MDT and was calculated for *Ae. albopictus* and *Ae. triseriatus* independently. Any differences in the MDTs were compared using a Student’s t-test. The maximum observed distance travelled (ODT$_{\text{max}}$) was determined as the linear distance from the ASMO to the most distant positive sticky ovitrap and was determined for each year.

Linear regression was conducted on the distance traveled by self-marked mosquitoes from the ASMO in relation to the trapping period in which the mosquito was subsequently captured. Linear regression analysis was conducted on the MDT data to determine the impact of distance dispersed during oviposition on mosquito population change. As such, for each mosquito species, the MDT of marked female mosquitoes within the 4-day capture period and the overall 20-day MDT was regressed upon the total number of marked and unmarked conspecific female mosquitoes captured at each site during each replication. A one-way ANOVA was completed to determine if the slope of the regression line was greater than zero to assess the relationship of MDT and population change using the statistical software JMP® Version 8 (SAS Institute 2009). The slope was considered significantly different from zero if the p-value was below 0.05. Linear regression analyses were performed for both field sites on the MDTs that were converted using the CF of Lillie et al. (1981) over the six replications.

**Results**

**ASMO Evaluation Studies**

Preliminary testing of the ASMO demonstrated a high rate of gravid mosquitoes being self-marked in a caged environment (Table 3-1). The average proportion of
mosquitoes marked was 81.3, 76.2, 78.5, and 74.6% when using ASMOs containing fluorescent dusts of the following colors red, blue, yellow, and green; respectively. No significant difference was found when comparing the average proportion of mosquitoes marked by each color. Fluorescent dusts had no significant impact on the daily mortality rate (Table 3-1).

In outdoor screened enclosure studies, 69, 72, 70, and 66% of the *Ae. albopictus* gravid females released were found to have self-marked with red, blue, yellow and green dusts, respectively, and subsequently captured in sticky ovitraps (Table 3-2). Thus, demonstrating that in a caged setting a majority of the mosquitoes visited multiple ovisites within one gonotrophic cycle. The MDT traveled, in meters, for the different colored dusts was 2.49 blue, 2.66 red, 2.47 yellow, 2.57 green and 2.49 for those mosquitoes exposed to an ASMO that contained no fluorescent dust. No significant difference was found in the distance traveled by mosquitoes marked with the different colored dusts and those not marked with a dust.

**Field studies**

During the summers of 2011 and 2012, 2,067 gravid *Ae. albopictus* and 501 gravid *Ae. triseriatus* were collected from sticky ovitraps (Table 3-3). The trends in the number of gravid females collected across the 2011 collection period differed from the patterns observed in 2012 (Figs. 3-5 and 3-6). Throughout the summer of 2011, the numbers of *Ae. albopictus* captured increased and peaked at 1.1 gravid females per trap in late August at the graveyard Site 2-1 (Fig. 3-5A). During this same time period at the University of Florida campus, Site 2-2, the number of captured *Ae. albopictus* remained at approximately 0.2 gravid females per trap throughout the sampling year.
(Fig. 3-6A). *Aedes triseriatus* captures peaked in late July and early August at Sites 2-1 and 2-2 in 2011.

In 2012, the number of gravid mosquitoes collected increased much earlier in the year than in 2011 (Fig. 3-5 and 3-6). In comparison to the 2011 collections, *Aedes albopictus* collections fluctuated at both sites throughout the 2012 sampling period peaking between 1 and 1.2 gravid mosquitoes per trap. At both sites in 2012, collections of *Ae. triseriatus* peaked in early July and then decreased to very few collected for the remainder of the sampling period.

Furthermore in 2012, *Tx. r. rutilus* eggs were regularly recovered in traps, whereas in 2011 only one collection of five *Tx. r. rutilus* eggs occurred. The average number of *Tx. r. rutilus* eggs per trap reached 4.6 at Site 2-1 and 2.4 at Site 2-2 in August and September of 2012, respectively. As collections of *Tx. r. rutilus* eggs increased, the numbers of gravid *Ae. albopictus* and *Ae. triseriatus* captured decreased.

Among the gravid mosquitoes collected, 37 *Ae. albopictus* and 17 *Ae. triseriatus* had visited an ASMO and were captured within four days of being self-marked (Table 3-3). The MDT in the first four days of being self-marked was 22.50 m and 31.33 m for gravid *Ae. albopictus* collected in 2011 and 2012, respectively. *Aedes triseriatus* had a MDT in the first four days of being self-marked of 56.11 m and 67.54 m in 2011 and 2012, respectively.

Within 20 days of being self-marked 153 gravid *Ae. albopictus* and 52 gravid *Ae. triseriatus* visited a second ovitrap and were captured (Table 3-3). The MDT for *Ae. albopictus* females within 20 days of being self-marked was significantly ($F_{1,8} = 6.95$, $P = 0.03$) different between 2011 (58.07 m) and 2012 (77.90 m). *Aedes triseriatus*, had a
MDT within 20 days of being self-marked of 83.85 m and 93.18 m in 2011 and 2012, respectively. The maximum observed distance traveled for self-marked mosquitoes was similar among the species and sites. The $ODT_{\text{max}}$ for both species was recorded in the farthest sampling annuli of 120-150 m.

A significant relationship between time and distance traveled was observed in self-marked and sticky ovitrap captured *Ae. albopictus* ($F_{1,153} = 299.92, P < 0.01$) and *Ae. triseriatus* ($F_{1,50} = 34.05, P < 0.01$) (Fig. 3-7). With each species, the greater the time from a self-marking event to subsequent capture, the greater the distance the mosquito traveled from the ASMO to the capture site.

A positive relationship was observed between conspecific mosquito population size and the distance traveled by captured self-marked mosquitoes (Fig. 3-8 and 3-9). As presented in these figures, an increasing slope documented an increase in marked mosquito captures as the overall conspecific mosquito population increased. As populations of *Ae. albopictus* increased the MDT during the 4-d post-marking period significantly ($F_{1,10} = 50.57, P < 0.01$) increased (Fig. 3-8A). The association of these two variables also was significant ($F_{1,6} = 27.56, P < 0.01$) for *Ae. triseriatus* during the 4-d post-marking period (Fig. 3-8B). During the 20-d post-marking period the relationship between the conspecific population increase and the MDT of marked mosquitoes was observed as significant for *Ae. albopictus* ($F_{1,10} = 31.75, P < 0.01$) but not for *Ae. triseriatus* (Fig. 3-9).

**Discussion**

The experiments in this study were designed to develop a technique that could determine the occurrence of *Ae. albopictus* skip oviposition in a field environment. Both
Ae. albopictus and Ae. triseriatus were self-marked and captured within a 4-d period, therefore, providing field-based evidence that these two species visit more than one oviposition site within a single gonotrophic cycle.

In the caged and open outdoor studies, Aedes albopictus responses measured as mark-capture percentages did not match, although in both studies, mosquitoes performed skip oviposition. In the caged arenas, 87% of captured Ae. albopictus released into the arena were found to have been self-marked and captured in a 24 h period. While in the field environment, only 40% of the captured Ae. albopictus within 10 m of the ASMO had been self-marked within a 4-d period mark and capture period. There are several reasons for this observed difference. The caged arena experiment had the gravid mosquitoes exposed to an ASMO with no other oviposition sites available before being exposed to the sticky ovitraps. In the field setting, competition from alternative oviposition sites diluted the impact of the ASMO and sticky ovitraps. Additionally, any unmarked mosquito that visited a sticky ovitrap was captured and thus removed from the population and not subject to marking. Finally, Aedes albopictus has demonstrated a preference for high-quality larval habitat when performing skip oviposition in laboratory experiments (Chapter 2). Therefore, alternative ovisites that varied in larval habitat quality may have influenced gravid mosquito ovisite selections.

In addition to examining skip oviposition, the field ovitrap results provided information on the population dynamics of three species Ae. albopictus, Ae. triseriatus and Tx. r. rutilus. The collections of gravid Ae. albopictus and Ae. triseriatus decreased with an increase in the number of Tx. r. rutilus eggs collected during 2012. However, in 2011 very few Tx. r. rutilus eggs were collected and collections of gravid Ae. albopictus
and *Ae. triseriatus* increased or remained stable throughout the sampling period. One hypothesis for this observation is that *Tx. r. rutilus* eggs were repellent to oviposition by *Ae. albopictus* and *Ae. triseriatus*. I was unable to find any examples of *Toxorhynchites* eggs affecting heterospecific mosquito oviposition behavior in the literature. However, the presence of predators such as fish (Angelon and Petranka 2002), tadpoles (Blaustein and Kotler 1993, Mokany and Shine 2003), and backswimmers (Blaustein et al. 2005) have resulted in mosquito oviposition avoidance. Another hypothesis is that *Tx. r. rutilus* populations had a suppressive effect on *Ae. albopictus* and *Ae. triseriatus* populations in 2012 as a result of predation. *Toxorhynchites r. rutilus*, in the larval stage, is predacious on other mosquito larvae and has been shown to control *Ae. aegypti* populations in Florida (Focks et al. 1980, Bailey et al. 1983). In 2011, the study area was under drought conditions (Keetch Byram Drought Index of 500-600) and this may be one factor in explaining the few *Tx. r. rutilus* eggs collected (Florida Forest Service 2013). In June of 2012, tropical storm Debby provided over 16 inches of rain to Alachua County where this study took place. This rain event may have provided the environmental conditions to stimulate both prey and predator populations. However, other environmental factors may have influenced the population dynamics of these three species.

In this study, it was found that marked mosquitoes dispersed greater distances from the self-marking ovisite as time progressed. This movement may be associated with behaviors associated with the seeking of ovisites, nectar meals, resting sites and blood meals. The work of Honorio et al. (2003) documented that laboratory reared and released gravid *Ae. albopictus* females distributed their eggs heterogeneously
throughout an environment and traveled over 800 m in six days during oviposition. In another study that marked and released laboratory reared and blood-fed Ae. albopictus, Marini et al. (2010) reported that in two of three trials the MDT from the release location increased as days after release increased. Our studies provide the first evidence that wild populations of Ae. albopictus disperse through the environment during the deposition of an egg batch and establishes an association that the distance traveled increases over time.

In addition to time affecting dispersal distance I found that population size also affects dispersal during oviposition. The number of gravid females collected per sticky trap is a representation of population size and a prediction of population dynamics. The positive association between population growth and increased dispersal may occur for several reasons. As populations increase competition for limited resources also may increase, specifically in the larval habitat, and result in density dependent larval mortality (Service 1985, Washburn 1995). Indirect and direct competition for access to larval nutritional resources has been shown to increase Ae. albopictus larval mortality, reduce adult size and female fecundity and trigger adult behavioral responses (Chan 1971, Mori 1979, Blackmore and Lord 2000, Yoshioka et al. 2012). Mori (1979) found Ae. albopictus larvae reared under crowded conditions dispersed further than those reared in uncrowded conditions. Aedes albopictus were found to perform more skip oviposition when presented low-quality habitats that contained larvae in crowded conditions than when provided high-quality larval habitats (Chapter 2). Therefore, during times when populations are high and competition for resources increases, ovipositing females may move greater distances seeking higher quality immature
habitats, as compared to the dispersal pattern when populations are low and habitats are more suitable.

The results of this study can be applied to the use of ovisites in surveillance programs. Ovipositing females that have acquired a vertebrate pathogen will disperse in the environment seeking suitable ovisites. These mosquitoes have the potential to transmit these pathogens to vertebrates after the extrinsic incubation period. Therefore, *Ae. albopictus* that have acquired a pathogen during a blood meal and are subsequently ovipositing will disperse further as time progresses. In addition, during times of high conspecific population densities these vectors may be induced to disperse further and have the potential to spread disease over a greater area than during times of low population densities. This density- and habitat-dependent behavior should be accounted for when completing vector surveillance, mosquito population management decisions, and assessment of pathogen transmission risk. Similarly, in assessing St. Louis encephalitis (SLE) virus transmission by *Culex nigripalpus* Theobald in Florida, Day et al. (1990) reported that this mosquito changed its behavioral response to environmental factors that resulted in the subsequent facilitation of pathogen transmission. Day et al. (1990) found that parous *Cx. nigripalpus* were positively associated with time and that gravid females were negatively associated with daily rainfall, especially during years in which SLE was detected with increased frequency. A periodic cycling of larval habitat quality (high-quality habitat - low-quality habitat - high-quality habitat) was observed to lead to enhanced SLE viral amplification and transmission; and the modeling of these habitat qualities assisted in predicting SLE risk (Day and Curtis 1994).
Our study results also can be applied to the use of ovisites as control devices. Devices have been developed to contaminate a skip ovipositing mosquito with an IGR so that the mosquito will transfer the chemical to other ovisites (Caputo et al. 2012, Gaugler et al. 2012). In order to maximize effectiveness, the placement of IGR treated ovisites in the environment should take into account *Ae. albopictus* population densities. During times of high population densities, ovipositing *Ae. albopictus* will disperse over greater distances and treated ovisites could be placed further apart. As opposed to the placement spacing of IGR-treated ovisites when *Ae. albopictus* populations are at low population densities. However, the exact placement distance of IGR treated ovisites may depend on other factors, such as suitable resting sites, nectar source availability and competing ovisites. Further studies that examine the effectiveness of IGR treated ovisites when employed at different spatial placements in association with *Ae. albopictus* population densities would improve the successful application of autodissemination control techniques.
Table 3-1. The average proportion of *Aedes albopictus* (Skuse) gravid females were self-marked by an Attractive Self-Marking Ovitrap and their average daily mortality rates (DMR).

<table>
<thead>
<tr>
<th>Dust Color</th>
<th>% Marked ± SEM</th>
<th>DMR ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>81.3 ± 1.34</td>
<td>24.2 ± 8.48</td>
</tr>
<tr>
<td>Blue</td>
<td>76.2 ± 3.33</td>
<td>24.9 ± 9.17</td>
</tr>
<tr>
<td>Yellow</td>
<td>78.5 ± 3.06</td>
<td>24.1 ± 9.11</td>
</tr>
<tr>
<td>Green</td>
<td>74.6 ± 3.20</td>
<td>24.7 ± 8.17</td>
</tr>
<tr>
<td>No Dust</td>
<td>0</td>
<td>25.7 ± 8.74</td>
</tr>
</tbody>
</table>

Cages (61 x 61 x 61 cm) held in the laboratory at room temperature. DMR was calculated as the number of deaths during a 24 h interval divided by the number of individuals alive during the start of that interval times 100.

1 No significant differences among the marking treatments, ANOVA $F_{3,48} = 1.04$, $P > 0.40$.
2 No significant differences, ANOVA $F_{4,60} = 0.30$, $P > 0.83$. 
Table 3-2. The average proportion of *Aedes albopictus* (Skuse) gravid females that were captured using sticky ovitraps following exposure to an Attractive Self-Marking Ovitrap and their mean distance traveled (MDT).

<table>
<thead>
<tr>
<th>Dust</th>
<th>Color</th>
<th>% Marked and Captured ± SEM</th>
<th>MDT ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td></td>
<td>69 ± 5.99</td>
<td>2.49 ± 0.16</td>
</tr>
<tr>
<td>Blue</td>
<td></td>
<td>72 ± 4.63</td>
<td>2.66 ± 0.22</td>
</tr>
<tr>
<td>Yellow</td>
<td></td>
<td>70 ± 4.18</td>
<td>2.47 ± 0.24</td>
</tr>
<tr>
<td>Green</td>
<td></td>
<td>66 ± 6.58</td>
<td>2.57 ± 0.14</td>
</tr>
<tr>
<td>No Dust</td>
<td></td>
<td>0</td>
<td>2.49 ± 0.16</td>
</tr>
</tbody>
</table>

Mosquitoes self-marked in a small cage (61 x 61 x 61 cm), then released into a screenhouse (18.3 x 9.2 x 4.9 m (l/w/h), peaking at 6.1 m along the length of the cage ceiling), Gainesville, FL.

1 No significant differences among the marking treatments, ANOVA $F_{3,19} = 0.21$, $P > 0.88$.

2 No significant differences, ANOVA $F_{4,16} = 0.23$, $P > 0.91$. 
Table 3-3. Number of gravid female *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say) that were captured in sticky ovitraps at a graveyard and on the University of Florida campus in Gainesville, FL. Mosquitoes were self-marked by visiting an Attractive Self-Marking Ovitrap before being captured in sticky ovitraps during the period June 5 through September 5, 2011 and 2012.

<table>
<thead>
<tr>
<th></th>
<th><em>Ae. albopictus</em></th>
<th><em>Ae. triseriatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total captured</td>
<td>2,067</td>
<td>501</td>
</tr>
<tr>
<td>Self-marked/captured in first 4 days</td>
<td>37</td>
<td>17</td>
</tr>
<tr>
<td>Self-marked/captured in 20 days</td>
<td>155</td>
<td>52</td>
</tr>
<tr>
<td>MDT in first 4 days 2011</td>
<td>22.50</td>
<td>56.11</td>
</tr>
<tr>
<td>MDT in first 4 days 2012</td>
<td>31.33</td>
<td>67.54</td>
</tr>
<tr>
<td>MDT in 20 days 2011</td>
<td>58.07</td>
<td>83.85</td>
</tr>
<tr>
<td>MDT in 20 days 2012</td>
<td>77.90</td>
<td>93.18</td>
</tr>
<tr>
<td>ODT&lt;sub&gt;max&lt;/sub&gt; 2011</td>
<td>149</td>
<td>148</td>
</tr>
<tr>
<td>ODT&lt;sub&gt;max&lt;/sub&gt; 2012</td>
<td>149</td>
<td>148</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean distance traveled (MDT) in the first four days is measured in meters and the distances traveled during each year per species were not different.

<sup>b</sup>Mean distance traveled (MDT) in a 20 day period is measured in meters and the distances traveled during each year for *Ae. albopictus* were significantly ($F_{1,8} = 6.95$, $P = 0.03$) different but were not different for *Ae. triseriatus*.

<sup>c</sup>Maximum observed distance traveled (ODT<sub>max</sub>) is measured in meters.
Figure 3-1. An attractive self-marking ovitrap (ASMO) and sticky ovitrap used to monitor *Aedes albopictus* (Skuse) skip oviposition. A) ASMO with the lid attached. B) ASMO with the lid removed to display the fluorescent dust coated interior. C) Sticky ovitrap with lid removed to display sticky panels and captured mosquitoes. Photos courtesy of Timothy J. Davis.
Figure 3-2. *Aedes albopictus* (Skuse) females exposed to an attractive self-marking ovitrap. A) Mosquitoes examined under fluorescent light. B) Mosquitoes examined under ultraviolet light. Photos courtesy of Timothy J. Davis.
Figure 3-3. *Aedes albopictus* (Skuse) females exposed to an attractive self-marking ovitrap inside a 61 x 61 x 61 cm cage and released into a large outdoor enclosure (9.2 m wide x 18.3 m long x 4.9 m high, peaking at 6.1 m along the length of the cage ceiling). Surrounding the small cage are 16 sticky ovitraps placed every 3.14 m² in four 1 m concentric rings to collect mosquitoes performing skip oviposition. Photo courtesy of Timothy J. Davis.
Figure 3-4. Aerial images of the two field sites used to evaluate *Aedes albopictus* (Skuse) skip oviposition located in Gainesville, FL. Each field site had 50 sticky ovitraps (represented by red dots) located in 30 m concentric rings originating from an attractive self-marking ovitrap. A) A graveyard. B) The University of Florida campus. Maps were based on screenshots from Google Earth Mapping Service (http://earth.google.com).
Figure 3-5. Mean number of gravid *Aedes albopictus* (Skuse) adults, *Aedes triseriatus* (Say) adults and *Toxorhynchites rutilus rutilus* (Coquillett) eggs per sticky ovitrap at a graveyard in Gainesville, FL. A) Collections were completed at 4-d intervals from June 5 until September 5, 2011. B) Collections were completed at 4-d intervals from June 5 until September 5, 2012.
Figure 3-6. Mean number of gravid *Aedes albopictus* (Skuse) adults, *Aedes triseriatus* (Say) adults and *Toxorhynchites rutilus rutilus* (Coquillet) eggs per sticky ovitrap at the University of Florida campus, Gainesville, FL. A) Collections were completed at 4-d intervals from June 5 until September 5, 2011. B) Collections were completed at 4-d intervals from June 5 until September 5, 2012.
Figure 3-7. Linear regression of the captured distance of marked gravid female mosquitoes in relation to five successive four day trapping periods during 2011 and 2012, in Gainesville, FL. Data points above a trapping period represent mosquitoes marked during an initial 4-d period, and subsequently captured within the first period or over four, 4-d post marking trapping periods. A) *Aedes albopictus* (Skuse). B) *Aedes triseriatus* (Say).

\[ y = 24.209x - 1.724 \]
\[ R^2 = 0.6622 \]
ANOVA \( F_{1,153} = 299.92, P < 0.01 \)

\[ y = 17.584x + 55.032 \]
\[ R^2 = 0.4051 \]
ANOVA \( F_{1,50} = 34.05, P < 0.01 \)
Figure 3-8. Linear regression of the mean distance traveled (MDT) of marked mosquitoes that were captured in the first four days in relation to the mean number captured (marked and unmarked) per sticky ovitrap (ST) in 2011 and 2012 in Gainesville, FL. A) *Aedes albopictus* (Skuse). B) *Aedes triseriatus* (Say).

A

\[ y = 77.521x - 6.5546 \]
\[ R^2 = 0.8349 \]

ANOVA \( F_{1,10} = 50.57, P < 0.01 \)

B

\[ y = 215.51x + 33.74 \]
\[ R^2 = 0.8212 \]

ANOVA \( F_{1,6} = 27.56, P < 0.01 \)
Figure 3-9. Linear regression of the mean distance traveled (MDT) of marked mosquitoes that were captured in captured over five 4-day periods in relation to the mean number captured (marked and unmarked) per sticky ovitrap (ST) in 2011 and 2012 in Gainesville, FL. A) Aedes albopictus (Skuse). B) Aedes triseriatus (Say).
CHAPTER 4
ASSESSMENT OF *Aedes albopictus* CLUTCH SIZE IN WILD AND LABORATORY POPULATIONS

**Introduction**

The intrinsic rate of natural increase is an important parameter in management models for insect pest populations and is determined in part by measuring the egg production of a population (Southwood 1978). Many factors influence an insect’s ability to produce eggs, including the organism’s genetics, biotic factors, and abiotic factors (Mayr 1961). Of these factors, studies have determined that adult size, gonotrophic age, blood meal size, and blood meal source influence mosquito fecundity (Gubler 1970, Chan 1971, Mori 1979, Grimstad and Haramis 1984, Hawley 1985, Briegel 1990, Lounibos et al. 1990, Blackmore and Lord 2000).

Under optimal laboratory conditions, *Aedes albopictus* (Skuse), the Asian tiger mosquito, averages 42 to 88 eggs per blood meal for the first gonotrophic cycle and has an average lifetime fecundity of 300-345 eggs (Hawley 1988). Laboratory studies have found that limiting larval food will reduce adult size (Mori 1979) and reduced size is correlated with lowered egg production (Blackmore and Lord 2000). Populations of *Ae. albopictus* that develop in natural conditions may be influenced by the availability of larval resources and several other factors that could affect fecundity. Despite the potential influence of numerous variables on wild *Ae. albopictus* egg production, Hawley’s (1988) review of the Asian tiger mosquito’s biology notes that studies have failed to directly examine the number of eggs laid in wild populations.

A batch of eggs is the full complement of eggs matured by a female mosquito during an ovarian cycle and a clutch of eggs is the number of eggs oviposited at a single site (Clements 1999). Skip oviposition has been defined as the behavior in which
a mosquito distributes a batch of eggs singly in several clutches over multiple containers (Mogi and Mokry 1980). *Aedes albopictus* has been found to perform skip oviposition in a laboratory study (Trexler et al. 1998). Researchers conducting surveillance for the eggs of wild populations of *Ae. albopictus* using ovitraps exposed for 24 hour periods have found that 50% of the traps contained fewer than 25 eggs and 22% had fewer than 10 eggs (Rozeboom et al. 1973). Because the observed number of *Ae. albopictus* eggs from these containers was less than the average batch size of laboratory reared *Ae. albopictus*, this species has been cited as conducting skip oviposition (Hawley 1988). However, a measure of the clutch size laid by individual mosquitoes from wild populations has not been completed. Such a study would allow for a proper comparison between field egg production capability and the surveillance data that associates skip oviposition with the number of eggs collected.

We measured the clutch size of wild populations of *Ae. albopictus* in order to obtain a more accurate parameter of the number of eggs laid in a given gonotrophic cycle. Collections of wild, previously fed Asian tiger mosquitoes were completed and the numbers of eggs deposited by individual mosquitoes was subsequently compared to the size of the mosquito. An assessment of oviposition differences between wild caught and laboratory reared *Ae. albopictus* was conducted.

**Materials and Methods**

**Mosquito Field Site Collections and Testing**

Mosquitoes were collected weekly from the third week in July until the end of September in 2011 and 2012. Collections took place at four suburban locations in Gainesville, FL (Appendix A). An aspirator, which employs a large fan to funnel insects into a mesh catch bag, was used to collect mosquitoes. The aspirator was built at the
United States Department of Agriculture Center for Medical Agricultural and Veterinary Entomology (USDA-CMAVE) as described in Ponlawat and Harrington (2005). Each sampling event consisted of three aspiration periods per site. An aspiration period involved aspirating foliage for five minutes. Only foliage in well-shaded areas was sampled. The collected mosquitoes were transported to the USDA-CMAVE, Gainesville, FL and examined on a chill table to determine species and blood fed status. *Aedes albopictus* with an enlarged red abdomen were considered blood fed.

The wild-caught blood fed *Ae. albopictus* were placed individually in 30 x 30 x 30 cm cages consisting of PVC pipe frame (2.54 cm diameter) and screening (18 x 14 mesh) (Fig. 4-1). Within each cage an ovisite consisting of a 473 mL black cup lined with seed germination paper and containing 200 mL of oak leaf infusion water was placed. The oak leaf infusion was formulated by adding 150 g of fallen live oak (*Quercus virginiana* Miller) leaves to 15 liters of well water in a container that was sealed, placed outside and allowed to ferment for seven days, as modified from Allan and Kline (1995). An individual flowering stem from a butterfly bush (*Buddleja davidii* Franchett cultivar ‘Guinevere’) was placed inside each cage as a carbohydrate food source. The flowering stems consisted of a flower spike with 3-5 leaves that was trimmed to weigh 2-3 g. The cutting was placed in a floral water tube pick (Panacea, Columbus, OH). Cages were held outdoors in a covered screened enclosure at the USDA-CMAVE in order to replicate local climatic conditions.

Females were allowed to oviposit for 15 days, after which they were frozen and subsequently examined later for retained eggs and wing length measurement. All females were examined for retained eggs by dissection and fully developed eggs were
assessed and recorded according to Christophers (1911) and Clements (1963). Those females that did not deposit any eggs were dissected and examined for egg development and the numbers of fully developed eggs recorded. Oviposited eggs were enumerated by removing the seed germination paper from the ovisite and counting the number of eggs under a stereo microscope. In addition, the ovisite infusion water was removed and examined for eggs that had been laid on the water surface. Wing lengths were recorded according to Lounibos et al. (1990) by measuring from the base of the costa to the wing margin, exclusive of fringes (Fig. 4-2). Measurements were completed by using a Keyence VHX-600E digital microscope (Keyence Corporation, Osaka, Japan).

**Laboratory Colony Rearing and Testing**

The laboratory colony of *Ae. albopictus* described in Chapter 2 was used as a comparison of clutch size. Eggs were hatched from the same generation within a replication (F5 - F14). Larvae were reared at five densities: 1 larva per 3 mL, 3 larvae per 3 mL, 5 larvae per 3 mL, 7 larvae per 3 mL, and 9 larvae per 3 mL. Each density was provided 0.5 g of ground TetraFin™ fish food per 1,000 mL initially and thereafter, every 48 hours. Upon pupation the mosquitoes were transferred to individual 46 x 46 x 46 cm screened cages based on rearing density and provided 5% sugar solution as a carbohydrate source. Cages were kept for seven days in a rearing room with a temperature of 25 ± 1°C, 75 ± 5% relative humidity, and 12:12 (L:D) photoperiod. After 10 days, the mosquitoes were provided a bovine blood-filled sausage and allowed to feed. Females that had a red swollen abdomen were transferred and placed individually into 30 x 30 x 30 cm cages. Cages were prepared in the same manner as
described for the wild-caught females. Females were allowed 15 days to oviposit and were examined in the same manner as the wild-caught females.

**Statistical Analysis**

A one-way analysis of variance (ANOVA) was conducted individually by mosquito source (wild-caught and laboratory-reared) to test the following hypotheses: 1) the mean clutch size was equal or alternatively not equal, 2) the mean eggs laid + eggs retained was equal or not equal, 3) the mean wing length was equal or not equal. For analysis of wild-caught mosquitoes, the four collection sites were considered fixed effects. For laboratory-reared mosquitoes, fixed effect variable was larval rearing density. In both analyses the dependent variables consisted of mean clutch size, eggs laid + retained and wing length. Tukey-Kramer Honestly Significant Difference (HSD) tests were completed to compare site effects and density effects for the respective source. Means were considered significantly different if the p-values were below 0.05 for each pairwise comparison. All statistical procedures were performed using the JMP® Version 8 software (SAS Institute 2009).

Linear regression analysis was utilized to determine if the relationship between the rearing density and wing length, rearing density and clutch size, rearing density and eggs laid + eggs retained, wing length and clutch size of laboratory reared females, wing length and eggs laid + eggs retained of laboratory reared females, wing length and clutch size of wild-caught females and wing length and eggs laid + eggs retained of wild-caught females. The regression functions of clutch size and wing length of the wild-caught and laboratory colony were compared by analyses of covariance (ANCOVA) according to Nasci (1990). All statistical procedures were performed using the JMP® Version 8 software (SAS Institute 2009).
Results

The total number of mosquitoes collected by aspiration was 9,988 (Table 4-1). The five most common species aspirated were *Ae. albopictus, Aedes infirmatus* Dyar and Knab, *Aedes vexans* (Meigen), *Culex nigripalpus* Theobald and *Psorophora ferox* (Humboldt). The numbers of *Ae. albopictus* collected were 483 at Site 4-1, 196 at Site 4-2, 113 at Site 4-3 and 197 at Site 4-4. Of the wild-caught *Ae. albopictus*, 90 were visibly blood-fed and thereafter developed eggs when placed in oviposition cages.

For wild-caught *Ae. albopictus* mean clutch size (F$_{3,86}$ = 10.36, P < 0.01), eggs laid + eggs retained (F$_{3,86}$ = 12.56, P < 0.01) and wing length (F$_{3,86}$ = 34.02, P < 0.01) were significantly different among collection sites (Table 4-2). Female *Ae. albopictus* from Site 4-2 had the smallest mean clutch size of 23 eggs and the smallest average wing length of 1.99 mm. Clutch sizes were similar for females originating from Sites 4-1, 4-3, and 4-4 with means of 46 eggs, 38 eggs and 43 eggs, respectively. Additionally, the wing lengths for *Ae. albopictus* collected from Sites 4-1, 4-3, and 4-4 were similar with mean lengths of 2.51 mm, 2.47 mm and 2.54 mm, respectively.

The *Ae. albopictus* reared in the laboratory at differing larval densities were found to significantly differ in mean clutch size (F$_{4,95}$ = 80.59, P < 0.01), number of eggs laid + eggs retained (F$_{4,95}$ = 83.50, P < 0.01) and wing length (F$_{4,95}$ = 44.30, P < 0.01) (Table 4-2). In these comparisons, each variable differed from one another with the exception of the two high-density treatments that had the smallest values. The mosquitoes reared at the lowest density of 1 larva per 3 mL of water had the largest clutch size, the largest number of eggs laid + eggs retained and the longest wings with means of 83 eggs, 84 eggs and 2.75 mm, respectively. The females reared at both 7 and 9 larvae per 3 mL of water had the smallest mean clutch sizes with 25 eggs and 20 eggs laid, respectively.
The *Ae. albopictus* reared at the two highest densities also had the shortest wing lengths of 2.23 mm and 2.04 mm. A negative correlation between rearing density and the following three measures: clutch size ($F_{1,98} = 266.69, P < 0.01$), the number of eggs laid + eggs retained ($F_{1,98} = 278.63, P < 0.01$) and the wing length ($F_{1,98} = 179.28, P < 0.01$) was observed (Figs. 4-3, 4-4).

An association was observed between wing length and the number of eggs developed by *Ae. albopictus* laboratory and field populations. Colony mosquitoes reared at different larval densities had a positive correlation between wing length and clutch size ($F_{1,98} = 167.78, P < 0.01$) and wing length and number of eggs laid + eggs retained ($F_{1,98} = 176.12, P < 0.01$) (Fig. 4-5). In addition, wild caught mosquitoes were found to have a positive correlation between wing length and clutch size ($F_{1,88} = 30.18, P < 0.01$) and wing length and number of eggs laid + eggs retained ($F_{1,88} = 37.54, P < 0.01$) (Fig. 4-6). However, the regression coefficients were significantly different ($F_{1,101} = 14.12, P < 0.01$) between the wild caught and the laboratory reared populations. The relationship of wing size and clutch size in the laboratory population (slope = 68.50) increased at a greater amount compared with the wild-caught females (slope = 29.40).

**Discussion**

The wild-caught *Ae. albopictus* that were captured at four residential sites were found to vary between collection sites for both clutch size and wing length. A possible explanation for the size difference is that interspecific competition affected *Ae. albopictus* larval production in the tank bromeliads that were abundant at Site 4-2. While a formal survey of larval habitats was not accomplished at the field sites, Site 4-2 was visually assessed to have many more bromeliads than the other sites. The production of *Ae. albopictus* from bromeliad phytotelmata has been found to be
regulated by *Wyeomyia* mosquitoes (O’Meara et al. 1995, Lounibos et al. 2003). Site 4-2 was the only site to have *Wyeomyia mitchellii* (Theobald) present in aspiration collections, providing further support for interspecific competition-induced effects on reproductive capacity. Microorganisms associated with the decay of leaf litter in tank bromeliads provide a food source for developing mosquito larvae (Frank and Lounibos 1983) and the growth of *Ae. albopictus* first instars is negatively affected by competition with *Wyeomyia* spp. fourth instars for this larval food (Lounibos et al. 2003). Such species interaction may account for the reduced egg production and wing sizes of Site 4-2 *Ae. albopictus*, however, other factors may have influenced Asian tiger mosquito developmental success at this site.

One factor that may affect the number of eggs an *Ae. albopictus* will produce is gonotrophic age. Chan (1971) found that the number of eggs produced per gonotrophic cycle decreased as the number of gonotrophic cycles increased. In contrast, Gubler and Bhattacharya (1971) found the number of eggs produced did not decrease in subsequent gonotrophic cycles for a strain of *Ae. albopictus* from Calcutta, India. Nonetheless, an objective of this study was to assess average clutch size of wild-caught *Ae. albopictus* populations in order to relate this measure to evidence of skip oviposition. Therefore, the comparison of *Ae. albopictus* clutch sizes should include a representation of the native mosquitoes, to encompass the wild population gonotrophic age distribution, especially when associations are made to field collections.

Egg collections of *Ae. albopictus* field populations have found small numbers of eggs per ovisite (Rozeboom et al. 1973). In this study, Site 4-2 had the lowest average clutch size of 23 eggs. Rozeboom et al. (1973) found 22% of the ovitraps to contain
less than 10 eggs. Only 10% of the 20 wild-caught females from site 4-2 developed fewer than 10 eggs. Therefore, the use of egg collection measures in comparison to laboratory egg production as evidence of skip oviposition may be a valid approach. However, the evidence provided by my study indicates that variations do exist in field population egg production and these fluctuations should be considered when assessing egg production.

Density-dependent effects have been found to impact container-inhabiting mosquito populations (Service 1985, Washburn 1995) and my results found a negative correlation between *Ae. albopictus* larval rearing density and egg clutch size in laboratory studies (Fig. 4-3A). Competition in the larval stage can be used to explain the variations in field population fecundities we observed. Mosquitoes from Site 4-1, 4-2, 4-3 and 4-4 had mean clutch sizes of 46, 23, 38 and 43 eggs, respectively; which were within the clutch size range of mosquitoes reared at larval densities of 9 larvae/3 mL of water (20 eggs) and 3 larvae/3 mL of water (53 eggs). None of the sample sites had an *Ae. albopictus* mean clutch size that was within the mean clutch size range of the mosquitoes reared at the two least crowded larval densities: 3 larvae/3 mL of water (53 eggs) and 1 larva/3 mL of water (83 eggs). These results indicate that *Ae. albopictus* from the sample sites are encountering density dependent conditions similar to what was generated during our rearing conditions of 3 larvae/3 mL of water or higher. Therefore, our indirect and comparative measures suggest that density dependent effects in the larval stage may have limited the egg production of field populations at the sites we sampled. In a similar study, *Aedes cantans* (Meigen) that developed in ponds with high conspecific densities had smaller egg clutches than those mosquitoes that
developed in ponds of low larval densities (Renshaw et al. 1994). Likewise, wild populations of *Wyeomia smithii* Coquillet were found to have a negative correlation of larval density and fecundity (Lounibos et al. 1982).

Another effect of density dependence on container-inhabiting mosquitoes is a reduction in body size (Livdahl 1982, Carpenter 1983, Broadie and Bradshaw 1991, Bradshaw and Holzapfel 1992). Body size in mosquitoes is often measured as wing length (Christophers 1960, Nasci 1990). Our findings from laboratory-reared mosquitoes found a negative correlation of rearing density and wing length (Fig. 4-3C). In a related study, Nasci (1990) reported that field collected *Ae. albopictus* were significantly smaller than mosquitoes reared under the uncrowded conditions of 0.75 larvae/3 mL of water with sufficient larval food. To further demonstrate that field populations experience density dependent effects, the laboratory findings can be applied to the results obtained from the wild caught mosquitoes. Mosquitoes from Site 4-1, 4-2, 4-3 and 4-4 had mean wing lengths of 2.51, 1.99, 2.47, and 2.54 mm respectively; which were less than the mean wing length of mosquitoes reared at larval densities of 3 larvae/3 mL of water (2.57 mm). These results provide further evidence that the mosquitoes collected from the field sites were experiencing density dependent effects similar to the laboratory larvae reared at 3 larvae/mL of water or higher larval density.

The congruence of the wing-length and egg production data documents an important correlation between the sampled field site and colony populations. Mori (1979) found that when *Ae. albopictus* were reared at high larval densities, the subsequent adults had reduced size and fecundity when compared to those larvae
reared at lower densities with the same level of food. Similarly, Blackmore and Lord (2000) found a positive and significant correlation in wing-length and reproductive capacity in *Ae. albopictus* reared at a range of larval densities. However, in our studies the colony population demonstrated a greater increase in egg production as wing length increased with a slope of 68.5 as compared to the correlation in field population egg production to wing-length measurements that had a slope of 29.4. Therefore, the wild *Ae. albopictus* sampled in this study may have experienced additional factors beyond larval population density that affected the association of size and fecundity. Larval food sources have been found to impact container-inhabiting mosquito size and reproduction (Bradshaw and Holzapfel 1983, Yee and Juliano 2006). *Aedes triseriatus* (Say) larvae that were reared with animal detritus (dead adult fruit flies and crickets) developed into larger adults than those larvae that developed with plant detritus (sugar maple and white oak leaves) (Yee and Juliano 2006). However, the authors were unable to determine the mechanism associated with benefits of animal detritus and hypothesized that larger adults were either an indirect result of greater larval food production through increased microorganism growth or a direct result of animal detritus ingestion. *Aedes albopictus* larvae were reported to have preferentially fed in areas of a container that had animal detritus when compared to locations that included plant detritus (Kesavaraju et al. 2007). The *Ae. albopictus* developing in the sampled field sites may have experienced larval habitats that primarily contained plant-based detritus. Our laboratory-reared larvae were fed ground TetraFin™ fish food that has a minimum crude protein level of 42% and contains fish meal as the primary ingredient, a form of animal detritus. If the laboratory reared *Ae. albopictus* directly consumed the animal
animal detritus then they may have had improved nutritional quality that lead to a greater increase in egg production as wing length increased compared to the field collected mosquitoes. In addition, parasitism by the gregarine, *Ascogregarina taiwanensis* (Lien and Levine), in combination with reduced nutrition also has been found to reduce *Ae. albopictus* wing length and egg production (Comiskey et al. 1999). *Aedes albopictus* parasitism by *A. taiwanensis* does occur in north central Florida, however, the parasitism rate fluctuates throughout the summer season (Becnel pers. comm.). Further research in the effects of density dependence on *Ae. albopictus* population dynamics should focus on the interactions of crowding, nutrition, and parasitism in field populations. Likewise, Legros et al. (2009) has made a similar call for further understanding density dependent effects in *Ae. aegypti*. 
Table 4-1. The total number of mosquitoes collected using a large aspirator at four residential locations in Gainesville, FL.

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Collection site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-1</td>
</tr>
<tr>
<td>Aedes albopictus</td>
<td>483</td>
</tr>
<tr>
<td>Ae. canadensis</td>
<td>43</td>
</tr>
<tr>
<td>Ae. infirmatus</td>
<td>711</td>
</tr>
<tr>
<td>Ae. sollicitans</td>
<td>8</td>
</tr>
<tr>
<td>Ae. triseriatus</td>
<td>17</td>
</tr>
<tr>
<td>Ae. vexans</td>
<td>286</td>
</tr>
<tr>
<td>Culex erraticus</td>
<td>12</td>
</tr>
<tr>
<td>Cx. nigripalpus</td>
<td>819</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>153</td>
</tr>
<tr>
<td>Psorophora columbiae</td>
<td>28</td>
</tr>
<tr>
<td>Ps. ferox</td>
<td>927</td>
</tr>
<tr>
<td>Wyeomyia mitchellii</td>
<td>0</td>
</tr>
</tbody>
</table>

Mosquitoes were collected weekly from the third week in July until the end of September in 2011 and 2012.
Table 4.2. The mean clutch size, number of eggs laid + eggs retained and wing length of *Aedes albopictus* (Skuse) females either laboratory reared at different larval densities or collected as blood-fed adults from four residential locations in Gainesville, FL.

<table>
<thead>
<tr>
<th>Source</th>
<th>n</th>
<th>Clutch size ± SEM¹</th>
<th>Eggs laid + eggs retained ± SEM²</th>
<th>Wing length (mm) ± SEM³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field collection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 4-1</td>
<td>30</td>
<td>46 ± 3 a</td>
<td>47 ± 3 a</td>
<td>2.51 ± 0.20 a</td>
</tr>
<tr>
<td>Site 4-2</td>
<td>20</td>
<td>23 ± 3 b</td>
<td>23 ± 3 b</td>
<td>1.99 ± 0.24 b</td>
</tr>
<tr>
<td>Site 4-3</td>
<td>18</td>
<td>38 ± 2 a</td>
<td>39 ± 2 a</td>
<td>2.47 ± 0.04 a</td>
</tr>
<tr>
<td>Site 4-4</td>
<td>22</td>
<td>43 ± 3 a</td>
<td>43 ± 3 a</td>
<td>2.54 ± 0.04 a</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F₃,₈₆ = 10.36 P &lt; 0.01</td>
<td>F₃,₈₆ = 12.56 P &lt; 0.01</td>
<td>F₃,₈₆ = 34.02 P &lt; 0.01</td>
</tr>
<tr>
<td>Laboratory reared</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 larva/3 mL</td>
<td>20</td>
<td>83 ± 4 a</td>
<td>84 ± 4 a</td>
<td>2.75 ± 0.03 a</td>
</tr>
<tr>
<td>3 larvae/3 mL</td>
<td>20</td>
<td>59 ± 3 b</td>
<td>60 ± 3 b</td>
<td>2.57 ± 0.04 b</td>
</tr>
<tr>
<td>5 larvae/3 mL</td>
<td>20</td>
<td>39 ± 3 c</td>
<td>39 ± 3 c</td>
<td>2.35 ± 0.05 c</td>
</tr>
<tr>
<td>7 larvae/3 mL</td>
<td>20</td>
<td>25 ± 2 d</td>
<td>25 ± 2 d</td>
<td>2.23 ± 0.04 c</td>
</tr>
<tr>
<td>9 larvae/3 mL</td>
<td>20</td>
<td>20 ± 2 d</td>
<td>20 ± 2 d</td>
<td>2.04 ± 0.05 d</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F₄,₉₅ = 80.59 P &lt; 0.01</td>
<td>F₄,₉₅ = 80.59 P &lt; 0.01</td>
<td>F₄,₉₅ = 44.30 P &lt; 0.01</td>
</tr>
</tbody>
</table>

Field collections occurred from the third week in July until the end of September in 2011 and 2012.

¹The mean clutch sizes were compared separately by source using Tukey-Kramer HSD multiple means comparisons. Means followed by the same letter are not significantly different (α = 0.05) for the reared mosquitoes and the field collected mosquitoes.

²The mean eggs laid + developed eggs retained were compared separately by source using Tukey-Kramer HSD multiple means comparisons. Means followed by the same letter are not significantly different (α = 0.05) for the reared mosquitoes and the field collected mosquitoes.

³The mean wing lengths were compared separately by source using Tukey-Kramer HSD multiple means comparisons. Means followed by the same letter are not significantly different (α = 0.05) for the reared mosquitoes and the field collected mosquitoes.
Figure 4-1. A 30 x 30 x 30 cm cage in which an individual *Aedes albopictus* (Skuse) was placed to complete oviposition. Inside the cage was a 473 mL black cup lined with seed germination paper that contained 200 mL of oak leaf infusion water. A cutting from a butterfly bush (*Buddleja davidii* Franchett cultivar ‘Guinevere’) was placed inside each cage as a carbohydrate food source. Cages were held outdoors in a covered screened enclosure in order to replicate local climatic conditions. Photo courtesy of Timothy J. Davis.
Figure 4-2. *Aedes albopictus* (Skuse) wing lengths were measured from the base of the costa to the wing margin, exclusive of fringes, using the Keyence VHX-600E digital microscope (Keyence Corporation, Osaka, Japan). Photo courtesy of Timothy J. Davis.
Figure 4-3. Linear regression analysis of emergent adult *Aedes albopictus* (Skuse) adult clutch size, number of eggs laid + eggs retained, and wing length in relation to larval rearing density. A) Clutch size. B) The number of eggs laid + eggs retained. C) Wing length.

**A**

\[ y = -8.07x + 85.6 \]

\[ R^2 = 0.7313 \]

ANOVA \( F_{1,98} = 266.69, P < 0.01 \)

**B**

\[ y = -8.1075x + 86.008 \]

\[ R^2 = 0.7398 \]

ANOVA \( F_{1,98} = 278.63, P < 0.01 \)

**C**

\[ y = -0.0879x + 2.8277 \]

\[ R^2 = 0.6456 \]

ANOVA \( F_{1,98} = 179.28, P < 0.01 \)
Figure 4-4. Linear regression analysis of clutch size and number of eggs laid + eggs retained on the wing-length of laboratory reared *Aedes albopictus* (Skuse). A) Clutch size. B) The number of eggs laid + eggs retained.
Figure 4-5. Linear regression analysis of clutch size and number of eggs laid + eggs retained on the wing-length of wild-caught *Aedes albopictus* (Skuse). A) Clutch size. B) The number of eggs laid + eggs retained.

\[
y = 29.404x - 31.628 \\
R^2 = 0.256 \\
\text{ANOVA } F_{1,88} = 30.18, P < 0.01
\]

\[
y = 30.599x - 34.023 \\
R^2 = 0.2995 \\
\text{ANOVA } F_{1,88} = 37.54, P < 0.01
\]
CHAPTER 5

*Aedes albopictus* Oviposition Preference as Influenced by Container Size and *Buddleja davidii* Plants

**Introduction**

Ovisite selection is a behavioral decision that is a critical component of mosquito life history (Bentley and Day 1989). A selection pressure exists for females to choose a container that will provide the highest offspring survival rates (Harrington et al. 2008). *Aedes albopictus* (Skuse), the Asian tiger mosquito, is a container-inhabiting mosquito in the larval stage. Container habitats are often resource limited and consequently density-dependent factors can influence mosquito productivity (Walker et al. 1991). Mori (1979) found that when *Ae. albopictus* were reared at high larval densities, the larvae had decreased survivorship and the subsequent adults had reduced size and fecundity when compared to those larvae reared at lower densities with the same level of food. Therefore, *Ae. albopictus* may preferentially oviposit in containers that have high levels of limited resources.

Several factors influence the availability of resources, such as water and vegetation, in the container habitat. Vegetation, predominantly leaf litter, which collects inside a container will decay and provide nutrients for microorganisms that mosquito larvae eat (Merritt et al. 1992). Larger-sized containers may provide more resources to offspring due to the increased opening diameter and depth allowing for the catch and retention of falling leaf litter and rainwater in comparison to smaller containers. In concordance with this theory, *Aedes aegypti* L. another container inhabiting mosquito, displays a positive association of increasing egg numbers with increasing container volume (Harrington et al. 2008). Conversely, Reiskind and Zarrabi (2012) found that *Ae. albopictus* displayed an oviposition preference for containers with a smaller volume.
(11.4 liter) over containers with a larger volume (47.3 liter) in a binary choice assay. Although both studies were conducted in screened enclosures, the Reiskind and Zarrabi (2012) study included only oviposition cups and mosquitoes, while the Harrington et al. (2008) study included a house and yard along with the oviposition cups and mosquitoes within the enclosure. These site differences may have influenced mosquito oviposition outcomes.

In addition to selecting the ovisite itself, factors associated with the terrestrial adult habitat may influence the deposition of an egg clutch by the Asian tiger mosquito. In north central Florida, ovipositing *Ae. albopictus* were much more common in suburban habitats than in sylvatic habitats (Obenauer et al. 2009a). Suburban habitats may support larger populations of *Ae. albopictus* and thus more ovipositing females. However, it has been shown that marked gravid *Ae. albopictus* traveled 1000 meters to reach a suburban environment when released in a secondary growth forest in Brazil (Maciel-de-Freitas et al. 2006). The availability of water holding containers, blood meal sources, and plant sugars may explain the concentrations of ovipositing *Ae. albopictus* in suburban habitats.

One feature that may make suburban habitats more attractive is the availability of carbohydrate food sources from landscape plants. Suburban landscapes often provide floristic diversity because they contain cultivated native and non-native plants (McKinney 2002). Thompson et al. (2003) reported that private suburban gardens contained twice as many flowering plant species as any other habitat in Sheffield, U.K. Mosquito flights to locate oviposition sites require the use of metabolic energy and nectar sources are a potential source of energy. A survey of wild caught *Ae. albopictus*...
in Japan showed that over 59% of the females collected contained plant sugars (Harada et al. 1976) and another field survey in Connecticut found several mosquito species to be positive for fructose in all stages of follicular development (Magnarelli 1978b). Therefore, sugar availability in the terrestrial habitat may direct *Ae. albopictus* oviposition (Bentley and Day 1989).

The current study was completed to examine the oviposition preference of *Ae. albopictus* for different sized containers and the influence of flowering landscape plants. Large screened enclosures were used in choice studies to examine *Ae. albopictus* oviposition preferences. In addition, a field study was conducted to examine *Ae. albopictus* oviposition outcomes in suburban neighborhoods.

**Materials and Methods**

**Mosquito Nectar Feeding Observation**

An observation study was completed to determine if gravid *Ae. albopictus* would attempt to nectar feed on flowering butterfly bushes and to photograph the occurrence. The F12 generation of a laboratory-reared strain of *Ae. albopictus* originally collected from Gainesville, FL were used in this observation. The rearing of *Ae. albopictus* was adapted from the United States Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology (USDA-CMAVE) protocol (Chapter 2). *Aedes albopictus* that had emerged as adults seven days prior were provided a bovine blood-filled sausage. Females that had a distended red abdomen were visually assessed as engorged. Sixty engorged females were transferred by a mouth aspirator (John W. Hock, Gainesville, FL) to a 61 x 61 x 61 cm cage and held for five days in a rearing room with a temperature of 25 ± 1°C, 75 ± 5% relative humidity, and 12:12 (L:D) photoperiod. After five days, 10 females were frozen and dissected to determine if
developed eggs were present according to Lounibos et al. (1990). The fifty gravid females were released at 0700 inside a greenhouse at the USDA-CMAVE. The mosquitoes were fed a 5% sucrose solution ad libitum prior to release. A flowering butterfly bush (*Buddleja davidii* Franchett cultivar ‘Guinevere’) was placed approximately 3 m away from the released mosquitoes. A Nikon D40 camera (Nikon Corporation, Tokyo, Japan) with a Nikon 105 mm f/2.8G ED-IF AF-S VR Micro-Nikkor lens (Nikon Corporation, Tokyo, Japan) was used to record all digital images. Mosquitoes images were recorded as they alighted on the butterfly bush florets.

**Large Enclosure Studies**

Three studies were conducted in three large outdoor screened enclosures located at the United States Department of Agriculture Center for Medical, Agricultural, and Veterinary Entomology (USDA-CMAVE). The enclosures have the following dimensions: 9.2 m wide x 18.3 m long x 4.9 m high, peaking at 6.1 m along the length of the cage ceiling (Kline 1999). As part of each study, a four choice arena was provided in the enclosure with the choices randomly assigned to the corners of an 8 x 8 m square (Fig. 5-1). In the first two studies, each choice consisted of a single oviposition container with or without a flowering butterfly bush adjacent to the container. In the third study, the oviposition containers were either adjacent to a flowering butterfly bush or a butterfly bush that had had the flowers removed. Butterfly bushes were 0.5 - 1 m tall and planted in 3.79 liter pots. The oviposition containers consisted of a large container with a volume of 1,892 mL, a surface diameter of 15.2 cm and a depth of 14.0 cm or a small container with a volume of 473 mL, a surface diameter of 9.9 cm and a depth of 10.8 cm. Both types of containers were black, 75% filled with an oak leaf infusion, and lined with seed germination paper. The oak leaf infusion was formulated by adding 150
g of fallen live oak (*Quercus virginiana* Miller) leaves to 15 liters of well water in a container that was sealed, placed outdoors and allowed to ferment for seven days, as modified from Allan and Kline (1995).

The F2-F7 generations of a strain of *Ae. albopictus* that originated from Gainesville, FL were used in this study and reared according to the USDA-CMAVE protocol (Chapter 2). One hundred gravid *Ae. albopictus* (12 days post adult emergence) were released into each enclosure. The *Ae. albopictus* used in the first study were not provided a 5% sucrose solution for 24 hr before release. In the second and third studies the mosquitoes were fed a 5% sucrose solution ad libitum prior to release. Mosquitoes were provided a blood meal using bovine blood-filled sausages seven days after emergence. After being blood fed, the *Ae. albopictus* were held for five days prior to release. The number of eggs laid in each container was recorded 48 hours after release into the cages. Each of the three large cage studies was replicated 24 times. Studies were conducted between 6 May, 2011 through 25 September, 2011 and 11 May, 2012 through 15 July, 2012.

**Field Study**

A field study was conducted in four suburban backyards in and near Gainesville, FL (Fig. 5-2). Field sites were chosen based on the presence of *Ae. albopictus* biting adults and sufficient shaded habitats to incorporate the study design. Three sizes of black containers were filled 75% full with the oak leaf infusion and lined with seed germination paper. The container sizes included the small and large containers described previously in addition to a medium sized container with a volume of 946 mL, a surface diameter of 11.4 cm and a depth of 13.3 cm. The containers were placed in a shaded area and randomly assigned positions in a 3 x 3 Latin square. Each container
was approximately one meter away from the nearest container. A second Latin square was established in the same backyard at least 10 m from the first Latin square arrangement. The second Latin square was constructed in the same manner as the first, but flowering butterfly bushes were adjacent to each of the infusion-holding containers.

The seed germination paper was replaced daily, the container inspected for eggs and the level of infusion maintained in each container. Seed germination papers were returned to the USDA-CMAVE for enumeration and identification. Eggs were identified based on appearance (Obenauer et al. 2009b) and 10% of eggs were reared to the fourth instar larva for identification confirmation using the keys of Darsie and Morris (2003). Each study was conducted over a seven day period and repeated once per month from June to September in 2011 and 2012, for a total of eight replications.

**Statistical Analysis**

In the large enclosure studies, the numbers of eggs per container were transformed into the proportion of eggs from each enclosure experiment per container. This conversion was accomplished to account for variations that occurred in female mosquito egg production among the three outdoor screened enclosures utilized in this study. A two-way analysis of variance (ANOVA) was conducted in JMP® Version 8 (SAS Institute 2009) to test the hypothesis that the fixed effects of container size and the presence or absence of flowering butterfly bushes did not affect the mean proportion of eggs laid in containers by mosquitoes that had a 5% sucrose solution withheld for 24 hr before release. This analysis was repeated on the data collected from mosquitoes provided a 5% sucrose solution ad libitum prior to experimentation. In addition, a two-way ANOVA was completed to examine the fixed effects of container size and the
occurrence of flowering or not flowering butterfly bush presence on the mean proportion of eggs laid.

In the field study, a multivariate ANOVA examined the fixed effects of container size, the presence of butterfly bushes, collection site, and year of collection (2011 or 2012) on the mean number of eggs per container. Tukey-Kramer Honestly Significant Difference (HSD) tests were completed to compare the means in each study. All statistical tests completed in this study were accomplished using the statistical software JMP® Version 8 (SAS Institute 2009).

Results

Mosquito Nectar Feeding Observation

*Aedes albopictus* were observed attempting to feed on butterfly bush florets within two minutes of release (Fig. 5-3). Individual females were observed to probe among florets for over five minutes before leaving.

Large Enclosure Studies

In the first study, the presence or absence of flowering butterfly bushes was found to significantly ($F_{3,92} = 24.63, P < 0.01$) affect oviposition by sugar-starved mosquitoes. Of all eggs deposited in containers within the enclosures, those 71.45% were collected from containers with adjacent flowering butterfly bushes (Fig. 5-4). Container size and the interaction of container size and the presence of butterfly bushes did not impact oviposition. Mosquitoes provided a 5% sucrose solution behaved similarly to mosquitoes in the first study (Fig. 5-5).

Containers near flowering butterfly bushes contained 69.65% of *Aedes albopictus* eggs deposited in this study, which was significantly more ($F_{3,92} = 19.70, P < 0.01$) than in containers near butterfly bushes that had their flowers removed (Fig. 5-6).
Similar, to the first two studies, container size and the interaction of container size and flowering/non-flowering butterfly bushes did not affect oviposition.

**Field Study**

Analysis of the field study data demonstrated that container size ($F_{2,191} = 17.77, P < 0.01$), the presence or absence of flowering butterfly bushes ($F_{1,191} = 11.06, P < 0.01$), field site ($F_{3,191} = 12.83, P < 0.01$), and the interaction of sampling year and field site ($F_{3,191} = 3.20, P = 0.03$) significantly affected the number of eggs laid per ovisite. Large containers had $30.64 \pm 2.31$ eggs per container which was significantly more than the $21 \pm 1.48$ and $16.55 \pm 1.46$ eggs in medium and small containers, respectively. The mean number of eggs per container was $24.64 \pm 1.53$ in containers adjacent to butterfly bushes which was significantly greater than the $19.68 \pm 1.40$ in containers without butterfly bushes. Large containers with a flowering butterfly bush had significantly more eggs than the medium and small containers with or without flowering butterfly bushes (Fig. 5-7). The number of eggs did not significantly differ in large containers that were positioned next to or did not have flowering butterfly bushes. The significant interaction in mean number of eggs deposited in ovisites between study year and field site was attributed to site 5-4 where the eggs per container dropped from 25 in 2011 to 15 in 2012 (Fig. 5-8).

**Discussion**

The availability of flowering butterfly bushes resulted in an increased number of eggs per ovisite in enclosure studies and field studies. This is the first study to demonstrate an increased mosquito oviposition response at ovisites near flowering plants. The *Ae. albopictus* oviposition response observed in these studies is most likely associated with an attempt by gravid females to locate floral nectar (Fig. 5-3). In
Foster’s (1995) review of mosquito sugar feeding, the most commonly reported source of mosquito sugar feeding was from floral nectaries. Floral nectar is an important source of mosquito energy. Mosquitoes expend energy in locating oviposition sites through the act of flying. Therefore, the seeking of floral nectar may direct oviposition outcomes (Bentley and Day 1989).

Mosquito sugar feeding often has been viewed as only necessary for males and an optional dietary supplement for blood fed females (Foster 1995) and in some mosquito populations, sugar meals may be taken infrequently (Edman et al. 1992). Evidence however, proves this hypothesis incorrect in most situations with wild-caught female mosquitoes in all stages of follicular development testing positive for fructose (Magnarelli 1977, Magnarelli 1978b, Nasci and Edman 1984, Reisen et al. 1986, Andersson and Jaenson 1987, Smith and Gadawaski 1994, Martinez-Ibarra et al. 1997).

In studies where *Ae. albopictus* were fed a blood meal containing RbCl, which can be detected in oviposited eggs; the mosquitoes were released in sylvatic habitats and were found to fly 1,000 m to suburban locations in which to oviposit (Maciel-de-Freitas et al. 2006). These gravid mosquitoes may have been seeking floral nectar sources that are often associated with suburban landscapes. This would imply that suburban landscapes contain quantitatively more floral nectar sources or that the floral nectar sources present were more attractive than sylvatic landscapes. We were unable to find any studies within the literature that compared the availability of nectar sources utilized by mosquitoes in sylvatic and suburban landscapes; however Opler (1983) indicates that nectar availability differs by habitat type and season. A survey of *Aedes cantator* (Coquillett), found this mosquito to be 19.6% positive for fructose in inland
forests and 57% positive for fructose in salt marshes suggesting that nectar sources were more available in the salt marsh habitat, the oviposition habitat of this mosquito species (Magnarelli 1978a).

Container size did not affect *Ae. albopictus* oviposition in outdoor screened enclosures, but did impact the oviposition pattern in the field. These results may have been influenced by several factors including the availability of flowering butterfly bushes. Several studies have concluded that floral nectar sources utilized by mosquitoes are limited (Bidlingmayer and Hem 1973, Harada et al. 1976, Magnarelli 1977, Magnarelli 1978a, Martinez-Ibarra et al. 1997, Müller and Schlein 2006). Consequently, the availability of floral nectar may influence mosquito responses to additional stimuli (Stone et al. 2012). Flowering butterfly bushes were used in both the enclosure and field studies; however, in the field studies competing flowering plants or other nectar sources were likely more available than in the enclosures. Thus, in the field study, the greater availability of flowering plants may mitigate the impact of butterfly bushes resulting in container size having a greater effect on oviposition than was observed in the enclosure studies. Therefore, the availability of flowering plants may have been a more dominant factor than container size in *Ae. albopictus* oviposition outcome. However, during this study a formal survey of flowering plants and nectar sources was not completed in the enclosures or the field sites in order to confirm their differential relative availability. Nonetheless, the flowering landscape plants that were planted in all of the screened enclosures included seven species: Japanese boxwood (*Buxus microphylla*), yaupon (*Ilex vomitoria*), burford holly (*Ilex cornuta*), Japanese privet (*Ligustrum japonicum*), India-hawthorn (*Rhadiolepis indica*), and sweet viburnum (*Viburnum odoratissimum*);
with one of the enclosures also having had golden dewdrop (*Duranta erecta*) planted (D. Kline and J. Hogsette, pers. comm). While a typical model of a suburban landscape contains 10-14 species of flowering plants (Watkins and Sheehan 1975). Another hypothesis that may explain the observed difference between the screenhouse and the field site data lies with my use of a laboratory-reared mosquito that may have behaved differently than wild mosquitoes. Selection pressure and conditioning during mass rearing can influence insect behavior (Boller 1972). However, our study attempted to mitigate colony selection pressures by using a recently colonized (F2-F7) strain of *Ae. albopictus* that originated from Gainesville, FL. Future studies are required to further explore the relative availability of nectar sources and their potential impact on oviposition outcomes.

A survey of *Ae. albopictus* larvae occupying treehole habitats found a higher prevalence of larvae in habitats with a higher volume of water (Tsuda et al. 1994). The results of our field study confirm *Ae. albopictus* preference for a larger container (1,892 mL) in comparison to the two smaller (946 and 473 mL) containers. In laboratory studies, Dieng et al. (2003) found similar results with *Ae. albopictus* displaying an oviposition preference for 312 mL containers when compared to 53 mL containers. Container dimensions are associated with mosquito larval nutrient levels by means of larger containers catching more leaf litter, being more resistant to desiccation, and being more resistant to water overflow flushing of nutrient content (Bradshaw and Holzapfel 1983, Walker and Merrit 1988). However, containers of varying sizes differ with respect to species richness (Mori and Wada 1977, Frank and Lounibos 1983, Sota et al. 1994), with larger-sized containers having increased diversity (Washburn 1995).
and the potential for increased predator encounters (Sunahara et al. 2002). Reiskind and Zarrabi (2012), found an oviposition preference for 11.4 liter containers when compared 47.3 liter containers. It may be that *Ae. albopictus* displays an oviposition preference for containers of increasing size, but containers of too large a size are not preferred because of increased risk of predation. Nonetheless, container size is dependent on both opening diameter and depth and these variables should be considered together when examining oviposition preferences. Harrington et al. (2008) reported in their examination of *Ae. aegypti* oviposition that container attributes effected oviposition preference in the following rank order: volume > water surface area > container opening diameter.

The mean number of *Ae. albopictus* eggs collected fluctuated at one field site from 2011 to 2012 (Fig. 5-8). At site 5-4 the number of eggs significantly declined from 2011 to 2012. At the other three sites the mean egg numbers did not significantly differ, although at site 5-1 and site 5-3 opposing decreasing and increasing trends were observed, respectively. The decreased number of eggs collected at field site 5-4 may be accounted for by an increase in a mosquito predator population, *Toxorhynchites rutilus rutilus* (Coquillett), which may have reduced the sites’ *Ae. albopictus* population (Chapter 3). Sites 5-1 and 5-4 were observed to have more *Tx r. rutilus* eggs in the oviposition containers than Sites 5-2 and 5-3 (unpub. data). *Toxorhynchites r. rutilus*, has been shown to control *Ae. aegypti* populations in Florida (Focks et al. 1980, Bailey et al. 1983) and may impact *Ae. albopictus* populations. In addition, the *Tx. r. rutilus* eggs may have had a repellent effect on *Ae. albopictus* oviposition. We are unable to find any evidence in the literature that *Tx. r. rutilus* eggs are repellent to heterospecific
oviposition; however other studies have found the presence of predators to be repellent to ovipositing mosquitoes (Blaustein and Kotler 1993, Angelon and Petranka 2002, Mokany and Shine 2003, Blaustein et al. 2005). Further investigation is needed to explore the population dynamics of *Ae. albopictus* in regards to predator-prey interactions that result from decreases in *Ae. albopictus* populations.

Our findings can be applied to improve *Ae. albopictus* surveillance and control plans. Ovitraps for *Ae. albopictus* surveillance often have been based on the designs used during the *Ae. aegypti* Pan American Health Organization eradication program (Soper 1965). This design consists of a 450-500 mL black cup or black painted jar with an opening diameter of 6.4-8.9 cm and a depth of 10.8-12.7 cm containing a paddle or lined with paper that is removed and the number of eggs enumerated. Similarly, lethal ovitraps have been designed using this same sized container (Zeichner and Perich 1999, Perich et al. 2003, Sithiprasasna et al. 2003), while others have used lethal ovitraps of 1.2 liters with an opening diameter of 15 cm and a depth of 12 cm (Ritchie et al. 2008). The results of our study indicate that a container of 1.8 liters with an opening diameter of 15.2 cm and a depth of 14.0 cm would be more attractive than a smaller container for *Ae. albopictus* surveillance and control.

Additionally, placement of ovitraps may enhance their attractiveness. Mosquitoes in the present study more often selected ovisites adjacent to a flowering butterfly bush than ovisites lacking the bush. Little work has examined the placement of ovitraps in association with nearby vegetation (Hawley 1988). One study examining ovitraps placement for three container-inhabiting species of mosquito (including *Ae. albopictus*) found more eggs in traps located in deep shade as opposed to those in
more open locations (Lambrecht 1971). Shaded areas often have a more humid and cooler microclimate than open areas and such areas appear to be preferred by mosquitoes. However, no studies have looked at specific plant associations in regards to Ae. albopictus. Our study placed ovitraps in shaded areas and examined the association of flowering plants (Figs. 5-1 and 5-2). The preference for butterfly bush was negated when the flowers were removed indicating that the Ae. albopictus were attracted to these plants as a potential nectar source, since Buddleja davidii does have extrafloral nectaries (Keeler 2008). Some studies report that Ae. albopictus expresses nectar feeding preferences for certain species of plants; such as dogwood (Cornus controversa), snowbell (Styrax japonica), fleabane (Erigeron annuus), and sumac (Rhus chinensis) (Harada et al. 1975). Nectar feeding preferences should be taken into account when ovitraps are used in order to enhance success. Furthermore, the aspects of flowering plants that are used as cues in host finding should be further explored to improve ovitrap efficacy. Olfactory cues associated with plant phytochemicals are utilized by mosquitoes in locating nectar sources (Foster 2008). Incorporation of phytochemicals that are produced by butterfly bush may enhance ovitrap effectiveness and warrants further study.
Figure 5-1. Gravid *Aedes albopictus* (Skuse) females exposed to a four choice arena inside an outdoor screened enclosure. A) A 1,892 mL ovisite with a flowering butterfly bush (*Buddleja davidii* Franchett cultivar ‘Guinevere’). B) A 473 mL ovisite with a flowering butterfly bush. C) A 473 mL ovisite without a butterfly bush. D) A 1,892 mL ovisite without a flowering butterfly bush. Photos courtesy of Timothy J. Davis.
Figure 5-2. Suburban yard in which *Aedes albopictus* (Skuse) oviposition preference was examined for three different sized black containers (473, 946, 1892 mL). A) Containers adjacent to flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’). B) Containers without butterfly bushes with a container denoted by a red arrow. Photos courtesy of Timothy J. Davis.
Figure 5-3. Gravid *Aedes albopictus* (Skuse) females attempting to feed from flowering butterfly bush (*Buddleja davidii* Franchett cultivar ‘Guinevere’). Photos courtesy of Timothy J. Davis.
Figure 5-4. Mean proportion of *Aedes albopictus* (Skuse) eggs recovered from different sized containers, 473 mL (S) and 1,892 mL (L), that occur with (BD +) and without (BD -) flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’). The mosquitoes used in this study were not provided with sucrose solution for 24 hr prior to experimentation. Columns with the same letter are not significantly (*F*$_{3,92}$ = 24.63, *P* < 0.01).
Figure 5-5. Mean proportion of *Aedes albopictus* (Skuse) eggs recovered from different sized containers, 473 mL (S) and 1892 mL (L), that occur with (BD +) and without (BD -) flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’). The mosquitoes used in this study were provided a 5% sucrose solution ad libitum prior to experimentation. Columns with the same letter are not significantly ($F_{3,92} = 20.97, P < 0.01$).
Figure 5-6. Mean proportion of *Aedes albopictus* (Skuse) eggs recovered from different sized containers, 473 mL (S) and 1892 mL (L), which occur with butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’) that retain flowers (F) or with the flowers removed (NF). Columns with the same letter are not significantly ($F_{3,92} = 19.70, P < 0.01$).
Figure 5-7. Mean number of eggs recovered from containers with (BD +) and without (BD-) flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’) and different sized containers: 473 mL (S), 946 mL (M), 1892 mL (L). Columns with the same letter are not significantly (*F*47,144 = 2.29, *P* < 0.01). Samples were taken from four Gainesville, FL suburban yards in the summers of 2011 and 2012.
Figure 5-8. Mean number of eggs per ovitrap at each field site. Samples were taken from four Gainesville, FL suburban yards in the summers of 2011 and 2012. Means within each field site that have the same letter are not significantly different ($F_{3,144} = 3.20$, $P = 0.03$).
CHAPTER 6
FUTURE DEVELOPMENT OF SURVEILLANCE TECHNIQUES AND CONTROL MEASURES THAT EXPLOIT Aedes albopictus OVIPOSITION BEHAVIORS

Introduction

*Aedes albopictus* (Skuse) is an invasive mosquito species that readily adapts to human habitations (Enserink 2008) and has been the primary vector of chikungunya and dengue outbreaks (Russell et al. 1969, Chan et al. 1971, Effler et al. 2005, Reiter et al. 2006, Pages et al. 2009). Traditional integrated mosquito control strategies have proven both ineffective at controlling *Ae. albopictus* in suburban settings and resource-intensive in urban settings (Fonseca et al. 2013).

The sustainable control of vector populations will require integrated approaches that employ novel techniques (Knudsen and Sloof 1992, Nam et al. 2000). One such recently developed novel approach for *Ae. albopictus* control employs using the oviposition site as a control device (Perich et al. 2003, Caputo et al. 2012, Gaugler et al. 2012). However, the oviposition behavior of *Ae. albopictus* will influence how successful these techniques are.

Skip Oviposition

*Aedes albopictus* was found to perform skip oviposition in laboratory and field studies and this behavior was influenced by larval habitat quality. The use of control devices that are based on the auto-dissemination of insect growth regulators (IGR) (Caputo et al. 2012, Gaugler et al. 2012) should take into account competing larval habitat quality when assessing *Ae. albopictus* skip oviposition behavior. Areas where competing larval habitats are of high quality will reduce *Ae. albopictus* skip oviposition, while areas in which low quality larval habitats occur will promote skip oviposition. Mosquitoes that visit more containers during a gonotrophic cycle will move the IGR to
more ovisites compared to those mosquitoes that perform a lesser degree of skip oviposition. The use of lethal ovitraps that employ an adulticide, glue panel, or another method to kill the visiting female also will need to consider larval habitat quality effects on skip oviposition behavior. Mosquitoes that are visiting more than one oviposition container during a gonotrophic cycle will have an increased chance of encountering a lethal ovitrap than those mosquitoes that visit a single ovisite during a gonotrophic cycle. Therefore, in populations with little skip oviposition occurring, an increased density of auto-disseminating or lethal ovitraps will be required to cause a population reduction compared to the number of devices needed in populations that perform more skip oviposition. However, assessing larval habitat quality may be difficult due to this mosquito occupying numerous natural and artificial containers that include cryptic microhabitats (Hawley 1988).

An assessment of larval habitat quality may be done indirectly by measuring adult mosquito wing-lengths. Results from my studies found *Ae. albopictus* had reduced wing-length when reared under intraspecific competition for limited resources and that mosquitoes collected at field sites significantly varied in the female mean wing-length. Future studies are required to associate field collected female wing-length with local larval habitat conditions in order to confirm this approach as a valid surveillance technique.

My studies also found skip ovipositing *Ae. albopictus* and *Ae. triseriatus* (Say) dispersal increased when conspecific populations increased. Higher populations may result in increased interspecific larval competition and decreased habitat quality. A decrease in habitat quality will increase skip oviposition and result in greater dispersal.
during oviposition. Female *Ae. albopictus* that transmit pathogens are those females that have already acquired a blood-meal and their behaviors during oviposition can influence disease incidence. Therefore, examining the factors that affect mosquito dispersal during oviposition may assist in predicting disease outbreaks. Day and Curtis (1994) reported that outbreaks of St. Louis encephalitis (SLE) in Florida were dependent on *Culex nigripalpus* Theobald behavioral responses to environmental factors. A periodic cycling of larval habitat quality (high-quality habitat - low-quality habitat - high-quality habitat) was observed to lead to enhanced SLE viral amplification and transmission; and the modeling of these habitat qualities assisted in predicting SLE risk (Day and Curtis 1994). Similarly, these techniques may assist in predicting chikungunya outbreaks when the virus is transmitted by *Ae. albopictus*.

**Flowering Plants**

My study found that flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’) significantly influenced *Ae. albopictus* oviposition decisions. Flowering plant nectar serves as a carbohydrate food source for mosquitoes (Foster 1995). Mosquitoes in search of oviposition sites expend energy during flight and nectar sources may direct mosquito oviposition (Bentley and Day 1989). Our findings can be applied to several aspects of *Ae. albopictus* surveillance and control.

Ovitrap placement for surveillance and control should consider the availability of flowering plants. Placement of ovitraps near flowering butterfly bushes will improve their success. Previous studies have found that *Ae. albopictus* express nectar feeding preferences for certain species of plants; such as dogwood (*Cornus controversa*), snowbell (*Styrax japonica*), fleabane (*Erigeron annuus*), and sumac (*Rhus chinensis*).
Future studies are required to evaluate the attractiveness of flowering plants in order to identify those that are most attractive.

The components of flowering plants that are attractive to mosquitoes should be pursued, particularly plant volatiles associated with attracting pollinating organisms. Phytochemical attractants derived from floral extracts have proven attractive to mosquitoes (Foster 2008). Otienoburu et al. (2012) found that Culex pipiens L. was significantly attracted to a synthetic blend of chemicals that were based on a milkweed (Asclepias syriaca) floral extract. Extracts from butterfly bushes should be examined in the development of phytochemical attractants. Phytochemical attractants could enhance trap captures for both control and surveillance.

Toxic sugar baits are being pursued as a control method based on mosquitoes feeding on sugar as a carbohydrate source (Müller et al. 2010). *Aedes albopictus* biting counts were significantly reduced by boric acid sugar bait in outdoor enclosure studies (Xue and Barnard 2003). The efficacy of toxic sugar baits may be enhanced by the addition of phytochemical attractants. My study found that gravid females were attracted to flowering butterfly bushes and other studies have found mosquitoes positive for fructose in all stages of follicular development (Magnarelli 1977, Magnarelli 1978b, Nasci and Edman 1984, Reisen et al. 1986, Andersson and Jaenson 1987, Smith and Gadawaski 1994, Martinez-Ibarra et al. 1997). Therefore, toxic sugar baits that contain attractants from flowering butterfly bushes may aid in the control of females that have already taken a blood-meal and potentially acquired a pathogen.

In conclusion, *Ae. albopictus* oviposition behavior is influenced by density dependent effects and the availability of flowering landscape plants. A technique to
assess larval habitat quality in wild *Ae.* *albopictus* populations is needed in order to predict the degree to which skip oviposition behavior is expressed. In addition, *Ae.* *albopictus* gravid females responses to butterfly bush floral attracts should be examined in behavioral evaluations.
APPENDIX A
DIGITAL IMAGES OF THE FIELD SITES ASSOCIATED WITH THE ASSESSMENT OF
*AEDES ALBOPICTUS* CLUTCH SIZE IN WILD POPULATIONS

Figure A-1. Digital images of the field sites in Gainesville, FL, associated with the assessment of *Aedes albopictus* (Skuse) clutch size. A) Site 4-1 was a suburban yard. B) Site 4-2 was a suburban yard. C) Site 4-3 was a graveyard in a suburban neighborhood. D) Site 4-4 was on the University of Florida campus. Photos courtesy of Timothy J. Davis.
Figure A-1. Continued.
APPENDIX B
DIGITAL IMAGES OF THE FIELD SITES ASSOCIATED WITH THE DETERMINATION OF *Aedes albopictus* OVIPPOSITION PREFERENCE AS INFLUENCED BY CONTAINER SIZE AND *Buddleja davidii* PLANTS

Figure B-1. Digital images of the field sites associated with the assessment of *Aedes albopictus* (Skuse) oviposition preference as influenced by container size and flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’). Site 5-1 was a suburban yard in Gainesville, FL. A) Area in which containers were with flowering butterfly bushes. B) Area in which containers were without butterfly bushes. Photos courtesy of Timothy J. Davis.
Figure B-2. Digital images of the field sites associated with the assessment of *Aedes albopictus* (Skuse) oviposition preference as influenced by container size and flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’). Site 5-2 was a suburban yard in Gainesville, FL. A) Area in which containers were with flowering butterfly bushes. B) Area in which containers were without butterfly bushes. Photos courtesy of Timothy J. Davis.
Figure B-3. Digital images of the field sites associated with the assessment of *Aedes albopictus* (Skuse) oviposition preference as influenced by container size and flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’). Site 5-3 was a suburban yard in Gainesville, FL. A) Area in which containers were with flowering butterfly bushes. B) Area in which containers were without butterfly bushes. Photos courtesy of Timothy J. Davis.
Figure B-4. Digital images of the field sites associated with the assessment of *Aedes albopictus* (Skuse) oviposition preference as influenced by container size and flowering butterfly bushes (*Buddleja davidii* Franchett cultivar ‘Guinevere’). Site 5-4 was a suburban yard in Gainesville, FL. A) Area in which containers were with flowering butterfly bushes. B) Area in which containers were without butterfly bushes. Photos courtesy of Timothy J. Davis.
LIST OF REFERENCES


Bohart, R. M. 1956. Insects of Micronesia (Diptera: Culicidae), vol. 12 no. 1, Bernice P. Bishop Museum, Honolulu, HI.


Buxton, P. 1927. Researches in Polynesia and Melanesia, part I-V. London School of Hygiene and Tropical Medicine, London,UK.


Müller, G. C., and Y. Schlein. 2006. Sugar questing mosquitoes in arid areas gather on scarce blossoms that can be used for control. Int. J. Parasitol. 36:1077-1080.


BIOGRAPHICAL SKETCH

Timothy J. Davis was born in Oshkosh, WI. He spent his childhood working on his family’s dairy farm and enjoying the outdoors. He received a Bachelor of Science degree in natural sciences and a Master of Science degree in entomology from the University of Wisconsin, Madison, WI. He was commissioned as a First Lieutenant in the United States Air Force. Upon completion of his degree requirements at the University of Florida, he will be reassigned and continue to serve his country in the United States Air Force.