Master's Theses

8-2014

Effects of Water Parameters on Container Mosquito (Diptera: Culicidae) Oviposition and Performance

Stephanie Sue Schelble
University of Southern Mississippi

The University of Southern Mississippi

EFFECTS OF WATER PARAMETERS ON CONTAINER MOSQUITO

(DIPTERA: CULICIDAE) OVIPOSITION AND PERFORMANCE

by

Stephanie Sue Schelble

A Thesis
Submitted to the Graduate School
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

Approved:

Dr. Donald Yee_____
Director

Dr. Kevin Kuehn_____

Dr. Carl Qualls_____

Dr. Maureen Ryan_____
Dean of the Graduate School

August 2014

ABSTRACT

EFFECTS OF WATER PARAMETERS ON CONTAINER MOSQUITO

(DIPTERA: CULICIDAE) OVIPOSITION AND PERFORMANCE

by Stephanie Sue Schelble

August 2014

Water body parameters have a considerable effect on the communities that develop within them. In small container habitats like tires, the depth, surface area, and volume effect the development and success of the mosquito communities. This study investigated the choices of the adult female mosquitoes, *Aedes albopictus* and *Culex quinquefasciatus,* between different depths and surface areas. In addition, larval performance was determined under differing depth and larvae densities. Oviposition studies showed that *Ae. albopictus* had a preference for deeper habitats (χ^2= 14.2902, p= 0.0139) but did not prefer any surface areas (χ^2= 7.2321, p= 0.0649) though there was a trend that indicated that there could be a preference for larger surface area. Conversely, *Culex quinquefasciatus* was shown to be sensitive to surface areas (χ^2= 11.1419, p= 0.0110) but not depth (χ^2= 9.9828, p= 0.0757). Larval densities affected the population growth, represented by λ', of *Aedes albopictus* ($F_{3,15}$= 19.3786, p< 0.0001) where higher densities of larvae depressed λ' values. *Culex quinquefasciatus* had significant differences in the interaction of larval density and depth ($F_{9,15}$= 3.2870, p= 0.0204) between the low λ' 10:10 and the high λ' 0:5 densities. Within the 10:10 density, differences were found in λ' with higher growth in the 7 cm depth compared to the 14 cm depth. Additionally, the 14 cm

depth produced heavier female *Ae. albopictus* than 7 cm depths ($F_{3,15}$= 3.3160, p= 0.0488). Overall, it was shown that *Ae. albopictus* prefer deeper habitats while ovipositing and although this does not seem to confer greater population growth, it does result in larger female mosquitoes. In addition, *Ae albopictus* depressed the population growth of *Cx. quinquefasciatus* in high larval densities.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

       Significance of Study
       Mosquito Study Species
       Research Questions and Hypotheses

       Introduction
       Methods
       Results
       Discussion

       Introduction
       Methods
       Results
       Discussion

LIST OF TABLES

Table

LIST OF ILLUSTRATIONS

Figure

CHAPTER I

INTRODUCTION

Significance of Study

Information concerning how gravid mosquitoes select habitats with variable depths, surface areas, and volume has been understudied in small aquatic habitats. The author will investigate how different aquatic habitat parameters (i.e., depth, surface area) explain patterns of oviposition in gravid container mosquitoes to determine optimal aquatic habitat parameters for *Aedes albopictus* and *Culex quinquefasciatus* in tire habitats. Changes in depth and surface area certainly have far reaching effects on the distribution, population densities, risk of predation, and availability of resources to these mosquito species (Arav & Blaustien, 2006; Lester & Pike, 2003; Sunahara & Motoyoshi, 2002). Thus, it is also important to determine the effects of depth on the performance of mosquito larvae. This study will elucidate the link between patterns of oviposition and larval performance. The effect of depth on inter- and intraspecific competition, another topic with little data, will also be studied. By studying both oviposition choices and larval performance. The author will be able to link some aspects of fitness with the choices female mosquitoes make when laying their eggs.

Mosquito Study Species

Mosquitoes are dipterans in the family Culicidae. The requirement of many female mosquitoes for blood-feeding to produce viable eggs has made them both nuisances and a medically important family of insects (Clements, 2000). Their

larvae and pupae have an obligate aquatic phase that is usually located in small, isolated water bodies (Clements, 2000).

There are a number of different ways to categorize mosquitoes based on habitat type, but a relevant grouping used in research are container dwelling species. These species oviposit in small water bodies that generally do not include internal sources of nutrients (Carpenter, 1983). Therefore, allochthonous resource inputs, such as leaves and dead animal matter, are the major source of nutrients for these systems (Walker, Lawson, Merritt, Morgan, & King, 1991; Walker, Merritt, Kaufman, Ayres, & Riedel, 1997; Winters & Yee, 2012; Yee & Juliano, 2006). Examples of container types include bamboo stumps, tree holes, cemetery vases, and tires (Beier, Patricoski, Travis, & Kranzfelder, 1983a; Yee, 2008). Container-dwelling mosquitoes provide a useful study organism for researchers because of their ecological and medical importance as well as the relative ease on which manipulations on their systems can be done for both field and laboratory experiments. Among the most prevalent mosquitoes found in container environments in North America include *Aedes aegypti, Ae. albopictus, Culex pipiens pipiens, Cx. quinquefasciatus, Cx. restuans, Toxorhynchites rutilus,* and *Ae. triseriatus* (Vezzani, 2007; Yee, 2008).

Of all the different containers that mosquitoes can use, tires are considered to be major sites of production (Yee, 2008). In Mississippi, the most common tire mosquitoes are, in order, *Ae. albopictus, Cx. quinquefasciatus, Orthopodomya singifera, Cx. terriatans, Cx. coronator,* and *Ae. triseriatus,* (Yee, Allgood, Kneitel, & Kuehn, 2012). Rainfall is the major source of water in these

systems, and evaporation decreases water volume (Dudley, 2006). Seasonal precipitation events will dictate when these tires can be suitable habitats. Unlike natural aquatic habitats (i.e., ponds), tires do not lose water to the soil, but they are limited by the volume that they can hold depending on the size of the tire and the angle at which it is resting (Beier, Travis, Patricoski, & Kranzfelder, 1983b; Dudley, 2006).

This study focused on the mosquitoes *Aedes albopictus* and *Culex quinquefasciatus*. These two species represent two different strategies of oviposition and larval foraging behavior. *Aedes albopictus* is the most common tire species in the southern U.S. (Yee, 2008) and is also a medically important vector of multiple diseases including dengue, West Nile, Japanese encephalitis, and chickungunya (Thiboutot et al., 2010). Larval *Ae. albopictus's* competitive abilities over native species have likely resulted in this species being the dominant mosquito species found in tires (Lounibos et al., 2002; Yee, 2008). The larvae of *Ae. albopictus* mostly feed by browsing during later instars but will also be found to filter feed (Merritt, Dadd, & Walker, 1992; Skiff, 2013). *Aedes albopictus* generally lay their eggs on the sides of containers to take advantage of future habitats that form when water levels rise (Clements, 2000). In addition, this species exhibits skip oviposition, where a single females my distribute eggs among many different habitats (Clements, 2000).

Culex quinquefasciatus differ from *Ae. albopictus* by ovipositing eggs in clusters known as rafts whose larvae hatch within a day. Thus *Cx. quinquefasciatus* can take advantage of current habitat conditions (Clements,

2000). Larvae generally are found in large open water habitats and preferentially filter feed as they develop (Clements, 2000; Merritt et al., 1992; Skiff, 2013). This species, while not as medically important to humans as *Ae. albopictus*, is a vector of West Nile virus and St. Louis encephalitis in bird populations as well as an important bridge vector of these diseases to humans (Sardelis, Turell, Dohm, & O'Guinn, 2001; Reisen, Fang, & Martinez, 2005). Although *Cx. quinquefasciatus* is found mainly in open-water habitats, it can also be found in tires along with concurrently and both utilize tires as an important larval habitat (Costanzo, Mormann, & Juliano, 2005; Yee, Allgood, Kneitel, & Kuehn, 2012; Yee et al., unpublished).

<p style="text-align:center">Research Questions and Hypotheses</p>

The author examined how the depth and surface area of an aquatic environment may affect mosquito development and oviposition. The author hypothesized that surface area affects oviposition of gravid female mosquitoes. The author predicted that *Aedes albopictus* and *Culex quinquefasciatus* will oviposit where their offspring will have optimal performance (e.g., low development times, high mass). The author also predicted that *Ae. albopictus* will oviposit in containers with smaller surface areas as predicted by their preference for smaller volume habitats (Sunahara, Ishizaka, & Motoyoshi 2002), whereas *Cx. quinquefasciatus* will oviposit on larger surface areas as indicated by the fact that they more often oviposit in larger, non-container habitats (Subra, 1981).

The author also hypothesized that depth influences oviposition of gravid female mosquitoes. The author predicted that *Ae. albopictus* will oviposit in

shallower depths in anticipation of water levels rising enough for their eggs to hatch resulting in a more suitable future habitat (Clements, 2000). *Culex quinquefasciatus* will oviposit in deeper environments to take advantage of an already advantageous habitat that will have a low risk of desiccation (Clements, 2000).

  To determine the effect of oviposition choices on developing larvae, another study on the effect of water depth on larvae in container environments was performed. The author hypothesized that depth will affect the performance of *Culex quinquefasciatus* and *Aedes albopictus* larvae*.* It is expected that *Cx. quinquefasciatus* will be less affected by increasing depth than the browsing mosquito *Ae. albopictus.* The reason for this is because *Cx. quinquefasciatus* will have easier access to food by filtering and remaining near the surface whereas *Ae. albopictus* often forage on benthic detritus, which may be metabolically expensive. The author also predicted that in shallower depths, *Ae. albopictus* will be in direct competition with *Cx. quinquefasciatus* and will show better performance with lower development times, higher mass, and ultimately higher population growth.

CHAPTER II

OVIPOSITION AND AQUATIC PARAMETERS

Introduction

Animal communities located in small water bodies are periodically challenged by the limits of their aquatic habitat. Many of these ecosystems are constrained not by predators, but by resources, which results in communities with high densities but low richness. As a result, both intra- and interspecific competition can be intense (Blaustein & Chase 2007). These potentially negative effects are then multiplied if the system is transient, where nutrients and space become even more limiting over a period of time as the nature of the habitat changes (Blaustein & Chase 2007; Sunahara & Mogi 2002).

Despite constraints on these systems small aquatic habitats are a great boon to researchers, making it easier to parcel out specific interactions because communities are often easy to quantify. Other studies in these "microcosms" include competition (Barrera, 1996; Costanzo et al., 2005; Grill & Juliano, 1996; Lounibos et al. 2002; Sunahara & Mogi, 2002), predation (Alto, Lounibos, Higgs, & Juliano, 2005; Bradshaw & Holzapfel, 1983; Lounibos, Frank, Machado-Allison, Ocanto, & Navarro, 1987; Shaalan & Canyon, 2009; Yanoviak, 2001b), and commensalism (Pelz-Stelinski, Kaugman, & Walker, 2011). However, despite the low number of species that inhabit these ecosystems, there are a plethora of additional interactions that may be found (e.g., mutualism, indirect mutualism, and intraguild predation, Blaustein, Olsfeld, & Holt, 2010). Many of these unstudied interactions deal with the bottom-up effects of detritus, which are

important in these detritus based ecosystems (Walker et al., 1991; Walker et al., 1997; Winters & Yee, 2012; Yee et al., 2012).

Among many studies of abiotic parameters using container systems, investigations of water depth often focused on how increasing depth, surface area, and volume predict the increased presence of aquatic predators and how this may affect mosquito communities (Arav & Blaustien, 2006; Lester & Pike, 2003; Sunahara et al., 2002). New Zealand predator species like *Austrolestes colensoni* and *Xanthocnemis zealandica* (Odonata) inhabit deep water habitats with larger volumes, whereas the mosquito prey, *Culex pervigilans,* exhibits higher densities in lower depths and smaller volumes than their predators (Lester & Pike, 2003). Additionally, larger container sizes increased the mosquitoes' contact with predator species, mainly because the predators were found in the larger containers (i.e., volume of ≥ 0.1 m^2, Sunahara et al., 2002). Other work has shown that larger environments housed the mosquito species *Aedes japonicas*, *Culex kyotoensis, Cx. tritaeniorhynchus,* and *Anopheles sinensis* (Sunahara et al., 2002)*.* In these systems, mosquitoes have to avoid predators that are commonly found in their preferred habitat, thus, different levels of predation pressure is then the main cause of changes in population densities for these species (Sunahara et al., 2002). Other mosquito species, like *Aedes albopictus, Aedes flavopictus,* and *Tripteroides bambusa* preferentially colonize smaller environments and suffer competition that defines their population densities (Sunahara et al., 2002).

The size of aquatic systems is an important part of determining the development patterns, levels of predation, competition, and fecundity of aquatic organisms (Crump, 1991; Lester & Pike 2003; Sota, 1998; Sunahara & Mogi, 2002; Timmermann & Briegel, 1993). Aquatic parameters, such as depth, volume, and surface area, should have an important effect on oviposition choices for terrestrial females seeking appropriate habitats for their offspring. The choices by females for egg placement can be even more crucial when an aquatic environment has limited resources and a high propensity for desiccation. In low volume habitats, it is possible that the effects of abiotic and biotic factors on oviposition will change depending on water volume (Dudley, 2006; Sunahara & Mogi, 2002).

Organisms generally exist in aquatic ecosystems with a depth and volume that are more or less optimal for a species life history strategies (Bradshaw & Holzaphel, 1983; Egan & Paton, 2004; Lounibos et al., 1987; Mutero, Blank, Konradsen, & van der Hoek, 2000). Larger, permanent environments often support high predator richness because they provide ample prey (Bradshaw & Holzaphel, 1983; Lounibos et al., 1987). The opposite may occur for some prey species. For instance, mosquitoes and amphibians are found to be more successful in transient environments that dry and refill frequently with rainwater (Egan & Paton, 2004; Mutero et al., 2000). These environments tend to limit predators, and it is beneficial for the prey to preferentially change their development time to emerge faster to a non-aquatic adult that is less prone to desiccation (Denver, Mirhadi, & Phillips, 1998; Egan & Paton, 2004; Laurila &

Kujasalo, 1999; Wynn & Paradise, 2001). Thus, oviposition decisions by females are made between larger more stable aquatic environments versus smaller temporary ones that have little or no predators.

Depending on the species, ovipositing females use physical, chemical, or a combination of both cues to find and utilize different aquatic habitats (Bentley & Day, 1989). Because physical and chemical cues have been shown to be synergistic in selection of an appropriate oviposition habitat, it is likely that most species use a combination of the two rather than either alone (Beehler, Millar, & Mulla, 1993). Cues that species use to make their oviposition choices have been studied in some detail (e.g., water color, container color, water chemicals, larval density, presence of eggs, exposure to sunlight, and nutrient availability (Beehler, Lohr, & DeFoliart, 1992; Beier et al., 1983a; Wilton, 1968; Yanoviak, 2001a). It is important to note that some mosquitoes will oviposit in suboptimal habitat that is closely correlated to the chemical conditions they were raised in (McCall & Eaton, 2001). This chemical memory may introduce some noise into oviposition experiments where mosquitoes will lay in habitats that more closely resemble the shallow, wide habitats that could chemically resemble the large pans we raised our mosquitoes in for these experiments.

There are a variety of ways females in the terrestrial environment have an opportunity to assess water depth in the interest of providing an appropriate aquatic environment for her offspring. Studies on water strider females that dive into the water to ovipost show that this direct oviposition only occurs under shallow depths and is an artifact of egg parasitism risk (Harada, 1992; Hirayama,

2009; Hirayama, 2010). Even largely terrestrial amphibians also directly sample the depths of potential oviposition sites (Crump, 1991). It is unclear how other organisms determine water depth of potential oviposition sites, although these females may use secondary characteristics of depth, including conductance (Arav & Blaustein, 2006). Several studies have found that females preferentially oviposit in smaller, shallow containers where there is low predator presence and richness (Arav & Blaustein, 2006; Lester & Pike, 2003; Sota, 1996; Sota, 1998; Sunahara & Mogi, 2002; Yanoviak, 1999).

Mosquitoes respond to the properties of water bodies, including the presence of water through water vapor, chemicals, and color (Kennedy, 1942). *Culex* species use random "skim-hopping" behavior above the ground to detect the presence of water; this is followed by sitting and resting on the water for a time until oviposition begins (Kennedy, 1942). *Aedes* species detect water from directionless flight overhead, and when they locate water, they hop and hover frequently over the water before ovipositing on the water's edge (Kennedy, 1942). Contact with water seems to be crucial for the start of the pre-ovipositing process; however, cues to assess depth are less well known. *Aedes* and *Culex* species appear to take a different approach before ovipositing to assessing the suitability of aquatic habitats that may affect their strategy for depth detection. The types of oviposition strategies that *Aedes* and *Culex* genera utilize differ as well. *Culex* species lay eggs that are connected together in floating rafts that then hatch generally within 24 hours (Clements, 2000). *Aedes,* however, oviposit on the sides of aquatic habitats, where the substrate is moist, to take

advantage of future aquatic habitats that will occur when the water level rises to submerge the eggs (Clements, 2000). *Aedes also* lay eggs individually and often perform "skip oviposition" to spread their clutch over multiple habitats (Clements, 2000).

It is important to copy the tire structures such that mosquitoes oviposit under the same conditions and interact the same way with the tires as they would in a tire pile. Thus, containers were manipulated on the interior of the tire for comparison and testing. Gravid mosquitoes were released and exposed to six different depths or four different surface areas. The author hypothesized that the container species *Ae. albopictus* will lay a greater proportion of eggs in shallower depths and lower surface areas. *Culex quinquefasciatus* is expected to prefer deeper habitats and higher surface areas. The results of this work will further inform researchers about the contributions of depth and surface area to patterns of larval mosquitoes in nature.

Methods

Adult mosquitoes were generated separately from colonies of F2 and F3 *Aedes albopictus* and F5 *Culex quinquefasciatus* raised from wild adults collected in Hattiesburg, MS. *Aedes albopictus* larvae from both colonies were reared using an appropriate feeding schedule (Table 1) and kept in an environmental chamber at 27˚C in a large plastic pan. Pupae were collected and placed in large cages for each species until they emerged as adults. Adult mosquitoes were fed on a 10% sucrose solution and were kept in a 27˚C room with a 14:10 light to dark photoperiod. An additional short time for reduced light

was placed in between the switch between light and dark. When ~95% of larvae emerged, females were blood-fed. *Aedes albopictus* and *Cx. quinquefasciatus* were blood-fed with tethered northern bobwhite (*Colinus virginianus,* IACUC #11092207) 2-8 days and 5-12 days, respectively, after eclosion. After 3-6 days for *Ae. albopictus* and 7-14 days for *Cx. quinquefasciatus* (the period of time it takes the two species to incorporate blood-meals to provision their eggs) gravid females were rendered unconscious with CO_2 and counted. These females were brought to the field site in separate glass vials and released into their experimental enclosures (Allgood, 2011).

Table 1

Feeding schedule of puppy chow as mg per larvae.

	Cx. quinquefasciatus	Ae. albopictus
Day 0	0.1500	0.2000
Day 1	0.2500	0.2000
Day 2	0.2500	0.3000
Day 3	0.3000	0.4000
+yeast	+0.1100	$+4.5 \times 10^{-5}$
Day 4	0.3000	0.6000
Day 5	0.4000	0.6000
Day 6	0.5000	0.6000
Day 7 and on	0.7000	0.6000

To test the effect of water parameters on oviposition responses, tires were set up for the two focal species that contained variable depths with constant surface area and variable surface area with a constant depth. These tires (16 inch rim size) were located in an array of four cages (4' x 4 'x 8') on a concrete pad at The University of Southern Mississippi Science Park (31° 21' 11.78" N, 89° 21' 35.47" W). The cages were wood framed and covered with netting (20 openings per inch), and above each cage was an overhead shade

cloth to decrease direct sunlight. Tires were placed in an upright position at an angle approximately 110-100° from the ground within each cage. The oviposition attractant used to stimulate egg laying in female mosquitoes was a leaf infusion. The infusion was made by adding 40 g of dried, coarsely ground red maple leaves (*Acer rubrum*) in 1200 ml of reverse osmosis water and 400 ml of inoculum water, run through a 250 μm sieve, from a small pond located near the USM campus. The inoculum provided a source of bacteria and other microorganisms that would break down the leaves and result in the dissolved, volatile substances mosquitoes use as oviposition cues (Beier et al., 1983a; Bentley & Day, 1989; Clements, 2000).

For the depth water parameter, 6 depths were used: 1.0, 2.0, 5.0, 7.0, 10.0 and 14.0 cm with a constant surface area of 0.0050 m^2. A black plastic cup (600 mL) was placed in each tire to serve as an oviposition site. All depths were maintained by filling the cups with sand up to the depth needed. Black sand was also added as the top layer for each cup so it matched the color of the cup. The cups were held in place within the tire with black cardboard. For these trials, 30 gravid *Ae. albopictus* and 40 gravid *Cx. quinquefasciatus* were released separately into cages. A higher number of *Cx. quinquefasciatus* were released per cage because the infusion was not expected to be as strong of an oviposition cue for that species. Each of the 6 depths were replicated 3 times for *Ae. albopictus* and 4 times for *Cx. quinquefasciatus. Aedes albopictus* had lower cage replicates because not enough gravid females were produced to run a fourth cage.

In the second set of trials, 4 different surface areas were presented to each species, based on the approximate surface areas that were correlated for the depths of 1 cm, 5 cm, 10 cm, and 15 cm, 0.0135 m^2, 0.0363 m^2, 0.0685 m^2, and 0.0935 m^2, respectively. Instead of cups, lightweight aluminum trays that were spray painted black and folded to fit inside the rim of each tire were used. Infused water was added until the water level was 1 cm from the side of the container. A constant depth of 4 cm was used across all surface areas by filling up the container with black sand as necessary. If sand was not needed, a small amount that would not alter depth was added anyway to produce the water discoloration made by the sand. For this study there were 4 replicates for each species, and the same number of gravid females as the previous experiment were used. In the arrays, all treatment levels were available for females to choose for oviposition.

To collect eggs for *Ae. albopictus*, each container was lined with moist, brown paper towels to intercept eggs laid above the water line whereas rafts from *Cx. quinquefasciatus* females were collected directly from the water surface each day.

The first experimental run began on November 1, 2013, to test the effects of depth on oviposition of *Ae. albopictus*. Egg papers were collected four days later on November 4, 2013. The second experimental occurred on April 9, 2014, and tested the effects of surface area on *Ae. albopictus* whose egg paper were collected on April 12, 2014. *Culex quinquefasciatus'* response to surface area was tested on May 1 through May 3, 2014. The fourth run tested the effects of

surface area on *Cx. quinquefasciatus* oviposition, but due to two consecutive, heavy thunderstorms only five females laid so those results were discarded. The next run testing the same variable was more successful with more ovipositing females and began on May 12, 2013 and terminated on May 14, 2013.

After the assumption of normality was not upheld for any of the species or trials, non-parametric Kruskal-Wallis tests were run. Kruskal-Wallis tests were conducted on the number of *Cx. quinquefasciatus* rafts and *Ae. albopictus* eggs laid in tires over all cages separately. Depth and surface area were used as the independent factor and the number of eggs/rafts laid per tire as the dependent variable. Wilcoxon Each Pair post hoc tests were run to determine the differences among depths or surface areas that had a p value over the set α value of 0.05. Analyses were conducted with JMP® 10 (SAS Institute Inc., Cary, NC).

Results

Depth had a significant effect on *Ae. albopictus* oviposition (χ^2= 14.2902, df=5, p=0.0139). The Wilcoxon Each Pair test did not find a significant difference among depths in eggs laid; however, one or zero eggs were laid in the lowest depths (1-7 cm, Table 2) compared to the highest two depths, where most eggs were laid (Figure 1, Table 2). Surface area was not found to be significantly different for *Ae. albopictus* (χ^2= 7.2321, df= 3, p=0.0649). However, the p value found was very close to the α of 0.05 set for these tests. Further study, which would increase the power of the Kruskal-Wallis test, could result in a significant effect of surface area on oviposition preference. Based on the results from the

experiments, there is a likely preference for larger surface areas (Table 2, Figure 2).

Surface area affected the pattern of *Cx. quinquefasciatus* oviposition ($\chi^2 =$ 11.1419, df= 3, p= 0.0110) with females ovipositing in the largest surface area over all other smaller surface areas (Table 2, Figure 3). Depth did not have a significant effect for oviposition of *Cx. quinquefasciatus* ($\chi^2 =$9.9828, df= 5, p=0.0757). The number of rafts laid were evenly spread across all six depths indicating that even though the p value for this test was close to 0.05, it is unlikely father tests would increase the power enough, if the same pattern is found, to indicate a significant effect of depth on oviposition preference for *Cx. quinquefasciatus* (Table 2, Figure 4).

Table 2

Number of total eggs for Aedes albopictus and total rafts for Culex quinquefasciatus over oviposition depth and surface area treatments.

Depth	Aedes albopictus Eggs	Culex quinquefasciatus Rafts
1 cm	0	3
2 cm	0	4
5 cm	1	6
7 cm	1	6
10 cm	49	5
14 cm	67	13
Surface Area		
0.0135 m^2	1	0
0.0363 m^2	13	0
0.0683 m^2	60	7
0.0935 m^2	139	10
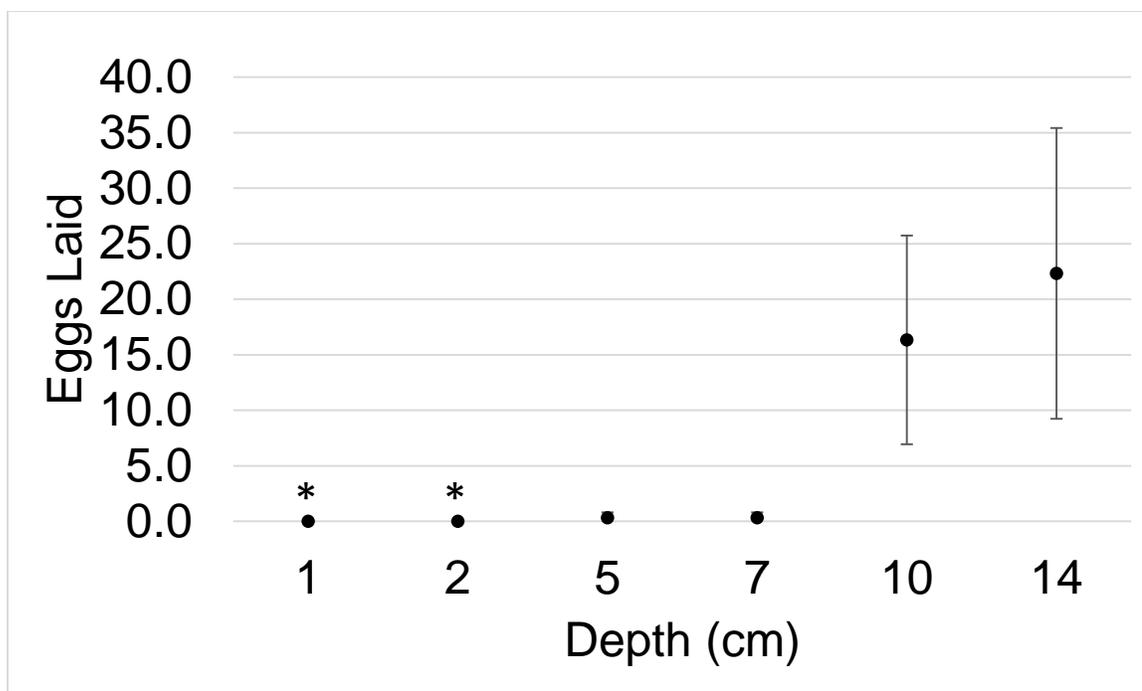
Figure 1. Average number of *Aedes albopictus* eggs laid in each depth. Points with an asterisk have no error bars because all values were zero.
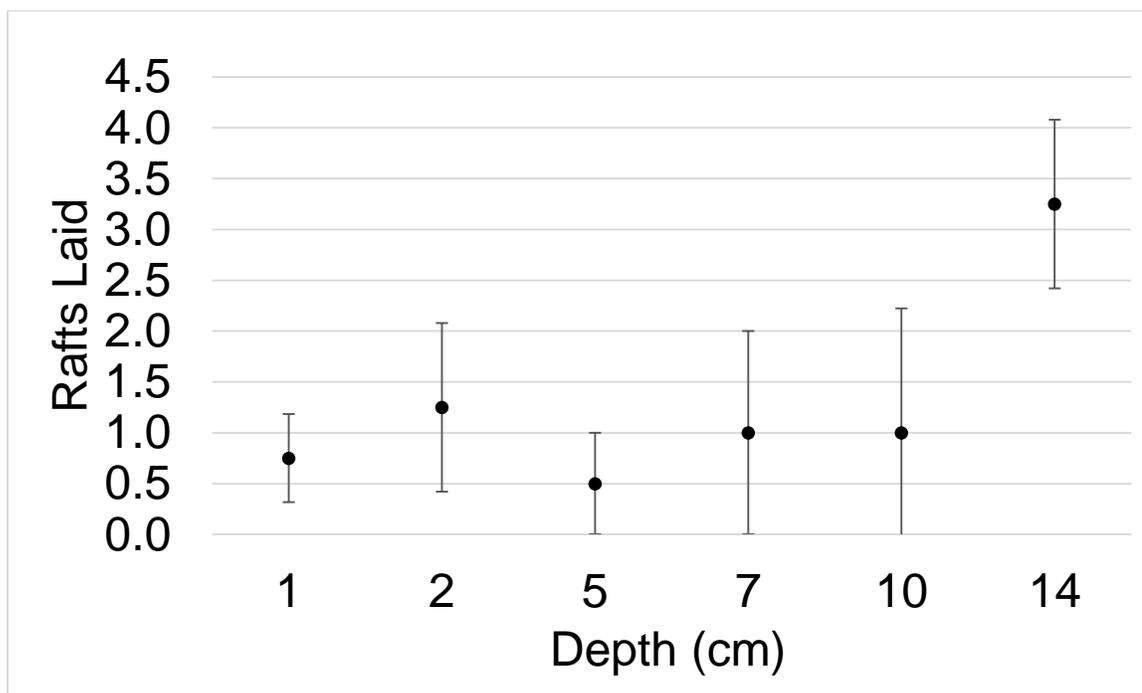
Figure 2. Average number of *Culex quinquefasciatus* rafts laid at each depth.
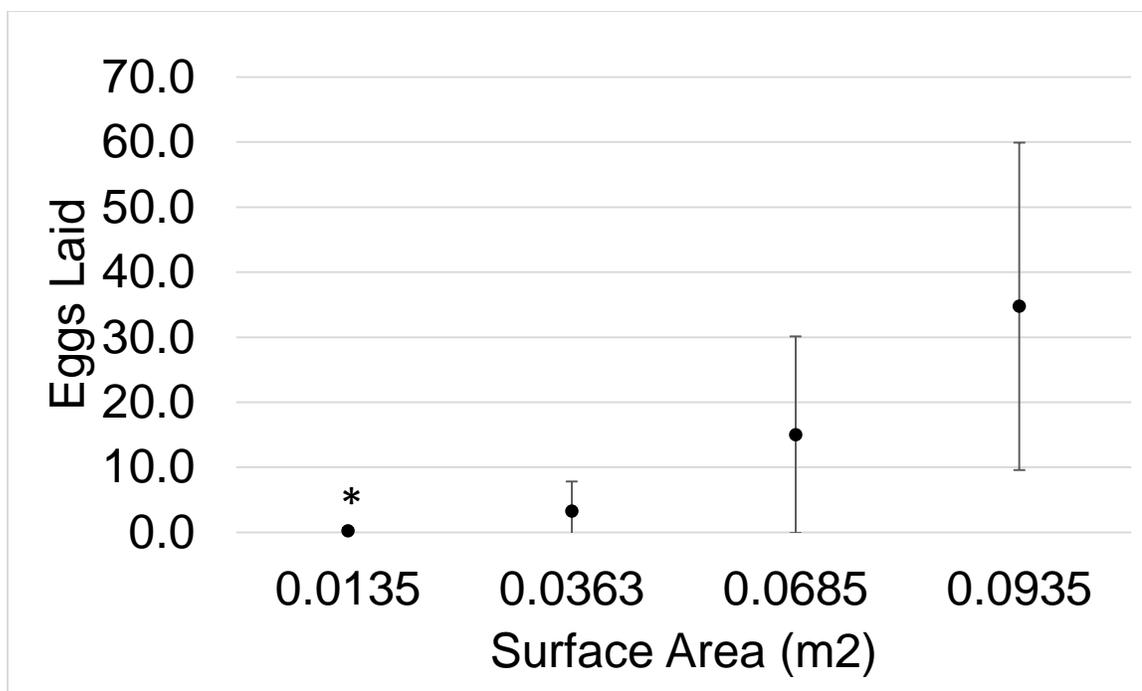
Figure 3. Aedes albopictus oviposition results from surface area experiment. Points with an asterisk have no error bars because all values were zero.

Figure 4. Culex quinquefasciatus oviposition results from surface area experiment. Post hoc Wilcoxon Each Pair results shown as letters. Each letter shared is a non-significant difference between surface areas. Points with an asterisk have no error bars because all values were zero.

Discussion

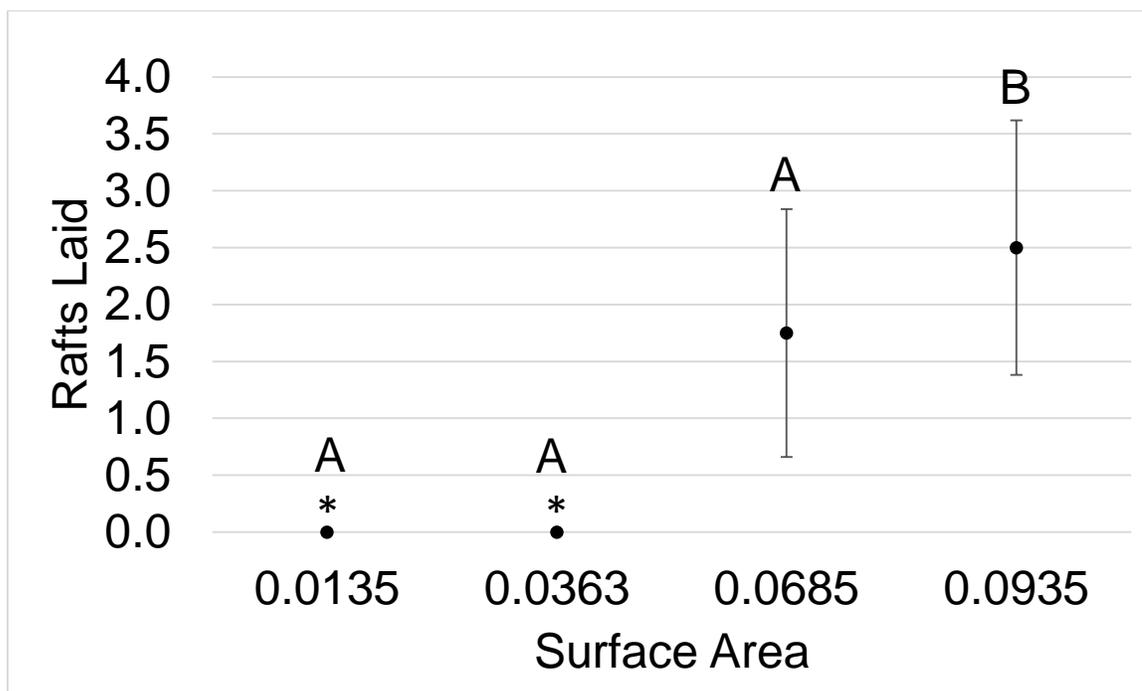Although *Ae. albopictus* showed a preference for deeper habitats, *Cx. quinquefasciatus* preferred large surface areas. Surface area was significant for *Cx. quinquefasciatus* even though there were only 17 females out of 160 that laid rafts. Additional studies would further confirm the significance of surface area on *Cx. quinquefasciatus* oviposition and would lower the variability of the oviposition signal (Figure 3).

This study does not exempt the importance of the effect of depth for *Cx quinquefasciatus* or the effect of surface area for *Ae. albopictus*, but it may indicate the priorities of each of these study species for different water parameters. *Aedes albopictus* is well-known for both its ravenous consumption of resources and high rate of survival (Costanzo et al., 2005; Sunahara & Mogi, 2002). Therefore, habitats that are deeper have a greater chance of holding more resources, but even if resources are not available, some larvae may survive (Sunahara & Mogi, 2002).

Another important distinction between *Ae. albopictus* and *Cx. quinquefasciatus* is that one female *Ae. albopictus* can skip oviposit, laying its eggs in multiple different habitats. By not laying all of her eggs in one metaphorical basket, she can spread her chances of offspring success. *Culex quinquefasciatus* needs to make only one choice, but with that choice, she needs to be certain of larval survival. Although females can lay more than one raft over a lifetime, a blood-meal needs to be taken before each raft is oviposited, thus, limiting the number of habitats she is capable of utilizing at any one time.

It is important to understand what effect parent generations have on their offspring. The decision of the female mosquito spells out the future of her larvae; choosing a poor habitat will result in poor performance of larvae that are otherwise well-suited genetically. The conditions of the habitat at the time of oviposition will not guarantee that habitat will be suitable in the future. For instance, an aquatic habitat that is deeper than other habitats in the vicinity may last longer if a drought occurs. It bears keeping in mind that the specific requirements of each species also needs to temper the female's choice as she prepares to oviposit. Such a decision between future suitability and species specific suitability is quite evident in the oviposition choices of *Ae. albopictus* and *Cx. quinquefasciatus*.

The hypothesized outcomes did not match the results for *Ae. albopictus*, but preference for larger surface area was predicted for *Cx. quinquefasciatus.* Owing to this fact, the oviposition results from this work are telling. For each significant variable there were instances of mosquitoes leaving an undesirable depth or surface area completely without eggs or rafts (Table 2). These instances show mosquitoes cueing in on specific water parameters, especially *Cx. quinquefasciatus* honing in on water parameter cues that are best for their offspring and forced to wait until another blood-meal to choose an alternate habitat for other offspring. The preference for higher surface areas and no preference for depth would indicate that the *Cx. quinquefasciatus* females could be ovipositing in habitats where their larvae would have a more successful time foraging.

CHAPTER III

LARVAL PERFORMANCE AND AQUATIC PARAMETERS

Introduction

Mosquito larvae often exhibit species-specific behaviors that are used to obtain food. Larvae vary in their trophic behaviors ranging from predators to collectors, the latter category including both filtering and gathering (Clements, 2000; Merritt et al., 1992). Filterers typically spend most of their time at the surface of the water in the neuston zone and obtain small particulate matter by either actively making currents or passively foraging on the organic particulates and microorganisms (Merritt et al., 1992). Collector-gatherers consume most of their food by obtaining small, non-attached particulate matter on underwater surfaces mostly located on the bottom of aquatic habitats or on submerged plant or detrital surfaces (Clements, 1999; Clements, 2000; Merritt et al., 1992).

Differences in the depth at which mosquitoes forage can vary based on instar and genus (Merritt et al., 1992). Both *Culex* and *Aedes* early instars tend to spend more time at the surface, and are both classified as filter feeders at this stage (Merritt et al., 1992). As *Aedes* mature they increase their time as collector-gatherers at the bottom in combination with some suspension feeding at the surface (Merritt et al., 1992*). Culex* species increase filtering in the water column and do some collecting-gathering toward the bottom as they reach later instars (Merritt et al., 1992).

Water depth can have a differential effect on mosquito developmental rates for different species. For instance, *Ae. aegypti* has a higher tolerance for

deeper depths than other species like *Cx. pipiens* (Briegel, 2002)*.* The successful development of more *Aedes* larvae to the adult stage in different depths may indicate that *Aedes* behavior may aid foraging success in deeper habitats compared to other species. Although all species have successful eclosion with shallow depths, some species may have more specializations for surface feeding (e.g., filtering) than other species; thus, these surface feeders may be more productive when detritus is closer to the surface (Briegel, 2002).

Changes in community structure affect the distribution of aquatic species. The size of aquatic habitat can change the patterns of colonization of many insect species including chironomids (midges), scirtids (marsh beetles), and ceratopogonids (biting midges) that readily exploit larger microcosms (Sota, 1996). Mosquitoes, on the other hand, are more likely to colonize smaller microcosms (Sota, 1996).

Depth also has the potential to change community structures and habitat permanence, which in turn may affect mosquito growth and development. Sunahara and Mogi (2002) found that *Aedes albopictus* prefer shallower bamboo microcosms due to the high desiccation resistance of *Ae. albopictus* eggs and their ability to rapidly develop under these severe conditions. The opposite trend was seen in *Ae. albopictus'* counterpart, *Tripteroides bambusa*, which utilizes slightly deeper waters that are generally more permanent and habitats where *Ae. albopictus* is competitively suppressed even though there are comparatively more resources in these larger microcosms (Sota, 1996). A similar trend was observed in tree-hole inhabiting mosquito communities in the

southeastern U.S., where *Aedes triseriatus*, a common tree-hole species with desiccation-resistant eggs were observed to inhabit smaller, less permanent tree-hole habitats that they can colonize rapidly compared to other mosquito species (Bradshaw & Holzaphel, 1983). From this study one can deduce that there can be a difference between species and genera in habitat preference as well as success in different types of habitats. Coping with small habitats as well as competing with co-occurring species in these habitats can affect aspects of mosquito performance in container systems.

Microcosm scaling, based on changes in depth and surface area, also affects the development and survival of *Culex pipiens* (Wynn & Paradise, 2001); lower surface areas negatively affected survival in this species. Development time was found to increase and larval size decreased when larvae were in low resources, deep depth, and low surface area microcosms (Wynn & Paradise, 2001). In deeper depths, larval survival was significantly lower than that of control conditions that were two times shallower (Wynn & Paradise, 2001). This difference in survival is attributed to the lower surface area to depth ratio that would have increased competition with other larvae (Wynn & Paradise, 2001). Thus, increasing depth along with lower surface area in a competitive environment can cause changes in larval survival.

Depth also has an effect on the relative amounts of energy that mosquitoes are able to accumulate during their larval stage (Timmermann & Briegel, 1993). In *Anopheles* species, such as *An. albiamanus, An. gambiae,* and *An. stephens -* all open water species, there was no survival past a depth of

2 cm (Timmermann & Briegel, 1993). Their greatest body calorie content was seen at their highest eclosion rates in depths of 1-2 cm (Timmermann & Briegel, 1993). In *Aedes aegypti,* maximal survival, eclosion success, and caloric content were observed at depths of 1 cm (Timmermann & Briegel, 1993). Unlike *Anopheles*, *Aedes aegypti* did survive in depths below 2 cm. Below 2 cm survival and eclosion success decreased for this species, but after an initial drop, caloric content and size increased with depth (Timmermann & Briegel, 1993). This finding indicates that *Aedes aegypti* may suffer higher mortality in deeper waters but that the larvae that do survive are larger.

My study will look into the effects of depth on *Cx. quinquefasciatus* and *Ae. albopictus* as well as the effect competition at different depths may have on larval performance. Shallower depths have been shown to increase the survival of mosquito larvae which may be due to resources laying closer to the surface of the water (Briegel, 2002; Timmerman & Briegel, 1993). Resource position close to the water surface is important because that is where *Culex* larvae mainly forage and where all mosquitoes are required to access air for respiration. The effects of depth may also be effected by the intra- and interspecific competition between larvae. The presence of *Ae. albopictus* larvae in smaller microcosm may show a competitive advantage these species exhibit when placed in these habitats (Bradshaw & Holzaphel, 1983; Sota, 1996; Sunahara & Mogi, 2002). Differences in these species may be partially explained by differences in foraging behaviors where *Aedes* tend to forage directly on unattached particles off the surfaces of detritus, but *Culex* filter close to the surface of the water and

only occasionally filter at deeper depths (Clements, 1999; Clements, 2000; Merritt et al., 1992).

Methods

Larvae for this study were taken from F2 eggs from an *Aedes albopictus* colony and F3 rafts from a *Cx.* quinquefasciatus colony. The colonies from where the eggs were obtained are raised according to the guidelines and feeding schedule previously described in Chapter II (Table 1). Newly hatched first instars mosquito larvae where transferred to the experimental microcosms. These microcosms were set up to assess mosquito performance with density combinations of larval *Aedes albopictus*:*Culex quinquefasciatus* ratio of 5:0, 0:5, 5:5, 10:0, 0:10, and 10:10. These densities are similar to high and average larvae to water volume densities of *Aedes albopictus* found in tires across Mississippi (Yee, unpublished data). Larvae were raised in 4 common water depths found in field tires 5.0 cm, 7.0 cm, 10.0 cm, and 14.0 cm (Yee, unpublished data). In addition, surface area was held constant at 0.0050 m^2 across different depths and larval densities. Surface area was controlled to reduce differences of the effects of hypoxia on larvae (Gerberg, 1970). Prior to the introduction of larvae, containers received 3 g of senescent red maple (*Acer rubrum*) leaves and 25.0 mg of cricket leg to provide food for developing larvae. Two replicates of each species combination (6) and by water depth (4) were used and housed in a 25°C environmental chamber on a 15:9 light day photoperiod cycle to simulate local summer conditions (n = 48 containers, 480 mosquitoes).

Larval mosquitoes were checked every day where pupation and eclosion dates were recorded along with the treatment and replicate information. After they emerged, adult mosquitoes were dried at 50 °C for ≥ 48 hrs. Adult mosquitoes were weighed using a XP2U Mettler Toledo ultramicrobalance (Columbus, Ohio) to the nearest 0.0001 mg.

To understand the effects of treatment combinations on population performance for both species, an index of population performance (λ') was calculated for each species. This index estimates the finite rate of increase and is derived from the per capita rate of increase (*r*) (Livdahl & Sugihara, 1984). This index is defined as follows:

$$\lambda' = \exp(r') = \exp\left[\frac{ln\left[\frac{1}{N_0}\sum_x A_x f(w_x)\right]}{D + \left[\frac{\sum_x x A_x f(w_x)}{\sum_x A_x f(w_x)}\right]}\right]$$

where N_0 is the initial number of females in a cohort (assumed 50%), *D* is the time from eclosion to first oviposition, A_x is the number of females that eclose on day *x*, w_x is the mean mass of females that eclose on day *x*, and $f(w_x)$ is a function that estimates fecundity from regressions based on female mass. In this case, *D* is assumed to be 5 days for *Cx. quinquefasciatus* (Subra, 1981) and 12 days for *Ae. albopictus* (Juliano, 1998). The equation for *Ae. albopictus* estimated fecundity is $f(w_x) = 19.5+152.7(w_x)$ (Lounibos et al., 2002), although for *Cx. quinquefasciatus*, a direct mass to fecundity relationship has not been established. Instead, the use of wing length (*l*) to fecundity ($f(l)= -123.88 + 90.31l$) was adapted by using regressions from female wing length to female

mass to solve for wing length ($l= [(w+ 0.162)/0.0021l]^{1/3}$) to give rise to the

fecundity mass relationship, $f(w)= -123.88+90.31*[(w + 0.162)/0.12]^{1/3}$ (McCann

et al., 2009). Values of λ' > 1 indicate that species populations are growing

whereas values λ' < 1 show negative growth. Values of λ' that equal 1 shows a

stable population with no growth or decline.

Values of λ' were generated for each container for each species.

Differences in λ' among treatment combinations (density, depth) were assessed

using a two way analysis of variance (ANOVA). An additional two-way ANOVA

was then used to determine differences for adult male and female mass

separately in response to treatment combinations (density, depth). For male *Cx.

quinquefasciatus* mass was found not to be normal but was normalized when

transformed with x^{-1}. All other masses were found to be normal and were not

transformed. For significant effects Tukey post hoc tests were run to resolve

differences. A non-parametric Kruskal-Wallis test was run for the non-normal

development time for each species and, males and females. A Wilcoxon Each

Pair post hoc test was run on each significant effect. Because it is likely that

mass and development time are dependent on one another, a Spearman's

Correlation was run for each species and sex. All analyses were conducted using

JMP® 10 (SAS Institute Inc., Cary, NC).

Results

All populations were shown to have positive growth (λ' >1.0). It was found that for *Ae. albopictus* population growth was significantly different among the densities used (Table 3). Population growth was higher in the high densities (10:10 and 10:0) compared to the low densities (5:5 and 5:0). Water depth and the interaction of density and depth were not significant (Table 3).

For *Cx. quinquefasciatus*, the density by depth interaction was found to be significant (Table 3). As seen in Figure 5, the densities 0:5 and 10:10 were significantly different from one another along with the difference between the 7 cm and 14 cm depth within the 10:10 density. In low densities for 10 and 14 cm (0:5 to 5:5) microcosms, there was no difference in the λ' of *Cx. quinquefasciatus.* As density increases there was a significant difference in the 10 and 14 cm λ' between inter and intraspecific competition densities (0:5 to 0:10; 5:5 to 10:10). At 5 and 7 cm there was a significant difference between low densities (0:5) with a higher λ' to the low interspecific competition density (5:5) as *Aedes albopictus* were added. This low λ' from the 5:5 density was significantly different from the higher intra (0:10) and interspecific (10:10) competition densities.

Table 3

A two way ANOVA on values of λ' for Aedes albopictus and Culex quinquefasciatus with depths spanning from 5 to 14 cm and density of the two species added to the microcosms. Significant variables are in bold.

Aedes albopictus	df	F Value	p Value
Density	3	19.3786	**<0.0001**
Depth	3	1.0582	0.3960
Density*Depth	9	1.4122	0.2664
Culex quinquefasciatus			
Density	3	86.4181	**<0.0001**
Depth	3	2.2139	0.1287
Density*Depth	9	3.2870	**0.0204**
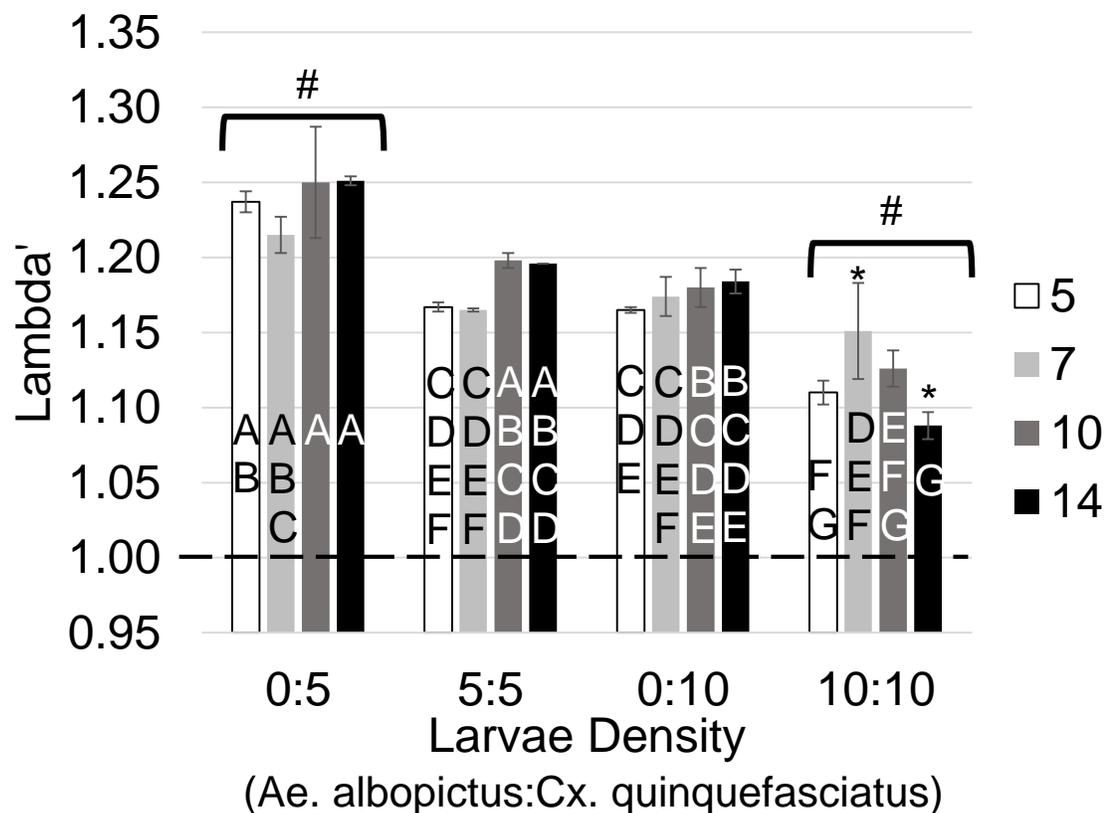
Figure 5. The average λ' for *Culex quinquefasciatus* in relation to depth and density of larvae in microcosms containing *Cx. quinquefasciatus*. All Tukey-post Hoc test results are shown. Bars that share a letter are not significantly different from one another. The symbol "*" denotes a significant between depths, and the symbol "#" denotes a significant difference among densities. The dashed line shows the λ' value where there is no population growth.

Mass and development time were also affected by density and depth. For *Ae. albopictus* females, there was a significant difference in mass (Table 4) between the 14 cm and 7 cm depth, with heavier females produced in the deeper containers; mass in other depths were not significant (Fig 6).

Density was also significant for female *Ae. albopictus* where the lower densities (5:0, 5:5) were significantly different from the higher densities (10:0,10:10; Table 4). For *Ae. albopictus* males, mass in the 10:10 density was significantly lower than other densities (Table 4). Male *Cx. quinquefasciatus* was significantly different between 0:5, 5:5, and 10:10 densities with mass decreasing as density increases (Table 4). The 0:10 density was not significantly different from 0:5 or 5:5 densities. Female *Cx. quinquefasciatus* mass was found to be significantly different between the higher 0:5 and the 5:5, 0:10, and 10:10 densities (Table 4).

Table 4

A two way ANOVA of mass for both species and sexes with density, depth, and interaction variables. Significant values are in bold.

Aedes albopictus Female		df	F Ratio	p Value
	Density	3	31.3164	**<0.0001**
	Depth	3	3.3160	**0.0488**
	Density*Depth	9	0.7980	0.6241
Aedes albopictus Male				
	Density	3	14.2856	**<0.0001**
	Depth	3	0.4236	0.7391
	Density*Depth	9	1.4303	0.2645

Table 4 (continued).

Culex quinquefasciatus Female

	Density	3	11.5197	**0.0004**
	Depth	3	1.0097	0.4158
	Density*Depth	9	0.7246	0.6810

Culex quinquefasciatus Male

	Density	3	12.4290	**0.0002**
	Depth	3	0.9050	0.4604
	Density*Depth	9	0.9142	0.5370
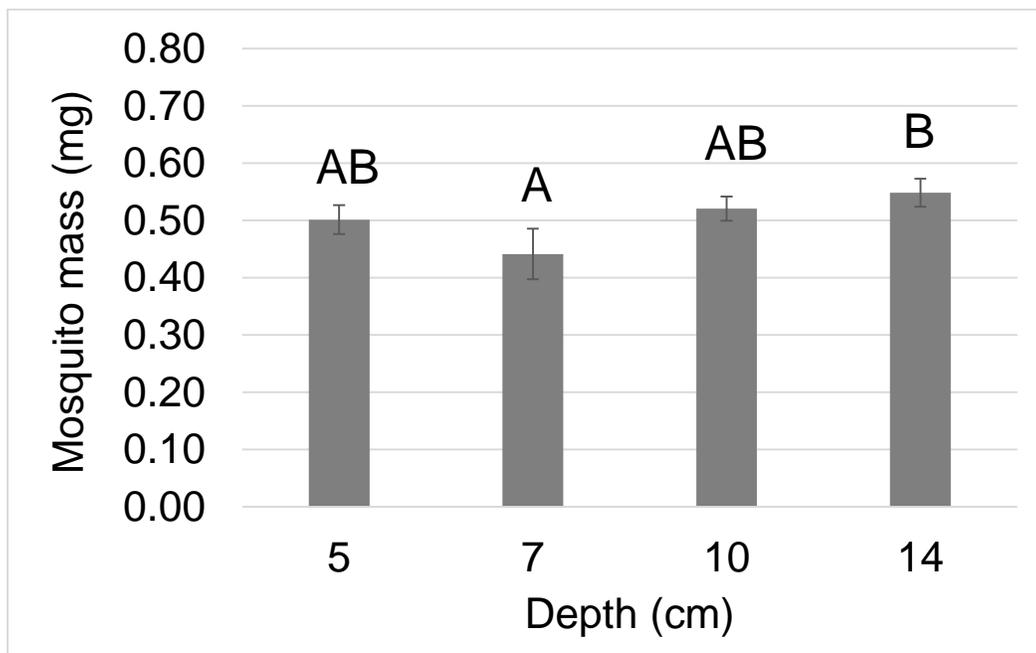
Figure 6. The effect of depth on mass of female *Ae. albopictus*. Tukey post Hoc test results are shown. Those depths that share a letter are not significantly different.

Density and depth did not affect development time of either male or female *Ae. albopictus*. However, density did affect male and female *Cx. quinquefasciatus* (Table 5)*.* The effects were the same for both sexes with a longer development times in microcosms that also housed *Ae. albopictus* (densities 5:5;10:10, Figure 7 and 8).

Table 5

Kruskal-Wallis test results for development time of Aedes albopictus and Culex quinquefasciatus with density and depth variables. Significant effects are in bold.

Aedes albopictus Female		df	X^2	p Value
	Density	3	2.1088	0.5501
	Depth	3	2.1088	0.5501
Aedes albopictus Male				
	Density	3	3.3229	0.3445
	Depth	3	4.8549	0.1827
Culex quinquefasciatus Female				
	Density	3	25.7650	**<0.0001**
	Depth	3	0.4771	0.9239
Culex quinquefasciatus Male				
	Density	3	24.4181	**<0.0001**
	Depth	3	1.1612	0.7623
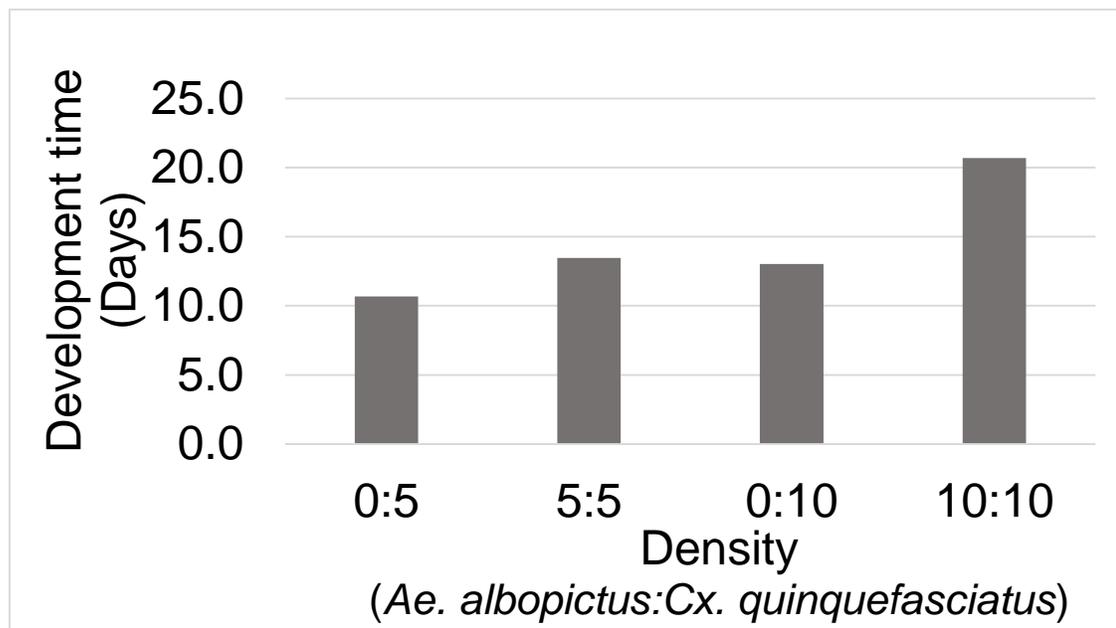
Figure 7. Culex quinquefasciatus female development time showing results from density. All development times from each density are significantly different from one another.
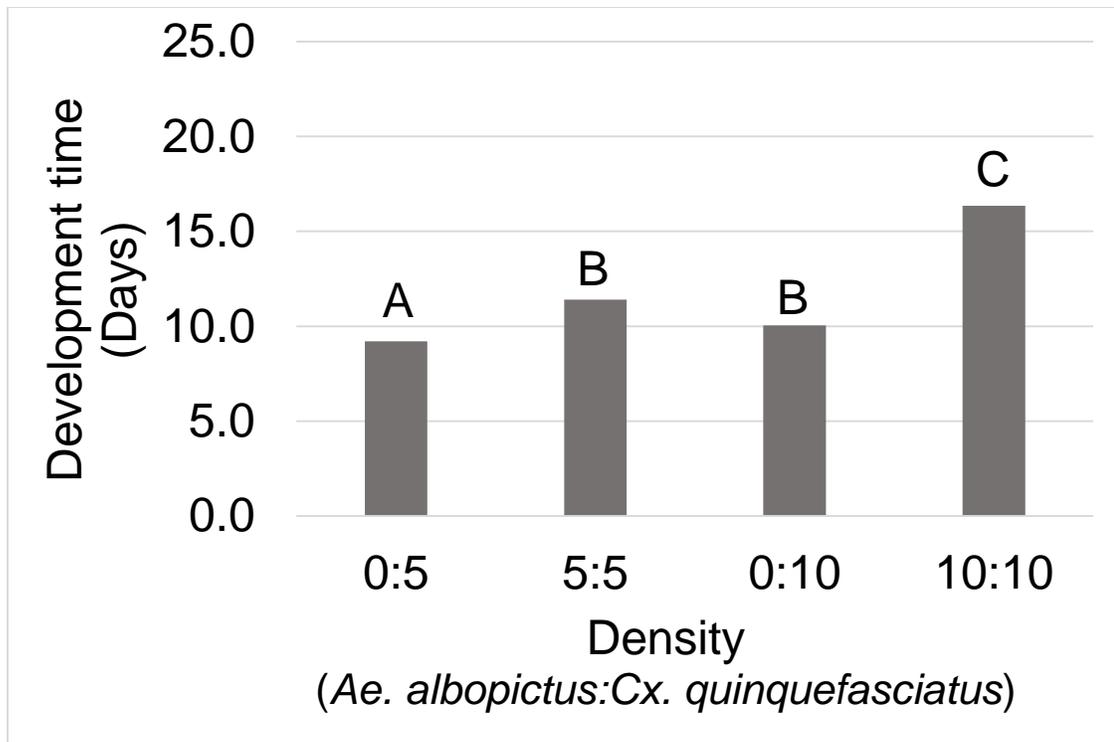
Figure 8. Culex quinquefasciatus male development time showing results from density. Post hoc Wilcoxon Each Pair results shown as letters. Each letter shared is a non-significant difference between densities.

For both male and female *Cx. quinquefasciatus* there was a correlation between development time and mass (female $\rho(30)=-0.5525$, $p=0.0013$; male $\rho(31)=-0.7282$, $p<0.0001$). From this analysis the negative correlation suggests an increased development time with decreasing mass. This correlation indicates that some of these effects could have been spurious, but it is likely not due to the very low p values for all significant effects. There was no correlation between development time and mass in *Ae. albopictus* (female $\rho(30)=0.0095$, $p=0.9597$; male $\rho(29)=-0.1417$, $p=0.4551$).

Discussion

Performance of larvae was mostly affected by density and less so by water depth. In most cases, both species at lower densities were found to have higher values of λ', higher, mass, and decreased development time. Thus, the hypothesis that depth will impact *Ae. albopictus* more than *Cx. quinquefasciatus* was not supported. The only effect of depth on *Ae. albopictus* was higher female mass at 14 cm compared to 7 cm. *Culex quinquefasciatus* did have a significant density by depth interaction indicating that at deeper depths interactions with more *Ae. albopictus* negatively impacts population growth. However, at shallower depths there was no impact of *Ae. albopictus* density (5:5 and 10:10) on the population growth of *Cx. quinquefasciatus* compared to similar densities of microcosms only containing *Cx. quinquefasciatus* (0:10, Figure 3). This observation does not support the hypothesis that *Cx. quinquefasciatus* will compete more with *Ae. albopictus* at shallower depths, as values for λ' were not different for intra and interspecific densities.

In the containers with the highest interspecific competition, λ' for *Cx. quinquefasciatus* was higher in the 7cm compared to the 14 cm depth. This may indicate that deeper depth with higher densities could be detrimental to populations for this species. High densities may have been stressful, especially in the deep depth environments. A likely reason for this effect of depth is indirect competition with *Ae. albopictus* where *Ae. albopictus* may have consumed resources in the lower portions of the microcosm before *Cx. quinquefasciatus* could access it in the upper portions of the microcosm.

Depth also affected *Aedes albopictus*' mass. Unlike *Cx. quinquefasciatus*, the smallest females were produced in the 7 cm depth, and largest females were produced in the 14 cm depth. This discrepancy between the effect of depth on these species can be explained because the presence of the *Ae. albopictus* effects the growth and development of *Cx. quinquefasciatus*. In habitats that are deep and that contain many *Ae. albopictus* those *Ae. albopictus* may negatively the development of *Cx. quinquefasciatus* which explains the *λ*' for *Cx. quinquefasciatus.* The reason *Ae. albopictus* seemed to perform poorly at 7 cm and best at 14 cm is not known, and mechanical reasons for this difference should be explored. It is possible at this depth, *Cx. quinquefasciatus* and *Ae. albopictus* may have had similar access to resources in a way that helped *Cx. quinquefasciatus* compete better against *Ae. albopictus.*

CHAPTER IV

CONCLUSION

In previous studies surface area has been shown to effect the performance of larvae; however, an oviposition response to depth and how it affects subsequent larval performance has not been investigated (Arav & Blaustien, 2006; Lester & Pike, 2003; Sunahara et al., 2002). Even individual studies on oviposition choice or larval performance with respect to depth are limited (e.g., Briegel, 2002; Reisen et al., 2005). Though there have been some studies done separating differences in depth and surface area on life history strategies in mosquitoes, they have not been done on both larval performance and oviposition (Reiskind & Zarrabi 2012; Wynn & Paradise 2001). These studies are few, and there is little overlap in species, food resources that were used, and competition treatments among larvae. In this study, the oviposition of both species and performance with depth was used along with intra- and interspecific competition. This study offers the first test that separates effects of depth and surface area on oviposition and the consequences of depth for larval performance.

It was shown that *Ae. albopictus* preferentially oviposit in deeper habitats, especially habitats that are deeper than 10 cm. However, population growth was not affected by depth; thus, it is unlikely that oviposition preference should affect the larvae for this species. Further studies on the effect of depth should also look at using a greater variation of food sources as the type of food may alter larval performance. For instance, Wynn and Paradise (2001) showed that when there

was a high level of food available, changes in biomass and development time were indistinguishable with changes in container size. When food resources were decreased, those differences became more pronounced (Wynn & Paradise, 2001). In this study, a highly nutritious food source was used (i.e., crickets) to ensure enough adult *Cx. quinquefasciatus* survived in the microsms. Animal detritus contains high amounts of nitrogen, and the amounts used were likely well above what would be needed to nutritionally stress *Ae. albopictus* and effect *Ae. albopictus* mortality (Winters & Yee, 2012). Thus, lower amounts of some nutrients, such as nitrogen, could have affected *Ae. albopictus* λ' at some depths.

Aedes albopictus* did appear to affect the performance of *Cx. quinquefasciatus* at different depths*, while having their own population performance unaffected. The nature of the mechanism for this interaction is unknown; however, *Ae. albopictus* is a superior competitor to other *Culex* species (Costanzo et al., 2005; Yee & Skiff, 2014). The likely reason for these observations are the differences between feeding behaviors between *Ae. albopictus* and *Cx. quinquefasciatus*. *Aedes albopictus* has been shown to forage mostly on the surface of detritus rather than filter at the surface of the water like *Cx. quinquefasciatus* (Yee & Skiff, 2014; Yee, Kesavaraju, & Juliano, 2004a; Yee, Kesavaraju, & Juliano, 2004b)*. By foraging on detritus and not filter feeding, *Ae. albopictus* is a more efficient forager and removes resources from the environment before the resources can enter the upper layer of the habitat. When only *Cx. quinquefasciatus* are competing against one another, they all have

equal chances to obtain resources. This interspecific competition does decrease

λ' but is not affected by depth like competition with *Ae. albopictus*.

Interestingly enough, the lowest mass for *Aedes albopictus* occurred at 7

cm, the same depth were the highest λ' value for *Cx. quinquefasciatus* was

obtained. It is important to note that mass was lower for *Ae. albopictus*

throughout all of the densities and not just those with *Cx. quinquefasciatus*. It is

possible that *Ae. albopictus* may be less effective at obtaining resources at this

depth or that feeding differences between the species were less pronounced in

this environment. Thus, depth may have mitigated the interaction between *Cx.*

quinquefasciatus and *Ae. albopictus* which allowed higher λ' values for *Cx.*

quinquefasciatus even at high larval densities (e.g., 5:5 and 0:10 (Figure 3).

Despite the effect of depth with the presence of *Ae. albopictus* larvae at

high densities, *Cx. quinquefasciatus* females had a high oviposition preference

for larger surface areas and no preference for depth. The absence of *Ae.*

albopictus adults while ovipositing may have resulted in female *Cx.*

quinquefasciatus placing their eggs in any depth without the threat of their larvae

being out competed by *Ae. albopictus.* However, interactions between

ovipositioning adults between these species have not been tested.

From these experiments it can be seen that oviposition and larval

performance are linked for *Ae. albopictus* but not for *Cx. quinquefasciatus*. *Aedes*

albopictus preferred deeper depths in the oviposition experiment, and while λ'

was not effected by depth, there was an effect of depth on adult mass. The effect

of depth as an oviposition cue benefited the larvae that occurred in those

habitats. Decisions in relation to depth by *Ae. albopictus* females in this study made sense from a fitness standpoint.

Culex quinquefasciatus did not have a preference for depth, but depth and density interacting together did affect λ' values for *Cx. quinquefasciatus*. Considering *Cx. quinquefasciatus* were housed with only other *Cx. quinquefasciatus* while ovipositing, it is easy to see why there was no discrepancy in oviposition. Larval performance with only *Cx. quinquefasciatus* in the microcosm showed no difference in λ' values over any one density. However, seeing that there was a difference when larvae were placed under high competition stress with *Ae. albopictus*, there could be effect on oviposition with depth that was not seen in this experiment. If this is the case, by not being able to cue in on appropriate depth environments *Cx quinquefasciatus* females could be placing their larvae in inappropriate habitats if another mosquito, like *Ae. albopictus,* entered the environment with their offspring. In Yee, Kneitel, and Juliano (2010) different environmental parameters like microorganisms predict the presence of early instar *Cx. restuans* and *Ae. albopictus* but do not predict the presence of late instars. The absence of interaction with *Ae. albopictus* in the oviposition cages could explain the discrepancy between *Cx. quinquefasciatus'* observed depth oviposition and negative impacts of depth on larvae performance. Early instars would have the same access to resources that *Ae. albopictus* early instar larvae would. As *Ae. albopictus* develop, they rely more on resources obtained from foraging on communities related to detritus giving them the upper hand and reducing the performance of *Cx. quinquefasciatus.* Future

work with water parameter studies could focus on how two interacting species would oviposit at different densities. One might predict that in the case of *Cx. quinquefasciatus* laying in the presence of *Ae. albopictus* that it is reasonable to assume that they may have a preference for shallow water where their offspring will be better able to compete with *Ae. albopictus* larvae. This prediction may be true especially if *Ae. albopictus* are in high densities similar to those found in the field, as this species females preferentially laying eggs in deeper habitats.

This work supports the conclusion that depth has an effect on the oviposition behavior of *Aedes albopictus* as well as the performance of their larvae. This observation is important as oviposition choices, for mosquitoes do effect trends we see in the field. The co-occurrence of *Ae. albopictus* and *Cx. quinquefasciatus* in the field cause decreased performance of *Cx. quinquefasciatus* (Costanzo et al., 2005; Lounibos et al., 2002; Yee, 2008). However, as seen from this study, it may not be that simple. High larval densities did lead to decreased population growth for *Cx. quinquefasciatus* as expected based on previous studies (Costanzo et al., 2005). The exception to this was their performance at 7 cm where *Cx. quinquefasciatus* exhibited the same *λ'* value as in medium densities (5:5, 0:10). It can be assumed that by some mechanism, *Ae. albopictus* does not perform well enough at this depth to affect the performance of *Cx. quinquefasciatus*. It is important to understand why there was less competition effect on *Cx. quinquefasciatus*. Behavior studies on *Cx. quinquefasciatus* and *Ae. albopictus* should also be done at this depth to determine the mechanisms for this observation.

APPENDIX

The University of
Southern Mississippi

Institutional Animal Care
and Use Committee

118 College Drive #5147
Hattiesburg, MS 39406-0001
Tel: 601.266.6820
Fax: 601.266.5509
www.usm.edu/spa/policies/animals

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of
Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in
accordance with regulations by the United States Department of Agriculture and
the Public Health Service Office of Laboratory Animal Welfare. The project
expiration date is noted below. If for some reason the project is not completed by
the end of the three year approval period, your protocol must be reactivated (a new
protocol must be submitted and approved) before further work involving the use of
animals can be done.

Any significant changes (see attached) should be brought to the attention of the
committee at the earliest possible time. If you should have any questions, please
contact me.

PROTOCOL NUMBER: **11092207**
PROJECT TITLE: **Environmental Filters and Medically**
Important Container Mosquitoes
PROPOSED PROJECT DATES: **10/01/2011 to 09/30/2014**
PROJECT TYPE: **Renewal/Continuation of a Previously Approved Project**
PRINCIPAL INVESTIGATOR(S): **Donald Yee, Ph.D.**
COLLEGE/DIVISION: **College of Science & Technology**
DEPARTMENT: **Biological Sciences**
FUNDING AGENCY/SPONSOR: **National Institutes of Allergy**
and Infectious Disease (NIH)
IACUC COMMITTEE ACTION: **Full Committee Review Approval**
PROTOCOL EXPIRATION DATE: **09/30/2014**

Jodie M. Jawor, Ph.D.
IACUC Chair

9/30/11
DATE

REFERENCES

Allgood, D.W. (2011). *Influence of detritus levels and organic pollution on interspecific resource competition, oviposition, behavior, and larval survival of two tire-inhabiting mosquito species (Diptera: Culicidae).* (Master's thesis). University of Southern Mississippi, Hattiesburg, Mississippi.

Alto, B.W., Lounibos, L.P., Higgs, S., & Juliano, S.A. (2005a). Larval competition differentially affects arbovirus infection in *Aedes* mosquitoes. *Ecology*, *86*, 3279-3288.

Arav, D., & Blaustein, L. (2006). Effects of pool depth and risk of predation on oviposition habitat selection by temporary pool dipterans. *Journal of Medical Entomology, 42*, 493-497.

Barrera, R. (1996). Competition and resistance to starvation in larvae of container-inhabiting Aedes mosquitoes. *Ecological Entomology*, *21*, 117-127.

Beehler, J.W., Lohr, S., & DeFoliart, G. (1992). Factors influencing oviposition in *Aedes triseriatus* (Dipera: Culicidae). *The Great Lakes Entomologist, 25*, 259-264.

Beehler, J.W., Millar, J.G., & Mulla, M.S. (1993). Synergism between chemical attractants and visual cues influencing oviposition of the mosquito, *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Chemical Ecology, 19,* 635-644.

Beier, J.C., Patricoski, C., Travis, M., & Kranzfelder, J. (1983a). Influence of

    water chemical and environmental parameters on larval mosquito

    dynamics in tires. *Environmental Entomology, 12*, 434-438.

Beier, J.C., Travis, M., Patricoski, C., & Kranzfelder, J. (1983b). Habitat

    segregation among larval mosquitoes (Diptera: Culicidae) in tire yards in

    Indiana, USA. *Journal of Medical Entomology, 20,* 76-80.

Bentley, M.D., & Day, J.F. (1989). Chemical ecology and behavioral aspects of

    mosquito aspects of mosquito ovipostion. *Annual Review of Entomology*,

    34, 401-421.

Blaustein, L., Olsfeld, R.S., & Holt, R.D. (2010). A community-ecology framework

    for understanding vector and vector-borne disease dynamics. Israel

    Journal of Ecology and Evolution, 56, 251-262.

Blaustein, L., & Chase, J.M. (2007). Interactions between mosquito larvae and

    species that share the same tropic level. *Annual Review of Entomology*,

    52, 489-507.

Bradshaw, W.E., & Holzapfel, C.M. (1983). Predator-mediated, non-equilibrium

    coexistence of tree-hole mosquitoes in southeastern North America.

    Oecologia, *57*, 239-256.

Briegel, H. (2002). Physiological bases of mosquito ecology. *Journal of Vector*

    Ecology, 28, 1-11.

Carpenter, S.R. (1983). Resource limitation of larval treehole mosquitoes

    subsisting on beech detritus. *Ecology, 64*, 219-223.

Clements, A. N. (1999). *The biology of mosquitoes: volume 2 sensory reception and behavior.* Oxfordshire, UK: CABI Publishing.

Clements, A.N. (2000). *The biology of mosquitoes: volume 1 development nutrition and reproduction.* Oxfordshire, UK: CABI Publishing.

Costanzo, K.S., Mormann, K., & Juliano, S.A. (2005). Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology, 42*, 559-570.

Crump, M.L. (1991). Choice of oviposition site and egg load assessment by a treefrog. *Herpetologica, 47*, 308-315.

Denver, R.J., Mirhadi, N., & Phillips, M. (1998). Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology, 79*, 1859-1872.

Dudley, W.D. (2006). The Biology of Temporary Waters. Oxford, UK: Oxford University Press.

Egan, R.S., & Paton, P.W.C. (2004). Within-pond parameters affecting oviposition by wood frogs and spotted salamanders. *Wetlands, 24*, 1-13.

Gerberg, E.J. (1970). *Manual for rearing and experimental techniques*. Selma, CA: American Mosquito Control Association, INC.

Grill, C.P., & Juliano, S.A. (1996). Predicting species interactions based on behaviour: predation and competition in container-dwelling mosquitoes. *Journal of Animal Ecology, 65*, 63-76.

Harada, T. (1992). The oviposition process in two direct breeding generations in a water strider, *Aquarius paludum* (Fabricius). *Journal of Insect Physiology, 38*, 687-692.

Hirayama, H., & Kasuya, E. (2009). Oviposition depth in response to egg parasitism in the water strider: high-risk experience promotes deeper oviposition*. Animal Behavior, 78*, 935-941.

Hirayama, H., & Kasuya, E. (2010). Cost of oviposition site selection in a water strider *Aquarius paludum insularis*: egg mortality increases with oviposition depth. *Journal of Insect Physiology, 56*, 646-639.

Juliano, S.A. (1998). Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? *Ecology, 79*, 255-268.

Kennedy, J.S. (1942). On water-finding and oviposition by captive mosquitoes. *Bulletin of Entomological Research, 32*, 279-301.

Laurila, A., & Kujasalo, J. (1999). Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. *Journal of Animal Ecology, 68*, 1123-1132.

Lester, J. L., & Pike, A.J. (2003). Container surface area and water depth influence the population dynamics of the mosquito *Culex pervigilans* (Diptera: Cuclicidae) and its associated predators in New Zealand. *Journal of Vector Ecology, 28*, 267-274.

Livdahl, T.P., & Sugihara, G. (1984). Non-linear interactions of populations and the importance of estimating per capita rates of change. *Journal of Animal Ecology, 53*, 573-580.

Lounibos, L.P., Frank, J.H., Machado-Allison, C.E., Ocanto, P., & Navarro, J.C.. (1987). Survival, development and predatory effects of mosquito larvae in Venezuelan phytotelmata. *Journal of Tropical Ecology, 3*, 221-242.

Lounibos, L.P., Suárez, S., Menéndez, Z., Nishimura, N., Escher, R.L., O'Connell, S.M., & Rey, R.R. (2002). Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*? *Journal of Vector Ecology, 27*, 86-95.

McCall, P.J., & Eaton, G. (2001). Olfactory memory in the mosquito *Culex quinquefasciatus. Medical and Veterinary Entomology, 15*, 197-203.

McCann, S., Day, J.F., Allan, S., & Lord, C.C. (2009). Age modifies the effect of body size on fecundity in *Culex quinquefasciatus* Say (Diptera: Culicidae). *Journal of Vector Ecology, 34*, 174-181.

Merritt, R.W., Dadd, R.H., & Walker, E.D. (1992). Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology, 37*, 349-376.

Mutero, C.M., Blank, H., Konradsen, F., & van der Hoek, W. (2000). Water management for controlling the breeding of *Anopheles* mosquitoes in rice irrigation schemes in Kenya. *Acta Tropica, 76*, 253-263.

Pelz-Stelinski, K., Kaugman, M.G., & Walker, E.D. (2011). Beetle (Coleoptera:Scirtidae) facilitation of larval mosquito growth in tree hole

habitats is linked to multitrophic microbial interactions. *Microbiology of Aquatic Systems, 62*, 690-703.

Reisen, W.K., Fang, Y., & Martinez, V.M. (2005). Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis Encephalitis virus transmission. *Journal of Medical Entomology, 42*, 367-375.

Reiskind, M.A., & Zarabi, A.A. (2012). Water surface area and depth determine oviposition choice in *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology, 49*, 71-76.

Sardelis, M.R., Turell, M.J., Dohm, D.J., & O'Guinn, M.L. (2001). Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. *Emerging Infectious Diesases, 7*, 1018-1022.

Shaalan, S.A., Canyon, D.V. (2009). Aquatic insect predators and mosquito control. *tropical Biomedicine, 26,* 223-261.

Skiff, J. J. (2013). *Interactions between potozoan prey (Phylum Protista) and their mosquito (Family Culicidae) predators: prey size and predator behavior effects*. (Master's thesis). University of Southern Mississippi, Hattiesburg, Mississippi.

Sota, T. (1996). Effects of capacity on resource input and the aquatic metazoan community structure in phytotelmata. *Researches in Population Ecology*, *38*, 65-73.

Sota, T. (1998). Microhabitat size distribution affects local difference in community structure: metazoan communities in tree holes. *Researches in Population Ecology, 40*, 249-255.

Subra, R. (1981). Biology and control of *Culex pipiens quinquefasciatus* Say, 1823 (Diptera: Culicidae) with special reference to Africa. *Insect Science and its Application, 1*, 319-338.

Sunahara, T., Ishizaka, K., & Motoyoshi, M. (2002). Habitat size: a factor in determining the opportunity for encounters between mosquito larvae and aquatic predators. *Journal of Vector Ecology, 27*, 8-20.

Sunahara, T. & Mogi, M. (2002). Variability of intra- and interspecific competitions of bamboo stump mosquito larvae over small and large spatial scales. *Oikos, 97*, 87-96.

Thiboutot, M.M., Kannan, S., Kawalekar, O.U., Shedlock, D.J., Khan, A.S., Sarangan, G., Srikanth, P., Weiner, D.B., & Muthumani, K. (2010). Chikungunya: a potentially emerging epidemic? *PLoS Neglected Tropical Diseases, 4,* 1-8.

Timmermann, S.E, & Briegel, H. (1993). Water depth and larval density affect development and accumulation of reserves in laboratory populations of mosquitoes. *Bulletin Society of Vector Ecology, 18*, 174-187.

Vezzani, D. (2007). Review: Artificial container-breeding mosquitoes and cemeteries: a perfect match. *Tropical Medicine and International Health, 12*, 299-313.

Walker, E.D., Lawson, D.L., Merritt, R.W., Morgan, W.T., & King, M.J. (1991). Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. *Ecology, 72,* 1529-1546.

Walker, E.D., Merritt, R.W., Kaufman, M.G., Ayres, M.P., Riedel, M.H. (1997). Effects of variation in quality of leaf detritus on growth of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae). *Canadian Journal of Zoology, 75,* 706-718.

Wilton, D.P. (1968). Oviposition site selection by the tree-hole mosquito, *Aedes trisderiatus* (Say). *Journal of Medical Entomology, 5,* 189-194.

Winters, A.E., & Yee, D.A. (2012). Variation in performance of two co-occurring mosquito species across diverse resource environments: insights from nutrient and stable isotope analyses. *Ecological Entomology, 37,* 56-64.

Wynn, G., & Paradise, C.J. (2001). Effects of microcosm scaling and food resources on growth and survival of larval *Culex pipiens. BMC Ecology, 1,* 3.

Yanoviak, S.P. (1999). Community structure in water-filled tree holes of Panama: effects of hole height and size. *Selbyana, 20,* 106-115.

Yanoviak, S.P. (2001a). Container color and location affect macroinvertebrate community structure in artificial treeholes in Panama. *Florida Entomologist, 84,* 265-271.

Yanoviak, S.P. (2001b). Predation, resource availability, and community structure in Neotropical water-filled tree holes. *Oecologia, 126,* 125-133.

Yee, D.A. (2014). [Macro invertebrate tire survey of Mississippi]. Unpublished raw data.

Yee, D.A., & Skiff, J. (2014). Interspecific competition of a new invasive mosquito, *Culex coronator*, and two container mosquitoes, *Aedes albopictus* and *Cx. quinquefasciatus*, across different detritus environments. *Journal of Medical Entomology, 51*, 89-96.

Yee, D.A., Allgood, D., Kneitel, J.M., & Kuehn, K.A. (2012). Constitutive differences between natural and artificial container mosquito habitats: vector communities, resources, microorganisms, and habitat parameters. *Journal of Medical Entomology, 49*, 482-491.

Yee, D.A., Kneitel, J.M., & Juliano, S.A. (2010) Environmental correlates of abundances of mosquito species and stages in discarded vehicle tires. *Journal of Medical Ecology, 47,* 53-62.

Yee, D.A. (2008). Tires as habitats for mosquitoes: a review of studies within the eastern United States. *Journal of Medical Entomology, 45*, 581-593.

Yee, D.A., & Juliano, S.A. (2006). Consequences of detritus type in an aquatic microsystem: effects on water quality, micro-organisms and performance of the dominant consumer*. Freshwater Biology, 51*, 448-459.

Yee, D.A., Kesavaraju, B., & Juliano, S.A. (2004a). Interspecific differences in feeding behavior and survival under food-limited conditions for larval *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *Annals of the Entomological Society of America, 97*, 720-728.

Yee, D.A., Kesavaraju, B., & Juliano, S.A. (2004b). Larval feeding behavior of

three co-occurring species of container mosquitoes. *Journal of Vector*

Ecology, 29, 315-322.