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Ecological Stoichiometry: What Role Does it Play in the Competition and Spatial Distribution Patterns of *Aedes Albopictus* and *Aedes Aegypti*?

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ECOLOGICAL STOICHIOMETRY: WHAT ROLE DOES IT PLAY IN THE COMPETITION AND SPATIAL DISTRIBUTION PATTERNS OF *AEDES ALBOPICTUS* AND *AEDES AEGYPTI?*

by

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ABSTRACT

Ecological stoichiometry is the balance of chemical substances within animal bodies through interactions and processes within their ecosystem. Though relatively underexplored, it provides a wealth of information linking interactions across different levels of organization. Detritus is the base of the food web within the small aquatic ecosystems occupied by the mosquitoes *Aedes albopictus* and *Aedes aegypti*. Nutrient content of detritus varies, but it can have a negative effect on mosquito growth and survival if nutrient thresholds are not met. I investigated nutrient environments and species abundance in cemetery vases in New Orleans, LA to assess detrital heterogeneity and its effect on coexistence patterns between *Aedes albopictus* and *Aedes aegypti*. Vases were found to contain a wide array of detrital environments, but I did not find support to suggest that it affects mosquito coexistence patterns. Under a laboratory experiment I also investigated whether *Aedes albopictus* would show greater survivorship in lower nutrient environments compared to *Aedes aegypti*, and whether coexistence would occur in higher nutrient environments. This hypothesis was supported which showed *Aedes aegypti* stoichiometry and survival to be negatively affected within the lowest nutrient environments in the presence of *Aedes albopictus*, but in the highest nutrient environments both species showed high survival rates. My findings contribute to our understanding of the process that affects potential coexistence and exclusion for *Ae. albopictus* and *Ae. aegypti*.

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CHAPTER I – INTRODUCTION

1.1 Ecological Stoichiometry

Ecosystems are comprised of organisms that interact with each other and their surrounding environment in ways that exchange energy and recycle elements. Ecosystems generally include complex food webs vital to the flow of matter and energy within them (Ellis, 2014). These ecological interactions and processes maintain a balance of chemical substances, referred to as ecological stoichiometry (Sterner, 2002). All organisms are made of many different elements, and this fundamentally important concept has far reaching implications into many areas of science (Sterner, 2002). The continued study and understanding of the chemical nature of organisms and how they interact with their surroundings, though relatively underexplored, provides valuable ecological information linking interactions across different levels of organization, from elements to ecosystems. There are eleven elements essential to all living things: C, N, H, O, S, Cl, Mg, P, Ca, Na, and K. Carbon, N, H, and O make up about 99% of living biomass, and these four elements, along with P and S, are the main constituents of macromolecules. Of these, C, N, and P are of relatively low abundance on the planet (Sterner, 2002). The idea that these three elements are so vital to living organisms, yet so scarce in relation to other elements, makes them the key stoichiometric elements studied. The elements making up an organism are often expressed as ratios, and are most often written as element content in relation to carbon (e.g., C:N, N:P, C:P) (Sterner, 2002). The most notable stoichiometric ratio is the Redfield ratio (Sterner, 2002). This ratio was computed by the oceanographer Alfred C. Redfield who found that marine particulate matter and dissolved nutrients to have the same C, N, and P ratios. This finding showed

there was a balanced flow of nutrients throughout marine ecosystems. Ecological stoichiometry relies on being able to recognize the abundance patterns of elements within living organisms, and these organisms require a certain amount of each of these elements in order to survive. If this amount is not met, growth cannot continue (Sterner, 2002).

In a biological sense, stoichiometry is broadened to include the transformation and conservation of energy as well as the conservation of matter. One of the key concepts of ecological stoichiometry is that of homeostasis, wherein an organism's negative feedback mechanism drives the internal concentration and maintenance of nutrient compositions in relation to its external environment and food resources (Sterner, 2002). This resistance to internal change is essential for life and can be maintained by various methods including body fluid regulation, pH balance, and gas concentrations. Chemical homeostasis of an organism influences its stoichiometric pattern to varying degrees, and can be applied ecologically to changes in growth (Sterner, 2002).

Each organism has a different level of homeostasis and this can be measured by obtaining the elemental composition of the organism and comparing to that of its food source (Sterner, 2002). The levels of homeostasis can range from heterostatic to strictly homeostatic. Heterostatic organisms do not have an internal regulation of nutrient content concentration in relation to their ingested resources (Fig 1.1 A), and as such, display a constant proportional change in relation to their food (Sterner, 2002). On the other end of the spectrum are strictly homeostatic organisms, which have an effective negative feedback mechanism that maintains a constant nutrient concentration regardless of their food resource consumption (Fig 1.1 B). Although an organism is strictly homeostatic does not mean there cannot be any variation in nutrient contents. Different life stages

often require different levels of nutrients, so the stoichiometry between an adult and larval form may differ. Therefore, if intraspecific variation in nutrient content is found, this does not preclude the presence of homeostasis in that organism (Sterner, 2002). Previous lab experiements with *Aedes albopictus* and *Culex quinquefasciatus* across different plant and animal detritus types have shown *Ae. albopictus* to be heterostatic with greater survivorship in comparision to *Culex quinquefasciatus*, which was homeostatic (Yee *et al.*, 2012).

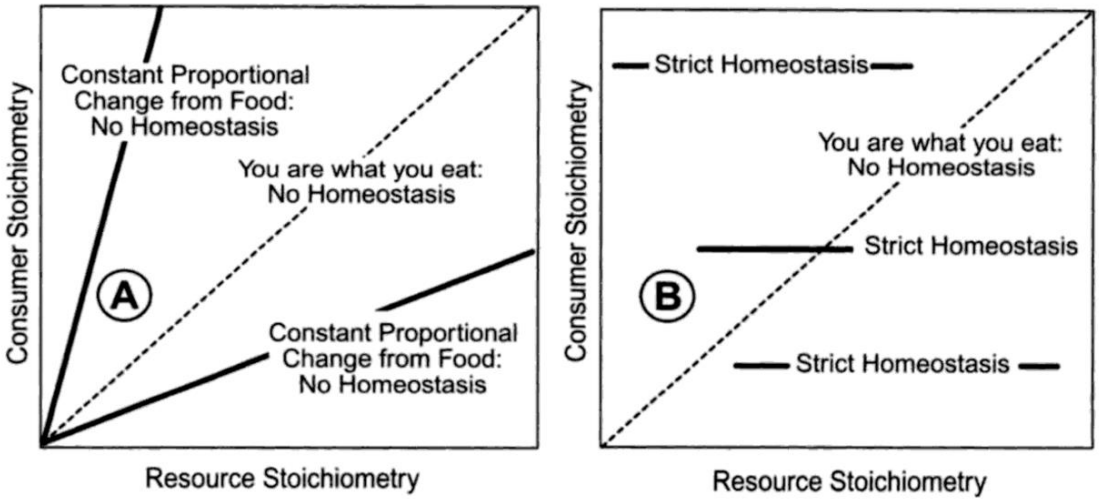

Figure 1.1 Generalized stoichiometric patterns, representing two extremes, relating consumer stoichiometry to resource stoichiometry.

Note: Horizontal and vertical axes are any single stoichiometric measure (e.g., C, N, C:P ratio). The dashed lines represent a consumer with stoichiometry that always matches that of its resources. A. Points on any line represent identical stoichiometry between the consumer and resources, showing a constant proportional change from food. B. Any horizontal line, representing strict homeostasis (adopted from Sterner, 2002).

In many aquatic ecosystems dominated by mosquitoes, the base of the food web is composed of detrital matter. Essential nutrients from this detrital matter pass to microbes (food for mosquito larvae). Detrital nutrient content varies among detritus types, and this stoichiometric difference supports varying concentrations of microorganisms (Murrell *et*

al., 2012). An organism that subsist on detritus and detritivores, e.g., the mosquito, can experience delayed growth and even death if its nutrient thresholds are not met.

1.2 Study Organisms

Mosquitoes belong to the family Culicidae, within the order Diptera, and contain about 3,500 species. These primitive, two-winged flies can be found throughout most of the planet, save permanently frozen areas (Clements, 2000). Adult females of many species require vertebrate blood as a protein source to generate eggs. Mosquitoes are known hosts of many pathogens (West Nile virus, dengue virus, Zika virus), which make them of high medical importance (Clements, 2000). Annually, mosquitoes attribute to over one million deaths and almost a billion new disease cases worldwide (WHO, 2016).

Two of the most important human disease vectors and problem invasive species to much of the world belong to the genus *Aedes* (Murrell *et al*., 2012). *Aedes aegypti* (yellow fever mosquito) is an introduced species that has been established in North America for centuries. *Aedes albopictus* (Asian tiger mosquito) is a relatively new invader, first recorded in the mid-1980s, but is now one of the most common species in the southeastern United States (Moore, 1999). Since the introduction of *Ae. albopictus* into the U.S., there has been a steady decline in the populations of *Ae. aegypti*, to local extinction in some areas. This decline of *Ae. aegypti* after the introduction of *Ae. albopictus* is most likely do to larval interspecific competition (Juliano *et al*., 2004). Although this competition has driven *Ae. aegypti* to local extinctions in some areas, there are areas in southern Florida peninsula where it persists (e.g., Miami, Key West). Areas of coexistence also occur in urban areas of the southern U.S. (e.g., New Orleans, LA, Savannah, GA, Houston, TX) (Juliano *et al.*, 2004). The mechanism for why coexistence

occurs between the two species in some areas is the focus of several studies, but is not fully understood (Juliano and Lounibos, 2005).

1.3 Container Systems

Mosquitoes exhibit complete metamorphosis, with juvenile and adult forms occupying different niches and relying on different nutrient sources. The juvenile stage (larvae through pupae) is completely aquatic, whereas adults emerge into the terrestrial environment. Because all mosquitoes are closely tied to an aquatic habitat, the habitat type is commonly used to classify species. Aquatic habitat types span a highly variable spectrum, from large to small. Containers are the smaller of the habitat types, and unlike larger habitats, containers usually rely on allochthonous nutrient input. Few species occupy containers, but those species that do are heavily influenced by densities and competition (Vezzani, 2007). Container habitats can be either natural (tree holes, rock-pools, bamboo) or artificial (discarded tires, bottles, cemetery vases) (Vezzani, 2007). Several medically important species (including *Aedes aegypti* and *Ae. albopictus*) breed almost exclusively in artificial containers (Vezzani, 2007). Because artificial containers are almost always directly linked to urbanization, the study of this type of habitat is of great importance to world health. With steady increases in the human population, human activity, and urbanization, there is a steady increase in the amount of artificial containers made available for disease spreading mosquitoes (Noori *et al.*, 2015).

Around the world, cemeteries are associated with almost every human establishment. As human populations continue to grow, the number of cemeteries may expand. Cemeteries are most often located very near, if not surrounded by, urban areas. In older cities (i.e., New Orleans) the oldest cemeteries are located within the city limits,

commonly adjacent to residential areas. Cemeteries located within city limits tend to be much more vegetated and resemble parks than their adjacent residential or commercial areas, which makes them a good place for insects to be found (Vezzani, 2007). In addition to the vegetation in cemeteries, there can often be found a high density of artificial containers, most notably flower vases. Cemetery vases vary from plastic, disposable vases, to stone or metal vases, to vases hanging off of mausoleums, to vases built right into the headstone itself. The intent for these structures is to be a place to hold flowers (live or artificial), yet serve a different purpose as an environment for aquatic invertebrates. Cemeteries are ideal environments for harboring mosquito populations. They provide all four basic requirements of mosquitoes: 1) an aquatic environment for egg laying and larval development, 2) shelter for adults, 3) an energy source (flower nectar), and 4) blood meals from humans and other vertebrates (Vezzani, 2007).

Flower vases without holes in the bottom collect the two main components needed for larval development and survival: water and detritus. Rainwater settles into the containers along with allochthonous materials (e.g., leaves, grass trimmings, dead insects) and provides an environment for females to lay their eggs. Larvae then feed directly on the decaying detrital matter, on the microbes in the water column, or both (Yee *et al.* 2012). In addition to larval containers, man-made structures (mausoleums, head stones, fences) as well as vegetation (tree trunks, bushes, grass) provide safe places for adult mosquitoes to seek refuge. Adults need places to rest when they are not being active. These shelters provide a safe place to avoid predators and harsh environmental conditions.

Both sexes of mosquitoes require sugar as their main source of energy. These sources are obtained via plant juices (mainly nectar, but also damaged fruits and other vegetative tissues) (Clements, 2000). Cemeteries provide ample sources of nectar for adults. Throughout the year, visitors bring fresh flowers to grave sites to place in vases. Also, cemetery landscaping and beautification efforts lead to the planting of many different plant species throughout, including flowering species. For example, one hectare of cemetery in Buenos Aires was found to have thirty-five plant species, including fifteen that flowered during the summer months (Vezzani, 2007). This constant access to flowers provides the mosquitoes with the life sustaining sugars they require.

Many species of container dwelling mosquitoes require blood for egg production because amino acid concentrations within nectar are insignificant. The proteins from the blood meal are the prime sources of nutrients for the formation of eggs, with some females requiring multiple blood meals for ovarian maturation and other only requiring one (Clements, 2000). The source of blood can vary between species, and can be specific or a combination between mammal, bird, reptile, or amphibian. There is a constant blood supply within the cemetery environment, including visitors, caretakers, stray animals, and natural fauna (Vezzani, 2007).

CHAPTER II – EXPERIMENT 1: CEMETERY VASES

2.1 Introduction

Aedes aegypti and *Aedes albopictus*, both invasive species and disease vectors, have larvae that inhabit small water-filled containers. Because of both species' affinity for humans, they breed almost exclusively in artificial containers, which can be found in proximity to human habitation. *Aedes albopictus* was introduced to the U.S. in the mid-1980s. Since then, the population of *Ae. aegypti* has been on a steady decline to local extinction in some areas, but in some areas of the southern U.S., including New Orleans, LA, coexistence occurs. This decline is thought to be the result of larval competition between the two species (Juliano *et al.*, 2004). Both species have similar foraging behaviors within their environment, feeding primarily on the bottom or middle of the container system by consuming microorganisms growing on detrital surfaces (Yee *et al.*, 2004). Coexistence between these species is the focus of several studies, but not fully understood (Juliano *et al*., 2004). Studies suggest that the nutrient environment within a container plays a key role in the survivorship of species. *Aedes albopictus* is superior in low nutrient (including nitrogen) environments, but when nutrients are plentiful, coexistence can occur (Juliano, 1998, Murrell *et al.*, 2012, and Yee *et al.*, 2004).

Containers are the small aquatic ecosystems. They have minimal internal productivity, relying heavily on allochthonous nutrient input and detrital decomposition (Vezzani, 2007). This detrital matter forms the base of the food web in these habitats. Microbes in the water break down the detritus, consuming essential nutrients, and are fed on by mosquito larvae. Artificial containers are almost exclusively used as breeding sites for several medically important mosquito species (Vezzani, 2007). The study of this type

of habitat is of great importance to world health because they are almost always directly linked to urbanization. Increasing human populations and urbanization gives rise to an increase in the number of artificial containers available for mosquito breeding (Noori *et al*., 2015).

Almost all human settlements have cemeteries, often resembling park settings, and are an ideal environment for harboring mosquito populations. Most cemeteries have a high density of artificial containers, most notably flower vases, which are often left uncared for. Rain or irrigation settles into the containers along with allochthonous materials (e.g., plant detritus) and provides an aquatic environment suitable for egg laying and larval development. Some of the more common detrital types found in cemetery vases are from plants (e.g., grass clippings, leaves, flowers, twigs, seeds) and animals (invertebrate carcasses). Depending on the cemetery's location, rules, vegetation, and overall care, the nutrient environments among vases within a cemetery or between cemeteries can vary greatly. This nutrient environment formed by detritus within the water is crucial to larval survival. For adults, vegetation and man-made structures serve as safe places to rest and avoid predation. Flowers brought in by visitors and landscaping efforts provide ample sources of energy for mosquitoes in the form of nectar. Lastly, females of these species require a blood meal for egg production, and cemeteries provide a constant supply (Vezzani, 2007). Cemeteries, with all these factors, serve as ideal locations for aiding in the spread and maintenance of *Aedes* mosquito populations.

This observational project focused on species abundance and container nutrient environments in cemetery vases in New Orleans, LA. The research objective was to test the hypotheses that, a) different cemeteries will yield different container environments

with respect to detritus types, C:N, and mosquito species abundance patterns, b) vases with detrital heterogeneity will foster different coexistence patterns between *Ae. albopictus* and *Ae. aegypti*, and c) C:N levels in vases will affect the production of adults. It was predicted that each cemetery would have different nutrient signatures based on the surrounding environment's allochthonous inputs which, in turn, will give rise to varying species abundance patterns among them. In addition to cemetery differences, it was predicted that individual vases with higher detrital heterogeneity would support higher coexistence between species, whereas vases with detrital homogeneity would produce more instances of competitive exclusion between species. Lastly, it was predicted that vases with a lower C:N (higher nitrogen levels) would produce a higher number of adults whereas vases with a higher C:N (higher carbon levels) would be less supportive of larval development and produce fewer adults.

2.2 Materials and Methods

In New Orleans, LA, eleven cemeteries across the metro area were sampled twice, in June and October, 2016. Seven vases within each cemetery were randomly chosen for sampling. New containers were selected during each sampling round because of the destructive nature of the sampling techniques. Several parameters, which have been shown to affect mosquito abundance and distributions (Yee *et al.*, 2012), were measured for each vase (i.e., canopy cover, vase detritus, water volume, water depth, and vase height above the ground). Water depth was measured by placing a thin, wooden dowel rod into the container in the deepest part (some vases had openings and bottoms too narrow for a regular tape measure) and marking the water line on the rod. A rule was used to measure the line on the dowel rod. Water volume (ml) was measured by pouring

the contents into a graduated cylinder, which was rinsed with reverse osmosis water between vases to prevent cross contamination. Height of each vase opening was measured from the ground using a tape measure. Canopy cover above each vase was measured using a spherical densitometer (range 0 = no cover to 37 = total cover). The densitometer was positioned above each vase opening at chest height and 12-18 in from the operator. For each vase, 50 mL of water was passed through a 150 micrometer sieve (to separate particulate detritus from the water), placed on ice, and frozen upon returning to the lab for water nutrient analysis (data not used). The remaining water containing the larvae and detritus was placed into individual containers, labeled, and taken back to the lab at The University of Southern Mississippi for quantification. Detritus from each container was separated into five categories: bark (including twigs), leaves, seeds (including fruit and flowers), fine particulate, and animal detritus. Inorganic detritus (e.g., rocks, Styrofoam, artificial flowers) was discarded. This detritus was dried at 50˚C for ≥48 hrs then weighed to the nearest 0.1 mg using a XP2U ultramicrobalance (Mettler Toledo, Ohio). Mosquito larvae were separated into classes of immatures: early instars (first and second), late instars (third, fourth), and pupae. Mortality in early instars can be high, therefore, I used a sampling protocol to assign individuals to species. All early instars were counted and raised to adults, and were allowed to develop in the vase water they were collected in to assure a similar nutrient environment for developing individuals. The adults were then identified to species and then the proportion of identified adults used to assign affiliations to the initial numbers of early instars (Yee *et al*., 2012). Late instars were immediately identified to species based on Darsie and Ward (2004). Pupae were placed into individual vials and allowed to emerge, then adults

identified to species. Once larvae pupated, they were placed in individual 0.25 dram shell vials and allowed to eclose and then identified, sexed, and dried at 50˚C for 48 hrs. Once dry, the mass of each mosquito was measured to the nearest 0.0001 mg. Each dried individual was analyzed for whole body carbon and nitrogen using an ECS 4010 Elemental Combustion System (Costech Analytical Technologies, California). Representative leaf and animal detrital samples were analyzed using the same combustion system. Carbon and nitrogen from the vase detrital environments and adults were analyzed. Detrital C:N levels and heterogeneity amongst vases were hypothesized to affect the production and coexistence patterns of adults mosquitoes. These C:N data were compared to the survival data of vases and this comparison was used to help better understand what controls adult production in nature. Vase nutrient environments allowed for a better understanding of the patterns of co-existence of these species within nature.

Stepwise multiple regression of log transformed data was used to assess the effect of vase variables (e.g., canopy, depth, height) on the stoichiometry or detritus in the vase. This method was also used to determine if mosquito stoichiometry was explained by these variables.

2.3 Results

2.3.1 Vase Stoichiometry

Leaf litter mass was positively affected by vase height ($R^2 = 0.028$, $F_{2, 144} = 3.20$, $P = 0.0441$) but no other factors affected it. In addition, fine detritus was positively affected by vase depth ($R^2 = 0.031$, $F_{1, 145} = 4.70$, $P = 0.0317$). Leaf litter %N, %C, and C:N were all affected positively by canopy cover and negatively by observation month. Specifically, canopy cover ($R^2 = 0.085$, $F_{3, 143} = 13.48$, $P = 0.0003$) and sampling month

(R^2 = 0.079, $F_{3, 143}$ = 13.54, P = 0.0003) combined explained 16.37% of variation in leaf %N. For leaf %C, canopy cover (R^2 = 0.132, $F_{3, 143}$ = 22.14, P < 0.0001) and sampling month (R^2 = 0.069, $F_{3, 143}$ = 12.45, P = 0.0006) explained 20.15% of the variation. Canopy cover (R^2 = 0.154, $F_{3, 143}$ = 26.34, P < 0.0001) and sampling month (R^2 = 0.059, $F_{3, 143}$ = 10.95, P = 0.001) explained 21.36% of variation in leaf litter C:N (Table 2.1).

Table 2.1

Results of stepwise multiple regression on log transformed values to determine variables that explained vase stoichiometry or detritus.

Dependent variable	Parameter	R^2	P
Leaf litter mass	Height	0.028	**0.044**
Leaf litter N	Canopy	0.085	**<0.001**
	Month	0.164	**<0.001**
Leaf litter C	Canopy	0.132	**<0.001**
	Month	0.202	**<0.001**
Leaf litter C:N	Canopy	0.154	**<0.001**
	Month	0.214	**0.001**
Fine detritus	Depth	0.031	**0.032**

Note: Significant contributors are shown.

2.3.2 Mosquito Stoichiometry

Canopy cover had a positive effect on *Aedes albopictus* tissue %N (R^2 = 0.083, $F_{1,145}$ = 13.1, P = 0.0004), %C (R^2 = 0.081, $F_{1, 145}$ = 12.79, P = 0.0005), and C:N (R^2 = 0.079, $F_{1, 145}$ = 12.48, P = 0.0006). *Aedes aegypti* C:N was positively affected by canopy cover (R^2 = 0.036, $F_{3, 143}$ = 5.45, P = 0.0209). In addition, canopy cover (R^2 = 0.038, $F_{3, 143}$ = 5.83, P = 0.0170) and amount of bark detritus (R^2 = 0.027, $F_{3, 143}$ = 4.21, P = 0.0421) combined explain 6.59% of variation in *Ae. aegypti* %N. For *Ae. aegypti* %C, canopy

cover (R^2 = 0.038, $F_{3, 143}$ = 5.67, P = 0.0186) and bark detritus (R^2 = 0.026, $F_{3, 143}$ = 3.99, P = 0.0475) explained 6.36% of variation (Table 2.2).

Table 2.2

Results of stepwise multiple regression on log transformed values to determine variables that explained mosquito stoichiometry.

Dependent variable	Parameter	R^2	P
Ae. albopictus N	Canopy	0.083	**<0.001**
Ae. albopictus C	Canopy	0.081	**<0.001**
Ae. albopictus C:N	Canopy	0.079	**<0.001**
Ae. aegypti N	Canopy	0.039	**0.017**
	Bark	0.066	**0.042**
Ae. aegypti C	Canopy	0.038	**0.019**
	Bark	0.064	**0.048**
Ae. aegypti C:N	Canopy	0.036	**0.021**

Note: Significant contributors are shown.

2.4 Discussion

My cemetery data show a wide array of vase detrital environments (e.g., water volumes 40-2000 mL, vases with no visible detritus to vases with several thousand mg of leaf or animal detritus) and differing mosquito abundance patterns (Table 2.3). These findings support my first hypothesis that different cemeteries will yield different container environments with respect to detritus types, nutrient signatures, and mosquito abundance patterns. However, the second (that detrital heterogeneity will foster different coexistence patterns) and third hypotheses (that C:N levels in vases will affect the production of adults) need further testing. Some vases were observed to have no measureable detritus yet produced both mosquito species, whereas others that contained

multiple detrital types produced no adults. There are several other variables that could have affected larval presence, such as the length of time a vase has been supporting larvae, the unknown effects of larvae feeding on detritus over a longer period of time, and how that may affect nutrient values, as well as the composition of the fine particulate matter that could have varying nutritional values when compared to other vases. Future testing of nutrients in vase collected water and fine particulate matter might give more insight on abundance patterns within a vase.

It has been well documented in previous studies that leaf litter comprises the majority of detritus in aquatic containers (Daugherty *et al.*, 2000, Yee and Juliano, 2006, Yee *et al.*, 2007, and Murrell *et al.*, 2011). The same applies to my observations, where leaf litter made up a majority of the identifiable detrital material throughout all the cemeteries (Table 2.3). Analyses showed that vase height positively affected leaf litter mass. Because falling leaves make up most of the detritus, it makes sense a higher vase is more likely to trap more litter because it is closer to the canopy. My data also showed that vases with higher canopy cover generally had greater vase leaf nutrients (C, N, and C:N) which were then passed on and measurable in the mosquitoes from those vases, as adult mosquitoes in vases from more shaded areas had higher %N and %C. A greater availability of nutrients, especially %N, decreases competitive intensity, leading to eventual coexistence. However, the statistical variation explained between environmental parameters and detritus was often low ($R^2 < 22\%$), suggesting that canopy cover and vase attributes were one but not the only factor important for explaining patterns of detritus in containers.

Varying environmental factors can affect and influence a cemetery as a whole. Cemeteries were sampled at the beginning of summer (June) and the end of summer (October). This was designed in an attempt to accurately represent the entire time frame under which both *Aedes* species reproduce. Eleven cemeteries were sampled and all of them produced both species at one time or the other (Table 2.3 and 2.4). Four of them produced both species of mosquito during both collection times, and the remaining seven produced only one of the two species on at least one sampling occasion. It is known that detrital environments do not remain constant in the field (e.g., Yee et al. 2015a). Nutrient consumption by microbes and larvae, introduction of more or different detritus, as well as climate can all alter the nutrient environment for larvae over time. With that in mind, it is feasible for an entire cemetery environment to change over the sampling period to become more or less supportive towards one species or the other. A cemetery could be nutrient poor during the beginning of the summer, and only support growth and development of *Ae. albopictus*, but over the course of the summer, obtain more nutrient rich detritus and evolve to an environment which supports coexistence of *Ae. albopictus* and *Ae. aegypti*. I did find that sampling month did affect leaf %C and %N, however modestly, suggesting some support for these ideas.

This study shows just how complex and dynamic a cemetery ecosystem can be in regards to mosquito production and distribution. At the vase and entire cemetery levels, environmental factors (like vase height and canopy cover supported by my data) have an effect on nutrient signatures within the aquatic ecosystem and potentially mosquito distribution. These data contribute valuable insight to our limited understanding of these two medically important species and how cemeteries have an effect. Until now, this type

of research had only been conducted a few times in cemeteries is south Florida (e.g., Murrell *et al*., 2011). Further research in other cemeteries within the range of *Ae. albopictus* and *Ae. aegypti* would lead to a more comprehensive understanding of the role of cemeteries in distribution which could aid in control and potential disease transmission. Within this study, testing the nutrient content of the water and it's suspended fine particulate matter will be another valuable layer. Cemeteries, while rarely studied, offer a grave wealth of information on artificial aquatic ecosystems.

Table 2.3

Cemetery vase measurements and species abundance of vases containing leaf and/or animal detritus and adult mosquito production for the month of June.

Cemetery	June Vases	June Depth (cm)	June Volume (mL)	June Height (in)	June Canopy	June Leaf (mg)	June Animal (mg)	June AA	June AE	June Both
CC	5	12.62 ± 1.75	744.00 ± 240.62	30.30 ± 4.21	14.80 ± 9.26	885.60 ± 390.45	163.9	0	4	1
FC	7	13.76 ± 1.12	565.71 ± 103.00	28.36 ± 4.97	24.86 ± 2.32	974.11 ± 275.02	54.52 ± 6.78	3	0	2
HC	2	14.25 ± 0.75	1360.00 ± 640.00	5.75 ± 1.75	8.00 ± 8.00	236.75 ± 157.35	0.00	4	0	0
HL	4	14.58 ± 1.35	445.00 ± 94.49	34.75 ± 2.29	0.00	138.00 ± 42.60	79.2	0	2	3
LF	4	13.38 ± 2.76	572.50 ± 161.11	32.13 ± 6.75	0.00	177.33 ± 112.05	4.24	0	6	0
LP	3	13.50 ± 3.40	495.00 ± 193.80	27.17 ± 8.15	0.00	978.20 ± 435.59	0.00	1	2	0
SB	3	10.63 ± 0.28	286.67 ± 29.06	43.63 ± 0.19	0.00	61.53 ± 39.50	0.00	2	1	1
SP	3	11.23 ± 1.12	436.67 ± 118.65	22.17 ± 2.46	0.00	267.80 ± 122.72	0.00	0	2	2
SR	5	15.00 ± 1.52	628.00 ± 73.38	29.50 ± 4.09	0.00	127.78 ± 56.08	53.10	0	4	1
SV	3	15.60 ± 1.20	496.67 ± 67.66	31.67 ± 11.01	0.00	440.10	70.70 ± 37.89	0	4	2
WM	5	8.68 ± 1.84	391.60 ± 68.92	18.16 ± 4.69	0.00	221.00 ± 102.71	69.02 ± 26.70	2	0	2

Note: Measurement values are averages ± SE. Canopy measurements were obtained using a spherical densitometer with values 0 (no canopy cover) to 37 (complete canopy cover). Cemetery codes and GPS coordinates: CC = Carrollton (29.9472, -90.1222), FC = Fleming (29.7444, -90.1347), HC = Holt (29.9842, -90.1052), HL = Hook and Ladder (29.9117, -90.0588), LF = Lafayette #2 (29.9347, -90.0525), LP = Our Lady of Prompt Succor (29.9078, -90.1467), SB = St. Bernard (29.8667, -89.8183), SP = St. Patrick (29.9775, -90.1100), SR = St. Roch (29.9747, -90.0525), SV = St. Vincent De Paul (29.9695, -90.0409), WM = Westlawn Memorial (29.9042, -90.0407).

Table 2.4

Cemetery vase measurements and species abundance of vases containing leaf and/or animal detritus and mosquito production for the month of October.

Cemetery	Oct. Vases	Oct. Depth (cm)	Oct. Volume (mL)	Oct. Height (in)	Oct. Canopy	Oct. Leaf (mg)	Oct. Animal (mg)	Oct. AA	Oct. AE	Oct. Both
CC	3	5.83 ± 2.89	176.67 ± 86.86	27.33 ± 4.06	21.67 ± 11.14	216.45 ± 121.05	45.50	0	4	0
FC	4	12.00 ± 1.62	535.00 ± 101.12	22.63 ± 6.45	13.50 ± 4.99	340.15 ± 176.44	0.00	6	0	0
HC	1	7.50	100.00	14.00	11.00	351.00	0.00	3	1	0
HL	-	-	-	-	-	-	-	0	5	0
LF	3	12.00 ± 1.50	216.67 ± 66.67	22.83 ± 4.59	17.33 ± 10.74	461.93 ± 283.12	0.00	0	4	1
LP	3	7.67 ± 1.41	245.00 ± 27.84	30.67 ± 0.33	22.33 ± 11.35	732.33 ± 225.86	0.00	0	5	0
SB	3	13.50 ± 0.87	485.00 ± 79.43	34.50 ± 9.76	0.00	283.93 ± 75.28	0.00	3	1	1
SP	2	5.75 ± 3.25	150.00 ± 50.00	15.50 ± 0.50	0.00	45.00	13.00	2	1	2
SR	1	6.00	100.00	12.00	0.00	0.00	42.80	0	3	0
SV	2	8.00	162.50 ± 62.50	32.00 ± 11.00	0.00	117.60 ± 86.40	164.30	0	3	2
WM	2	9.50 ± 1.00	287.50 ± 12.50	44.75 ± 0.25	0.00	112.30	33.75 ± 8.55	2	1	3

Note: Measurement values are averages ± SE. Canopy measurements were obtained using a spherical densitometer with values 0 (no canopy cover) to 37 (complete canopy cover). Cemetery codes and GPS coordinates: CC = Carrollton (29.9472, -90.1222), FC = Fleming (29.7444, -90.1347), HC = Holt (29.9842, -90.1052), HL = Hook and Ladder (29.9117, -90.0588), LF = Lafayette #2 (29.9347, -90.0525), LP = Our Lady of Prompt Succor (29.9078, -90.1467), SB = St. Bernard (29.8667, -89.8183), SP = St. Patrick (29.9775, -90.1100), SR = St. Roch (29.9747, -90.0525), SV = St. Vincent De Paul (29.9695, -90.0409), WM = Westlawn Memorial (29.9042, -90.0407).

CHAPTER III – EXPERIMENT 2: COMPETITION

3.1 Introduction

One of the main factors affecting species distribution patterns of organisms is competition. Resource competition (both interspecific and intraspecific) can have profound effects on community structure. For some mosquitoes, one species is often negatively affected to the point of competitive exclusion (Murrell and Juliano, 2008). Changing the resource types or amounts can sometimes alter the severity of the resource competition, leading to coexistence amongst species (Daugherty et al. 2000). In containers, *Aedes albopictus* has a competitive advantage over *Aedes aegypti* under certain restricted or low nutrient resources, such as pine needles and oak leaves (Murrell and Juliano, 2008), but detrital resources that include accumulations of dead invertebrates have been shown to lower interspecific competition and in some cases, support coexistence (Daugherty *et al.*, 2000).

Previous studies have shown stoichiometric differences among mosquito genera reared on similar detrital types (e.g., Yee et al. 2015b). When comparing *Culex* and *Aedes* mosquito C:N ratios, it was found that the ratio for *Culex quinquefasciatus* did not change across treatment levels, but those of *Ae. albopictus* and *Ae. aegypti* mosquitoes did (Yee *et al.*, 2015b). This suggests that some *Culex* mosquitoes are homeostatic and some *Aedes* are heterostatic. Many previous studies have examined how mosquito performance is affected by larval feeding patterns (Yee *et al*., 2004, Winters and Yee, 2012, Yee *et al*., 2015b). These studies found *Ae. albopictus* and *Ae. aegypti* to be browsers that spent more time feeding directly on detritus on the bottom or middle of the container, whereas *Culex* were observed filtering the water column of microorganisms.

Generally, detritus at the bottom of the container has a higher concentration of nutrients, such as nitrogen, and the water column has a less nutrient rich suspension of fine particulate matter. For this reason, *Aedes* should have a nutrient signature close to that of the detritus, whereas the nutrient signature of *Culex* should be a close to that of the microorganisms it feeds on. Stable isotope analysis supports this, showing *Aedes* with a greater amount of nitrogen when compared to *Culex* (Yee *et al*., 2015b).

Detrital nutrients are key in the survival of both of these *Aedes* species, and competitive exclusion can occur if nutrient requirements are not met. For container mosquitoes, nitrogen is most often the limiting element (Kaufman and Walker, 2006, Murrell *et al.*, 2011, and Yee *et al.*, 2015b). When in a competitive environment, the species that can acquire and assimilate the available nutrients, such as nitrogen, the most efficiently has the greater chance of survival. But nutrients are not always readily available. Nutrients in the container system come from allochthonous materials that need to be broken down by microorganisms. Certain detrital types (e.g., pine needles and oak leaves) take longer to break down and do not contain as much nitrogen as others. Insect carcasses, on the other hand, break down rapidly and contain greater stores of nitrogen (Yee and Juliano, 2006). Previous studies have shown nitrogen from insect carcasses to support greater survivorship of both *Aedes* species in competitive environments, but *Ae. albopictus* has greater survivorship in the lower nitrogen environments with the slower to decay plant detritus (Murrell and Juliano, 2008, and Daugherty *et al.*, 2000). Knowing which container detritus environments support greater survivorship between species will aid the understanding of species distributions.

This manipulative experiment was a lab based study that focused on competitive interactions based on different detrital resources. Carbon and nitrogen analysis was used to observe any stoichiometric effects of competition on species; survival was also measured. The research objective was to test the hypothesis that *Aedes albopictus* will show greater survivorship in lower nitrogen environments than *Aedes aegypti*, but species would coexist with higher nitrogen levels. This is based on results of a meta-analysis examining the effect of nutrients on competition between *Ae. albopictus* and resident species (Juliano, 2009).

3.2 Materials and Methods

Eggs were generated from lab populations of *Aedes albopictus* and *Aedes aegypti* raised from wild adults collected in New Orleans, LA. For hatching, eggs were submerged in nutrient broth solution for 12-24 hrs at 27˚C. This solution was made by dissolving 0.33 g of Difco nutrient broth powder in 750 mL of water that has been purified through reverse osmosis (RO). Hatched larvae were rinsed with RO water to remove any remaining broth and allocated into specific treatment microcosms (250 mL tripour beakers). Treatments contained ratios of dried animal (crickets (*Acheta domestica*)) and leaf (senescent live oak (*Quercus virginiana*)) detritus: 0:10, 0:20, 1:10, 2:20, 1:0 and 2:0 animal:leaf with one unit of detritus equaling 0.125 g. Crickets (lower C:N) and senescent live oak leaves (higher C:N) are used in many studies to represent animal and leaf detritus (Yee, 2016) and each of these are commonly found in artificial containers in New Orleans (H. Deerman, personal observation). The amounts were chosen based on previous research that suggests less animal detritus is required than leaf detritus for larval survival (Yee and Juliano, 2006, Yee *et al.*, 2007) and that individual

larvae need about 0.005 g per unit of detritus to survive (Yee *et al.*, 2015b). Each treatment was replicated three times and for each detrital treatment level there were eight larval ratios, with four intraspecific 0:10, 0:20, 20:0, 10:0 and four interspecific levels 10:10, 10:20, 20:20, 20:10 (*Aedes aegypti*:*Aedes albopictus*). These ratios were chosen to test both intraspecific and interspecific competition of both species at low and high larval densities (Murrell and Juliano, 2008).

Two days prior to larval introduction, the beakers were filled with 199 mL of RO water, detritus, and one mL of homogenized inoculum collected from field vases to allow for the growth of microorganisms. For the duration of the experiment, water levels were checked daily and RO water added to maintain a 200 mL water level. Beakers were kept in an environmental chamber (Percival Scientific, Inc., Perry, IA, USA) set to 27˚C on a 14h:10h light:dark cycle to duplicate average mid-summer conditions in New Orleans, LA (National Weather Service, H. Deerman, personal observation). Every day, the beakers were rotated and checked for pupae. If pupae were present, they were removed from the beaker and placed individually into 0.25 dram shell vials until adult eclosion. Once adults emerged, they were identified, sexed, freeze-killed, and placed into an oven set to 50˚C and remained there at least 48 hrs. Once drying was complete, the mass of each adult was measured to the nearest 0.001 mg using a XP2U ultramicrobalance (Mettler Toledo Inc., Columbia, Ohio). Three replicates of individual female mosquitoes from each treatment were placed into a stainless steel burner cup and analyzed in the Elemental Combustion System. Three replicates of the detrital food source were also analyzed to determine the carbon and nitrogen signatures.

Multivariate analyses of variance (MANOVA, PRO GLM; SAS Institute, Inc. 2004) was used to test if larval densities, detritus ratios, or their interaction affected mosquito stoichiometry (%C, %N, C:N) for each species, separately. Standardized canonical coefficients (SCC) were used to identify important dependent variables contributing to multivariate effects (Scheiner, 2001).

For each treatment, a composite index of mosquito population performance was used (λ'). This metric represents a finite rate of change in a population over a set period of time, and this rate of increase is often used in studies of interspecific competition. It was derived from an estimate of the per capita rate of population change ($dN/Ndt = r$) (Livdahl and Sugihara, 1984). Values of λ' have been used to quantify competitive population level effects for *Ae. aegypti* and *Ae. albopictus* (e.g. Juliano, 1998; Daugherty *et al*., 2000; Lounibos *et al*., 2002). The equation is:

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

where N_0 is the initial female number (assumed to be 50%), x is the average number of days to eclosion, A_x is the number of females eclosing on day x, and w_x is the average mass of females from a treatment on day x. The function $f(w_x)$ varies among mosquito species, but relates female fecundity to mass. D is the number of days a newly eclosed female requires to mate, obtain a blood meal, and oviposit. For *Ae. aegypti* and *Ae. albopictus*, this is assumed to be 12 and 14 days, respectively (Grill and Juliano, 1996). The fecundity to size relationships used: *Ae. aegypti,* $f(w_x) = 17.11 + 16.59(w_x)^{0.765}$ (Grill and Juliano, 1996) and *Ae. albopictus,* $f(w_x) = 19.5 + 152.7w_x$ (Lounibos et al., 2002).

A two-way analysis of variance (ANOVA, PRO GLM; SAS Institute, Inc. 2004), with density combinations and detritus ratios as independent variables, was used to assess the finite rate of increase (λ') for both species.

3.3 Results

3.3.1 Competition Stoichiometry

Raw data for *Ae. albopictus* %C and %N met assumptions of normality; C:N data did not meet assumptions of normality with transformations, but was not important based on SCCs in any analysis. There were significant effects of detritus ratios (Pillai's $Trace_{15, 162} = 0.793$ $P < 0.001$) but not for larval densities (Pillai's $Trace_{15, 162} = 0.287$, $P = 0.323$) or their interaction (Pillai's $Trace_{75, 162} = 1.024$, $P = 0.276$). Based on SCCs, %C (2.41) and %N (-1.83) contributed most to the significant effect (SCC C:N = - 0.67). Specifically, the 2:0 (cricket:oak) ratio produced mosquitoes with the highest %C compared to 0:10, 0:20, 1:10, and 1:0 ratios, with 2:20 intermediate to 0:10 and 1:10, but greater than 0:20 and 1:0 (Fig 3.1). *Aedes albopictus* reared in 2:20 cricket:oak had a significantly greater C:N than those reared in low oak alone (0:10), but mosquitoes reared in high animal alone (2:0) had the highest C:N, which was significantly greater than all other detrital ratios (Fig 3.2). Although SCCs were large for %N, mean separation failed to detect differences among means. The detritus level with the least %N was 1:10 (9.38

%N) and the highest was 1:0 (10.26 %N).

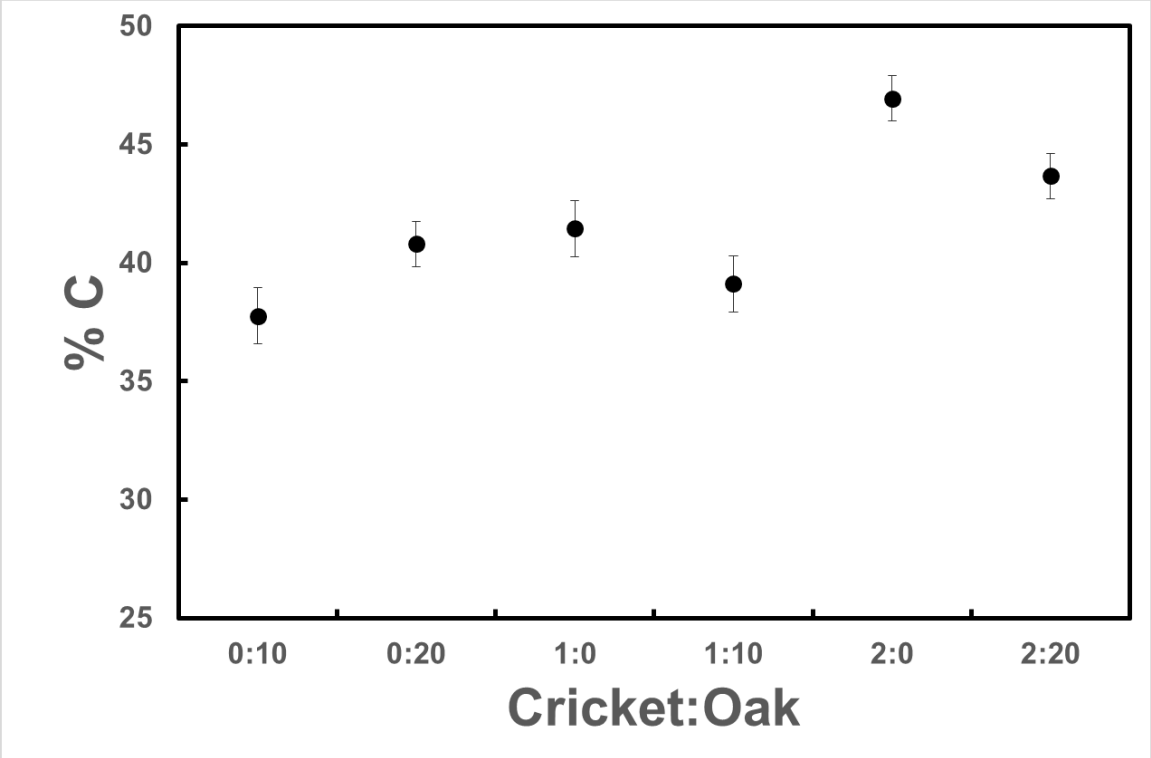

Figure 3.1 Percent carbon (C) for adult *Aedes albopictus* mosquitoes across different detritus ratios (cricket:oak).

Note: Values are means ± SE from three replicates (except 0:10, 1:0, and 1:10 which had two replicates). Detritus ratios are expressed in units, where one unit = 0.125 gram.

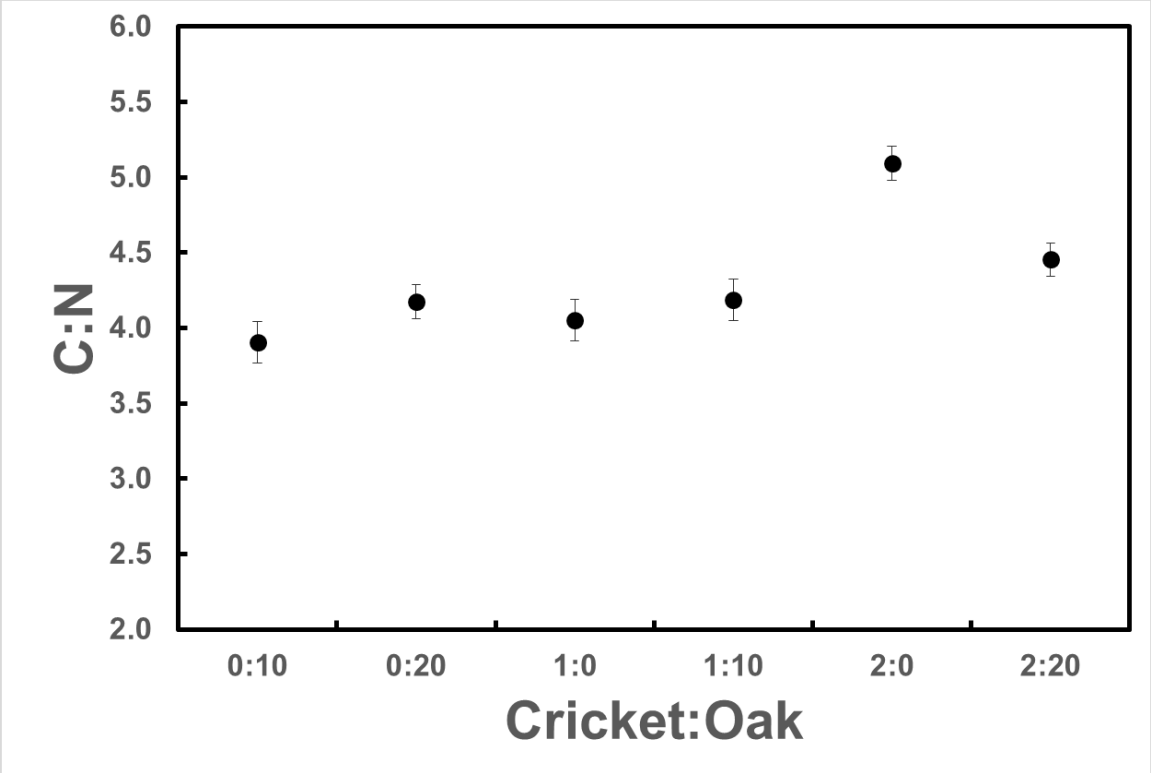

Figure 3.2 Ratio of percent tissue nitrogen (N) and carbon (C) for adult *Aedes albopictus* mosquitoes across different detritus ratios (cricket:oak).

Note: Values are means ± SE from three replicates (except 0:10, 1:0, and 1:10 which had two replicates). Detritus ratios are expressed in units, where one unit = 0.125 gram.

For *Ae. aegypti*, raw C:N data met assumptions of normality, but %C and %N were log(x) transformed to meet assumptions. To simplify interpretation, raw data are presented in figures. There were significant effects of larval densities (Pillai's $Trace_{15, 156} = 0.775$, $P < 0.001$), detritus ratios (Pillai's $Trace_{15, 156} = 1.107$ $P < 0.001$), and their interaction (Pillai's $Trace_{72, 156} = 1.326$ $P = 0.003$). For the interaction, SCCs were largest for %N (-33.83) and %C (35.97) and smaller for C:N (-12.25). The 10:20 *Ae. aegypti*: *Ae. albopictus* density reared in oak alone (0:10) had significantly less %C than all other combinations except for 20:10 *Ae. aegypti*: *Ae. albopictus* ratio in low oak alone (0:10) (Fig 3.3). These individuals also had significantly less %N than those from *Ae. aegypti*:

Ae. albopictus density 10:0 in detritus ratios 0:20, 2:20, and 2:0, 20:20 *Ae. aegypti*: *Ae. albopictus* density in detritus ratios 2:20 and 1:10, 20:10 *Ae. aegypti*: *Ae. albopictus* density in detritus ratio 0:20, 20:0 *Ae. aegypti*: *Ae. albopictus* in detritus ratio 2:0, and 10:20 *Ae. aegypti*: *Ae. albopictus* density in the 1:0 detritus ratio (Fig 3.4).

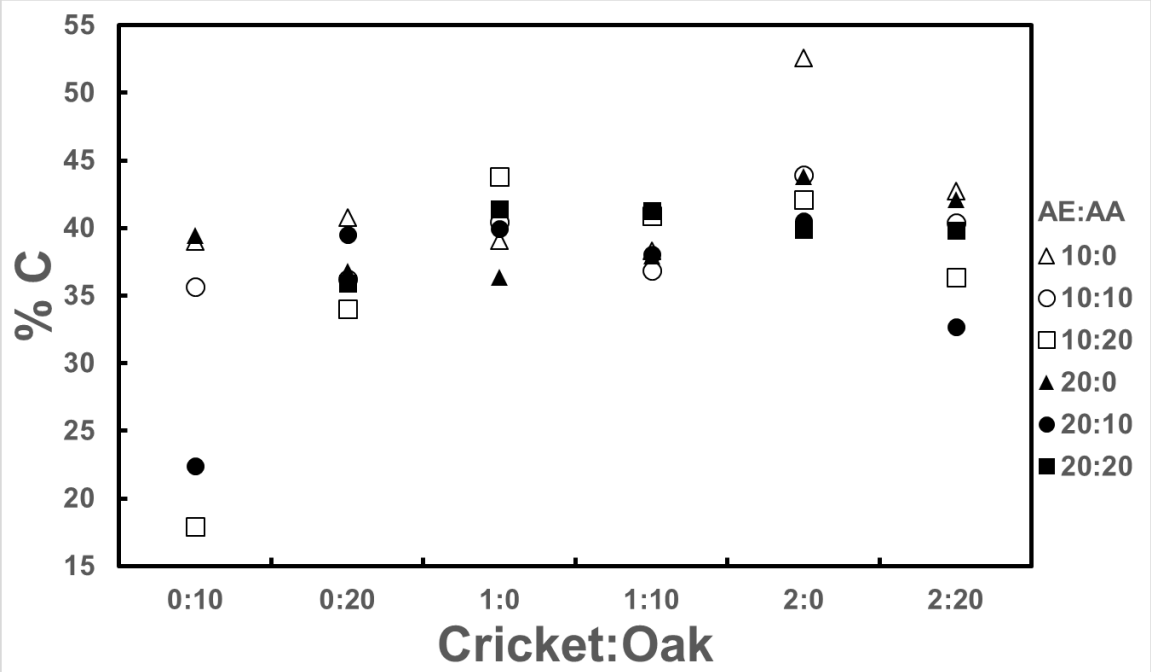

Figure 3.3 Percent carbon (C) for adult *Aedes aegypti* mosquitoes across different detritus ratios (cricket:oak) and mosquito densities (AE:AA).

Note: AE = *Aedes aegypti* and AA = *Aedes albopictus*. Values are means ± SE from three replicates (except 0:10, 1:0, and 1:10 which had two replicates). Detritus ratios are expressed in units, where one unit = 0.125 gram

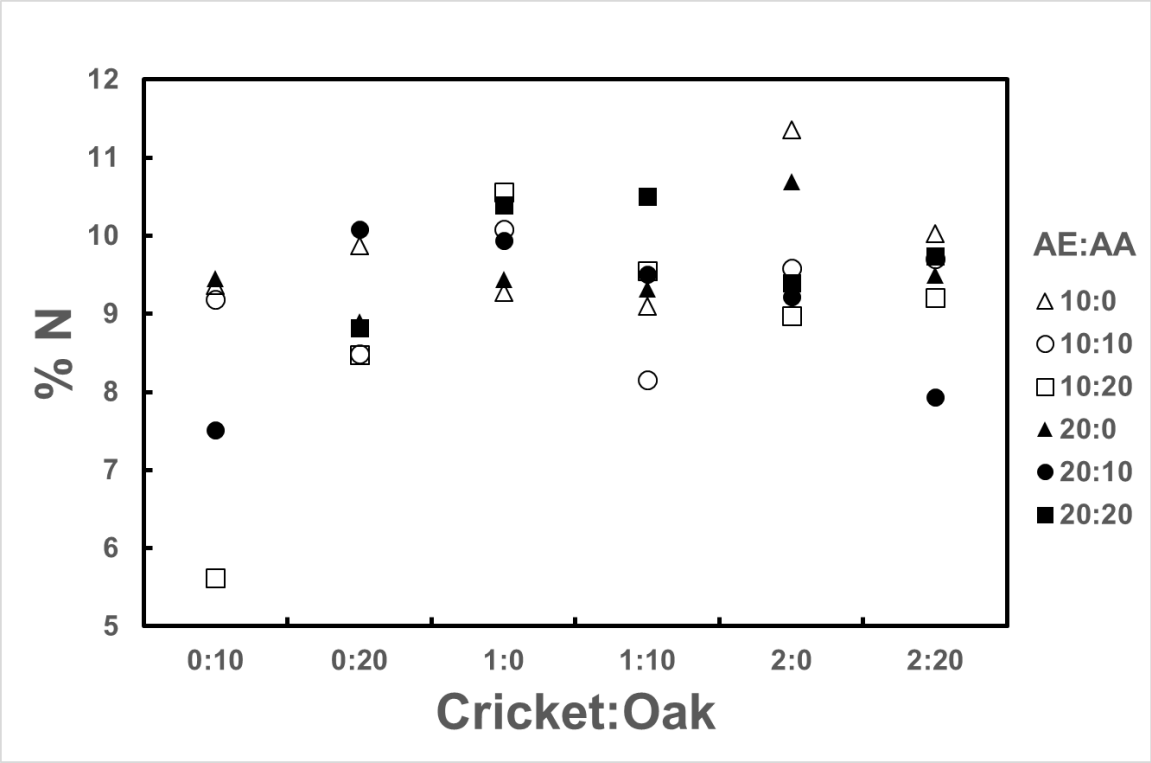

Figure 3.4 Percent nitrogen (N) for adult *Aedes aegypti* mosquitoes across different detritus ratios (cricket:oak) and mosquito densities (AE:AA).

Note:AE = *Aedes aegypti* and AA = *Aedes albopictus*). Values are means ± SE from three replicates (except 0:10, 1:0, and 1:10 which had two replicates). Detritus ratios are expressed in units, where one unit = 0.125 gram.

Aedes aegypti individuals reared in the 10:10 density in detritus ratios 2:0 and 1:10 had greater C:N than those from the 20:10 *Ae. aegypti*: *Ae. albopictus* ratio in low oak alone (0:10) (Fig 3.5). Those reared in the 10:20 *Ae. aegypti*: *Ae. albopictus* density in the high animal alone (2:0) had greater C:N than those from 10:20 and 20:10 *Ae. aegypti*: *Ae. albopictus* density in low oak alone (0:10). Within the 20:10 density, C:N ratio was significantly greater in the high animal alone (2:0) compared to the low leaf alone (0:10) (Fig 3.5).

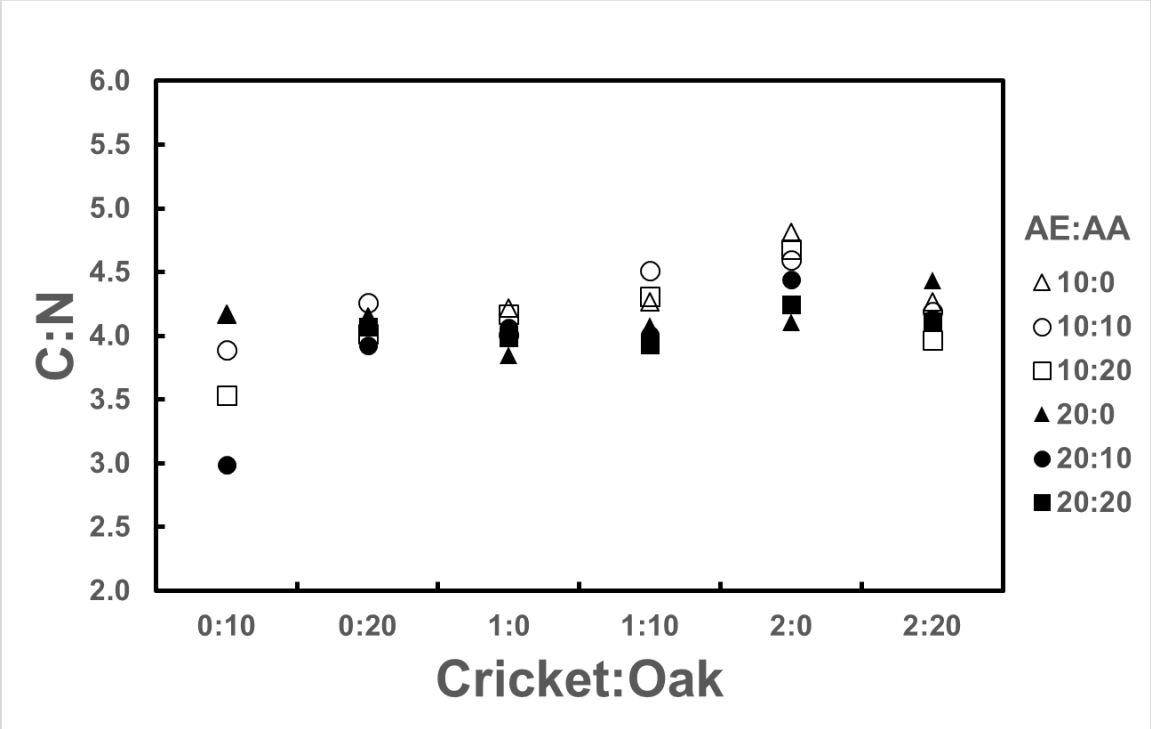

Figure 3.5 Ratio of tissue nitrogen (N) and carbon (C) for adult *Aedes aegypti* mosquitoes across different detritus ratios (cricket:oak) and mosquito densities (AE:AA)

Note: AE = *Aedes aegypti* and AA = *Aedes albopictus*). Values are means ± SE from three replicates (except 0:10, 1:0, and 1:10 which had two replicates). Detritus ratios are expressed in units, where one unit = 0.125 gram.

3.3.2 Population Growth (λ')

For *Ae. albopictus,* neither raw nor transformed data met assumptions of normality however variances did meet assumptions. There were significant effects of density combinations and detritus ratios on λ' for *Ae. albopictus,* but their interaction was not significant (Table 3.1). For the density effect, all larval densities except for 20:10 had significantly greater mean λ' than 20:20 (Fig 3.6). In addition, λ' for the 0:10 density was also significantly greater than 20:10. All other ratios were not significantly different from one another. For detritus ratios, the value of λ' in 0:10 Cricket: Oak was significantly lower than all other ratios, with high leaf alone (0:20) being significantly lower than 2:20,

and 2:0. The value of λ' for the 2:20 ratio was greater than 0:10, 1:10, and 1:0, with the 2:0 was greater than 0:10 and 1:0 (Fig 3.7).

Table 3.1

Results of two-way ANOVA (detritus ratio and density) on values for estimated population growth (λ') for *Aedes albopictus* and *Aedes aegypti* in experimental microcosms.

	Finite rate of increase (λ')					
		Ae. albopictus			*Ae. aegypti*	
Source	d.f.	*F*	*P*	d.f.	*F*	*P*
Resource (R)	5	58.90	**<0.0001**	5	140.74	**<0.0001**
Density (D)	5	21.99	**<0.0001**	5	27.35	**<0.0001**
R x D	25	1.20	0.2860	24	4.99	**<0.0001**

Note: Significant effects are shown in bold type.

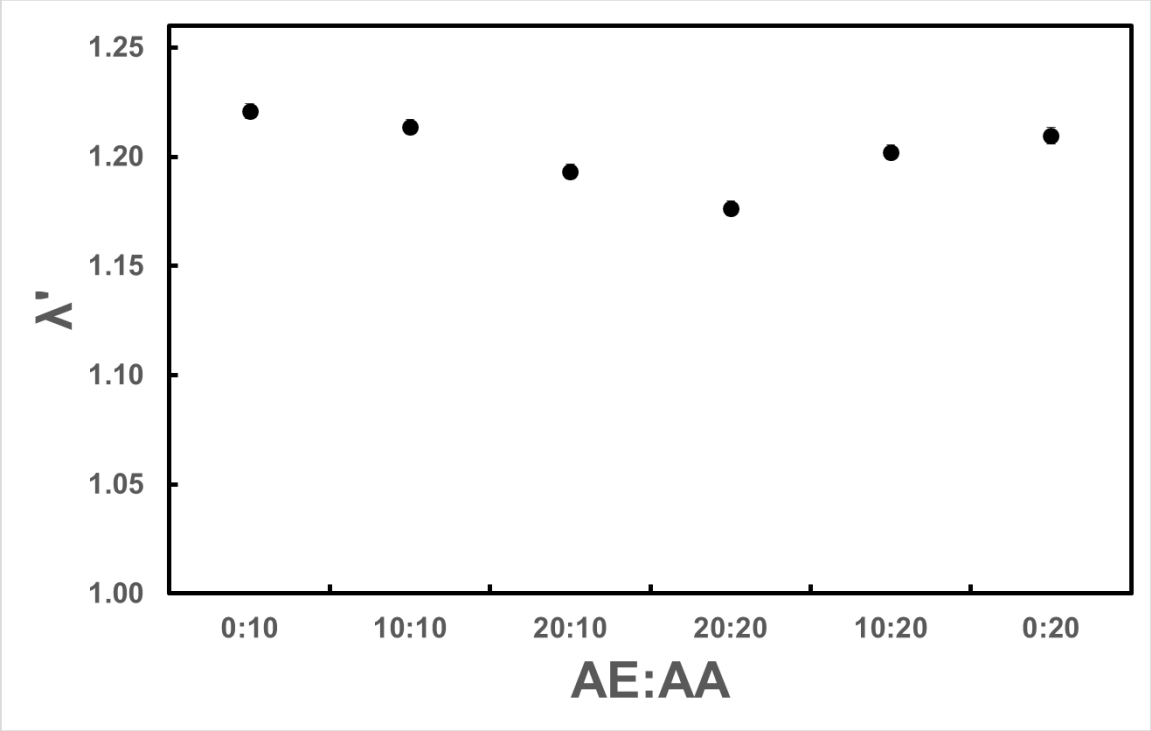

Figure 3.6 Estimated population growth (λ') of *Aedes albopictus* across mosquito densities (AE:AA).

Note: AE = *Aedes aegypti* and AA = *Aedes albopictus*). Values are means ± SE from three replicates (except 0:10, 1:0, and 1:10 which had two replicates).

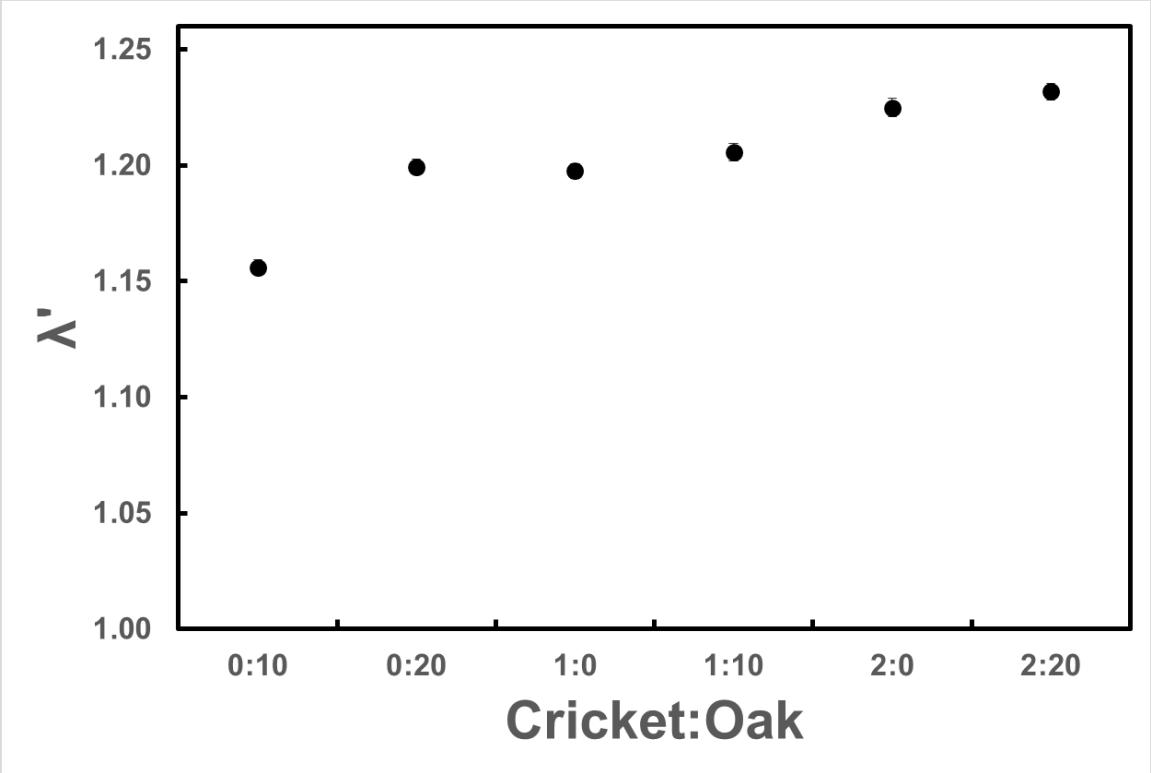

Figure 3.7 Estimated population growth (λ') of *Aedes albopictus* across different detritus ratios (cricket:oak).

Note: Values are means ± SE from three replicates (except 0:10, 1:0, and 1:10 which had two replicates). Detritus ratios are expressed in units, where one unit = 0.125 gram.

For *Ae. aegypti*, assumptions were met by the raw data. There were significant effects of density combinations, detritus ratios, and their interaction on λ' for *Ae. aegypti* (Table 3.1). Within detritus combinations 0:20, 1:10, and 1:0, the mean λ' of the 10:10 larval ratios were significantly greater than 20:20. Within combinations 0:20 and 1:0, the mean λ' of 20:20 ratios were significantly less than 10:0. In detrital combination 0:10, the mean λ' of larval ratio 10:10 was significantly greater than 10:20, but less than 20:10 and 10:0. There were no significant differences in the 2:20 or 2:0 detrital combinations.

Within larval densities 10:10, 10:20, 20:0 and 10:0, the mean λ' of *Ae. aegypti* in detrital combinations 0:20 were significantly greater than 0:10 combinations. In the larval

ratio 20:20, the mean λ' of detrital combination 0:20 was significantly less than 2:20, and there were no significant differences found in the 20:10 larval ratios (Fig 3.8).

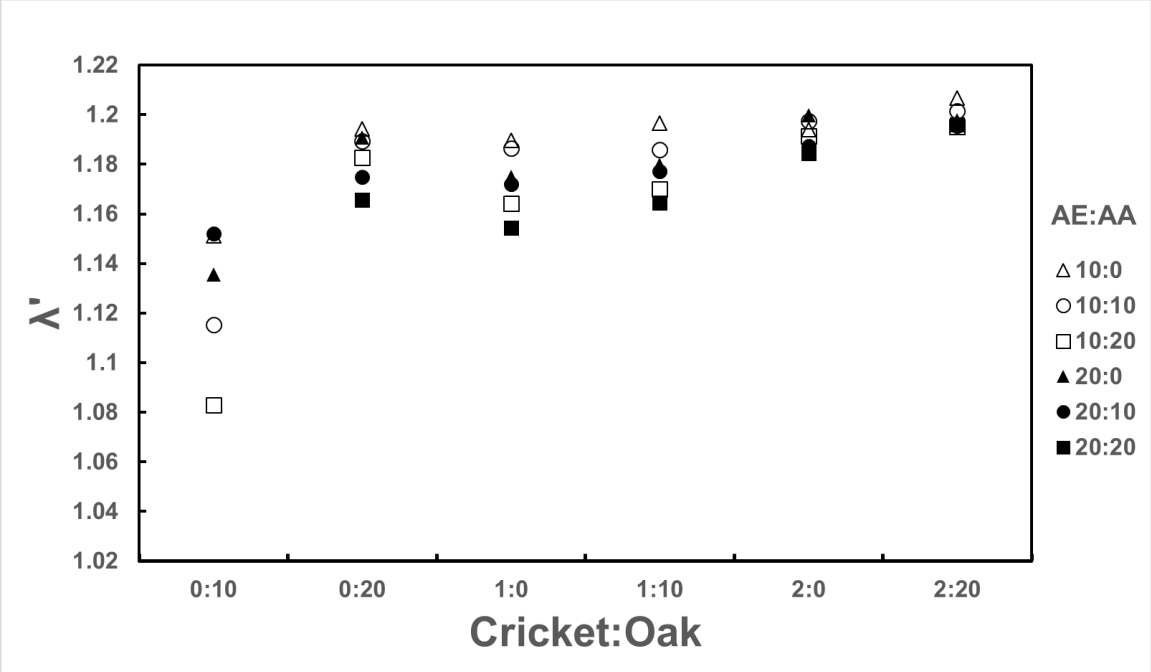

Figure 3.8 Estimated population growth (λ') of *Aedes aegypti* across different detritus ratios (cricket:oak) and mosquito densities (AE:AA).

Note: AE = *Aedes aegypti* and AA = *Aedes albopictus*). Values are means ± SE from three replicates (except 0:10, 1:0, and 1:10 which had two replicates). Detritus ratios are expressed in units, where one unit = 0.125 gram.

3.4 Discussion

My data show that detrital types and larval densities affect mosquito nutrients, population growth, and survival, which, in turn, affect competitive outcomes. *Aedes albopictus* stoichiometry values did not vary with respect to intraspecific or interspecific density combinations; this was not true for *Ae. aegypti*. Within the intraspecific density combinations, there was no variation in *Ae. aegypti* stoichiometry, but under more nutrient limited interspecific combinations (e.g., oak leaves only) there were significant decreases in both nitrogen and C:N of *Ae. aegypti*, suggesting that the presence of *Ae. albopictus* changed their stoichiometry in these nutrient limited environments. This is the

first report of competition affecting the nutrient composition of mosquitoes, and may help us understand how competition affects specific aspects of mosquito biology. Both stoichiometry and population growth data support my hypothesis that *Ae. albopictus* exhibits greater survivorship with lower nitrogen than *Ae. aegypti*, but species coexistence occurs under higher nitrogen levels (Juliano, 2010)

Within the most nutrient limited environment (0:10) were the most notable effects on *Ae. aegypti*. This detrital combination showed the lowest overall survival rates (Fig 3.8) for *Ae. aegypti* compared to the other detrital combinations. In *Ae. aegypti* only densities (10:0 and 20:0) average survival was greater than 80%. However, survival in the 10:10 larval density was 65%, and when *Ae. albopictus* numbers were increased (10:20), survival dropped to 55%, whereas in the 20:10 density, survival dropped to 40%, and at the highest density (20:20), no *Ae. aegypti* survived (Fig 3.9). *Aedes albopictus* survival was 20-45% higher than *Ae. aegypti* across all these interspecific environments. When compared to other detrital combinations, *Ae. aegypti* survival increased with more nutrients available, with the highest survival in combinations with the greatest nitrogen and animal content (i.e., 2:0 and 2:20). The nutrient signatures of *Ae. aegypti* within the 0:10 detrital combinations followed the same pattern as survival. Specifically, C:N and %N all decreased as more *Ae. albopictus* were present (Fig 3.4 and 3.5). These results suggest nitrogen to be the limiting nutrient compared to carbon and when faced with the presence of *Ae. albopictus* competition, *Ae. aegypti* are not as effective in obtaining or assimilating this nutrient, which may negatively affect survival. As nitrogen has been suggested to be a major limiting element in container systems (Kaufman and Walker, 2006) my findings could help to explain patterns of occurrence of these species in nature.

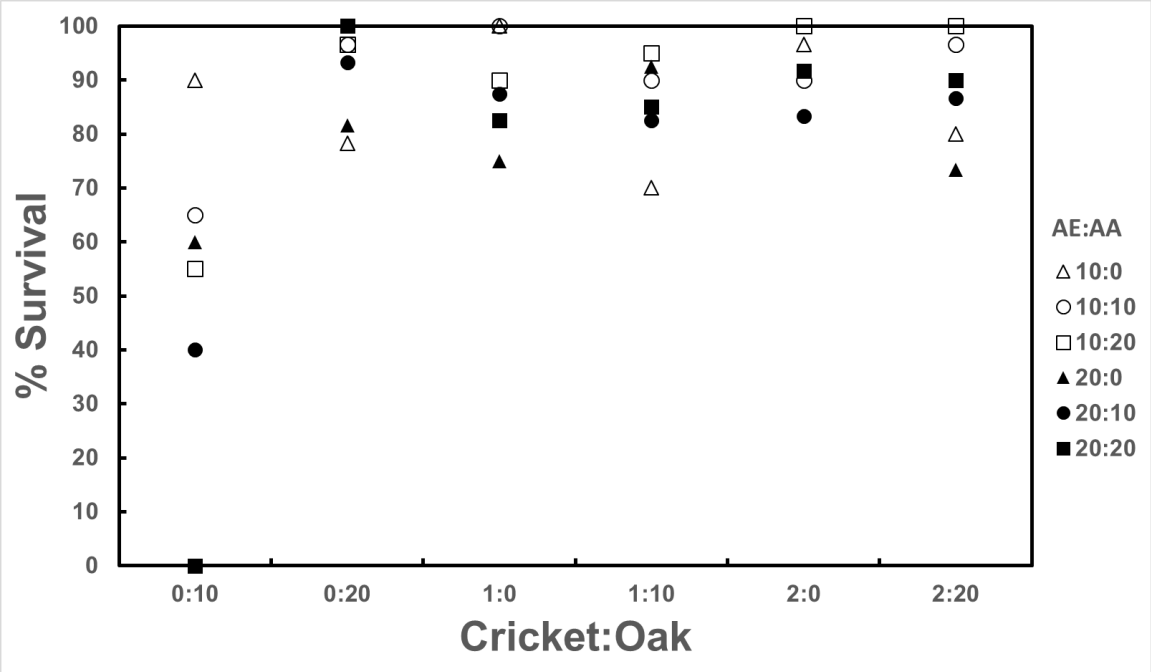

Figure 3.9 Survival percentage of *Aedes aegypti* mosquitoes across different detritus ratios (cricket:oak) and mosquito densities (AE:AA).

Note: AE = *Aedes aegypti* and AA = *Aedes albopictus*). Values are means ± SE from three replicates (except 0:10, 1:0, and 1:10 which had two replicates). Detritus ratios are expressed in units, where one unit = 0.125 gram.

Of the detrital types used in this experiment, cricket carcasses are quick to decay, producing a higher nitrogenous environment (Yee and Juliano, 2006). Crickets have a significantly greater nitrogen content than oak leaves, which are slower to decay and mostly made of carbon, resulting in a nitrogen poor larval environment (Yee et al. 2015a). This rapid decay along with larvae consuming cricket carcasses directly, allow for mosquitoes to obtain nutrients quicker, whereas leaf detritus must be broken down by decomposers before nutrients are available to larvae (Daugherty et al., 2000, Yee and Juliano, 2006, and Yee et al., 2007). Under intraspecific competition, both species generally had greater population growth and survival in higher nitrogen environments (e.g., 2:0), but lower survival and population growth in nitrogen poor environments (e.g., 0:10) (Fig 3.8). As with other studies, my work showed that different detrital types and

amounts can affect larval development, which affects competition intensity and potentially distribution patterns in the field (Juliano, 1998, Daugherty et al., 2000, Yee and Juliano, 2006, Murrell and Juliano, 2008, and Juliano, 2009).

Between the two *Aedes* species compared, *Aedes albopictus* appears to be the superior competitor. Within all intraspecific treatments, especially nitrogen poor environments, *Aedes albopictus* showed greater survival, and also had greater λ' values across all other treatment combinations. In treatments with higher available nutrients or with the introduction of cricket carcasses, *Aedes aegypti* survival increased, decreasing competitive intensity. These findings are consistent with previous studies that find *Aedes albopictus* to be a superior competitor within low nitrogen or low nutrient larval environments and a decrease in competitive intensity between *Aedes* species when more nutrients or animal detritus are available (Daugherty et al., 2000, Yee and Juliano, 2006, Yee et al., 2007, Murrell and Juliano, 2008, Murrell et al., 2011).

I also found that interspecific competition varied among detrital environments. *Aedes aegypti* was negatively affected in lower nitrogen environments in the presence of a competitor, however *Ae. albopictus* thrived in these environments. This suggests that *Ae. albopictus* is the more successful competitor within these more nutrient limited environments, also consistent with other laboratory studies (Yee et al., 2004, Daugherty et al., 2000, Murrell and Juliano, 2008). The mechanism for this superiority may be due to foraging behaviors. *Aedes* species feed by filtering and browsing, preferably the latter when substrate is available (Yee et al., 2004, Kesavaraju et al., 2007). Previous studies showed *Ae. albopictus* spends significantly more time browsing leaf surfaces than *Ae. aegypti*, which may contribute to its competitive advantage in environments with limited

nutrients (Yee et al., 2004, Yee, 2016). The suggestion that *Ae. albopictus* is a superior resource competitor because of foraging does not exclude it from being able to better assimilate limiting nutrient like nitrogen, however at present no data exist to support this notion.

In nature, these *Aedes* species occupy many types of containers. These container variations and locations provide for a wide array of potential larval environments (see Chapter II). The results of this study can be applied to nature as one could expect to find both species inhabiting containers, such as cemetery vases, that often see both plant and animal detrital input. However, I may predict that we would only find *Ae. albopictus* in a tire on the edge of a parking lot that may only have fallen leaves as detritus. Knowing the environmental conditions, including potential detrital input and container environments of an area can aid in predicting mosquito distribution patterns and potential areas at higher risk of arboviral disease outbreaks.

This study shows the effects of detrital inputs and larval densities on the survival, population growth, and nutrient signatures of *Ae. albopictus* and *Ae. aegypti*. *Aedes albopictus* was shown to be a superior competitor in low nitrogen environments, but when nitrogen was increased, competitive intensity decreases to a point of coexistence, as evidenced both species having higher λ' values under these conditions, especially *Ae. aegypti*, which showed a significant increase in survivability in comparison to treatments of the same density but lower nitrogen content. Understanding the competitive interactions that take place within the larval environment of these two container species is a key part of our knowledge of the distribution patterns of these *Aedes* species. These findings contribute to our understanding of the process that affects potential coexistence

and exclusion for *Aedes albopictus* and *Ae. aegypti*. Knowing these mechanisms will help in control measures and predicting sites of future arboviral disease outbreaks. In addition, future research into nutrient signatures and how they might affect adult life histories and viral competence would be of great importance. This is a relatively underexplored, yet medically important, research area.

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