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Carbon and Nitrogen Analysis To Determine Competitive Outcome For Three Species of Container Mosquitoes

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by

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Abstract

It is well documented that intense larval competition exists between species of container mosquitoes. Two of the main genera of mosquitoes found to inhabit containers are *Aedes* and *Culex*. This study sought to determine the effects that different detritus treatments and larva ratios would have on carbon and nitrogen content, mass, and survival of larvae of various species. The species used in this experiment were *Aedes albopictus, Aedes aegypti* and *Culex quinquefasciatus.* I hypothesized that *Ae. albopictus* would be more efficient in acquiring nitrogen then the competitor species *Ae. aegypti* and *Cx. quinquefasciatus.* Thus, I expected *Ae. albopictus* to have higher survivorship levels than the other species when competition took place in an environment with limited resources. I also hypothesized that survival would vary in all species between detritus types. I used single and mixed amounts of leaf and animal detritus: 2:0, 1:1, 2:10 and 0:10 animal:leaf, with one unit of detritus equaling 0.10 g. The detritus treatment levels were crossed with five larval densities: 0:20. 20:0, 20:20, 0:40, 40:0. My results showed that neither the detritus treatment nor larval intra- or interspecific densities had any effect on the survivorship of *Ae. albopictus. Aedes aegypti* showed no changes in survivorship across intra- or interspecific densities, but did show decreased survivorship in treatments that contained only leaf detritus compared to those with animal detritus. *Culex quinquefasciatus* showed changes in survivorship due to both larval density and detritus treatment levels. Survival was highest for *Cx. quinquefasciatus* in detritus treatments containing animal detritus and lowest in leaf only treatments. Their survival suffered in the high larval density treatments compared to the low density larva treatments. However,

survival for *Cx. quinquefasciatus* was unusually high in the high larva density animal only detritus treatment with *Ae. albopictus* present. Findings support the view that *Ae. albopictus* is the top competitor in container enviroments due to the lack of intra- and interspecific competitive effects across the detritus types and amounts used. Analysis of nitrogen, which is assumed to be limited in the systems studied, will allow for a better understanding of the mechanism by which *Ae. albopictus* is able to better survive.

Keywords: competition, container system, carbon, nitrogen, stable isotope

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Introduction

Problem Statement

There are approximately 3,500 species of mosquitoes worldwide (Knight and Stone 1977), some of which are capable of acting as vectors of important human diseases. The species that are capable of acting as disease vectors are particularly well researched due to their medical interest, including *Aedes albopictus* (Asian tiger mosquito), *Aedes aegypti* (yellow fever mosquito), and *Culex quinquefasciatus* (southern house mosquito).

It has been well documented that *Ae. albopictus* has expanded its range within the United States dramatically since its first recorded sighting in North America in 1985 (Hawley et al. 1987). As *Ae. albopictus* has invaded more regions of the United States, it has negatively affected resident species such as *Ae. aegypti,* and in certain regions this competition has even lead to regional extinction of *Ae. aegypti* (Juliano 1998). It is known that competition exist between native and non-native species of mosquitoes and that some species are more successful than others in competitive environments. However, the mechanism that causes one species to be more successful than the other is not always clear. It has been hypothesized that some species may be able to make better use of the nutrients available in a system (Juliano 2010).

Stable isotope analysis is a technique that can be used to determine how consumers interact with the food web and allows inferences to be made about a consumer's diet by tracking certain isotopes of common elements (e.g., ^{13}C and ^{15}N) (Post 2002). Stable isotope analysis has been used to determine how levels of ^{13}C and ^{15}N vary when mosquito larvae are reared in non-competitive environments with different nutrient levels for *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* (Winters and Yee 2012, Kaufman et al. 2012, Ezeakacha et al. unpublished data). However, it has not been

determined how these levels vary in a competitive environment. The purpose of this experiment is to determine how the total values of carbon and nitrogen for adult mosquitoes vary among *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* under interspecific competitive interactions, and in various detritus environments to see if these values can help to explain the outcome of competition.

Literature review

Metamorphosis

Mosquito exhibit a complex life cycle, with development having four distinct phases: egg, larva, pupa, and adult. Mosquitoes of the species *Culex* lay their eggs on the surface of the water in large masses called egg rafts. In the genus *Aedes* adult females also lay their eggs on the surface of the water, but their eggs are laid individually and do not group together in rafts. In most *Aedes*, eggs are laid above the water line on the sides of the container (e.g., tires, tree holes) or other open water system (e.g., pond). Eggs generally take 48 hours to hatch, but exact time depends on temperature and the species of mosquito. Once the eggs hatch, the larvae pass through four instars, with a molt separating each. Most larvae feed on heterotrophic microorganisms that grow on organic material (e.g., detritus). As the larva feed they begin to grow larger and subsequently molt into successive instars (2^{nd} thru 4^{th}). After the 4^{th} instar, the mosquito develops into a pupa. In the pupal phase the mosquito does not feed, so it is important that the mosquito feed heavily during the larval stage. After the pupal stage the mosquito then develops into an adult mosquito and leaves its aquatic habitat (Capinera 2008).

Mosquito Habitats

Many *Aedes* and *Culex* are referred to as container mosquitoes as they frequently are found to inhabit small bodies of water such as discarded vehicle tires, cemetery vases, and tree holes. In these environments the immature mosquitoes depend on the microorganisms living on the detritus found in the container to fuel their development (Hawley 1988). The detritus is often made up of dead leaves, flowers, and the bodies of dead invertebrates. Leaf input into the system is an important nutritional component, but it has been shown that the invertebrate carcasses offer more nutrients (Yee & Juliano 2007). It also has been shown that microorganisms, which are an important food source of mosquito larva, are capable of growing on both plant and animal detritus (Yee & Juliano 2006).

Identification of Adults

It is relatively easy to identify different mosquito to genus, but it becomes increasingly more difficult to differentiate between species within the same genus. *Aedes albopictus* are of medium body size and have a body length which typically ranges between 2 and 10 mm. In this species, the head, thorax, abdomen and wings are distinctively black in color (Fig.1)(Hawley 1988). Their legs have multiple large white bands that give them a distinctive look. Another notable feature is the broad white stripe that runs down the center of the thorax and head (Fig.1) (CDC 2012)

Figure 1: Photo of *Aedes albopictus* in the wild. Note the black coloration and patterning of white stripes on thorax and legs. Photo by S. Ellis, Bugwood.org

Aedes aegypti look very similar to *Ae. albopictus* unless viewed under magnification. *Aedes aegypti* is slightly smaller in size than *Ae. albopictus* with an average length between 4.0 and 7.0 mm. Adults are usually brown or black in body color although the legs contain the trademark white bands that all members of the *Aedes* genus possess (Fig. 2). Unlike *Ae. albopictus*, adult *Ae. aegypti* have two white stripes running parallel to each other down the middle of the dorsal side of the thorax. The two stripes running down the middle of the thorax are surrounded by two thin white stripes that curve away from each other and are located on the outer edges of the dorsal side of the thorax (Fig. 2) (Carpenter and LaCasse 1955).

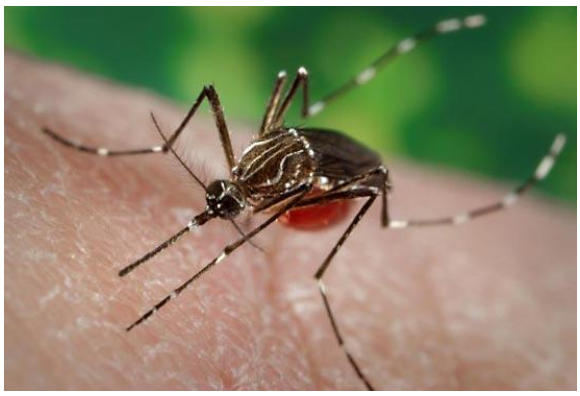

Figure 2: Photo of preserved *Aedes aegypti*. Note one of the two distinctive curving white lines located on the dorsal portion of the thorax can be seen. Note also the white bands located on the legs. Photo by Paul Howell and Frank Hadley Collin.

Adult *Culex quinquefasciatus* are small in body size with the average length being ~ 4.0 mm. The majority of its body is of light brown coloration, with the dorsal portion of the thorax and abdomen being of a darker shade of brown (Fig.3). The wings and legs are also dark brown in coloration (Fig. 3) (Sirivanakarn et al. 1978)

Figure. 3: Photo of preserved *Culex quinquefasciatus.* Note the dark brown coloration of the wings, legs, thorax and dorsal side of the abdomen. Photo by Pest and Diseases Image Library, Bugwood.org

Resource Competition

Competition between species for a shared limiting resource, such as food, often will lead to the competitive exclusion of one of the two species. This competition occurs

when the resources become limited, when the size of the environment decreases, or when the number of competitors increases (Tilman 1982).

One of the dominant factors that affects the success of a mosquito species in a competitive environment is the capability for its larvae to survive in a habitat that is experiencing reduced food levels due to increasing competition. Competition is not limited to multispecies interactions (interspecific competition), but also is affected by competition among individuals of the same species (intraspecific competition), especially as the total number of larvae in a container increases (Juliano 1998, Juliano et. al 2004). For example, *Ae. albopictus* larva has been shown to out compete many native species such as *Ae. aegypti* larva in numerous studies under various conditions(e.g. Daugherty et al. 2000, Juliano 1998, Juliano et al. 2004). Daugherty et al. (2000) showed that in leaf only containers *Ae. albopictus* successfully eliminates *Ae. aegypti*. However, when animal detritus was the resource, *Ae. aegypti* and *Ae. albopictus* appeared to coexist. In this case, *Ae. albopictus* is the superior competitor due to the fact that its larvae are more capable of acquiring the available nutrients in the container compared to *Ae. aegypti*. The reason that exclusion occurs in leaf detritus but not in animal detritus is that animal detritus has been shown to be much more nutrient rich; meaning that less of it is required to promote the healthy development of a specific number of mosquitoes when compared to the amount of leaf detritus that would be required for the same number of mosquitoes to develop into healthy adults. However, it has also been shown that in nature, leaf detritus is by far the most common source of nutrition and thus it is unlikely that environments that contain the unusually large amounts of animal detritus required for both species to develop to their maximum potential exist in large numbers (Kaufman et

al. 2010). Because leaf detritus produces poor nutrient environments compared to animal detritus, the limiting factor must be those low nutrients. This hypothesis was supported by Winters and Yee (2012), who found that *Ae. albopictus* appears to have a lower requirement for nitrogen across different resources environments (e.g., animal only, leaf only, mixed) based on stable isotope and nutrient analysis and that this may point to the mechanism for how *Ae. albopictus* is able to successfully outcompete other species.

Stable Isotope Analysis

Stable isotope analysis is the process by which the ratios of isotopic components of a compound are identified. The term isotope is used when comparing molecules of the same element that differ in the number of neutrons they contain (e.g., ^{13}C vs. ^{12}C.) Isotopes are named based on the number of neutrons found in that particular atom of the element. For example, a nitrogen ion containing twelve neutrons would be referred to as nitrogen 12 with the shorthand notation being ^{12}N.

Isotopic ratios can be used to determine food web interactions because different species of plants and animals have unique isotopic signatures based on factors such as species and environment. These signatures are passed on to consumers, which allow one to roughly determine the diet of the consumer. Isotopic signatures are the identifying ratios that isotopes of the same element occur in. For example plant species "A" may contain ^{34}S in a 20:1 ratio to ^{33}S. Thus, one would expect to find the high levels of ^{34}S and low levels of ^{33}S in the isotopic analysis of a consumer that feeds primarily on this plant. The elements for the experiment outlined below will focus on carbon and nitrogen. As a general average across all species carbon naturally occurs in ~99:1 ratio of ^{12}C to ^{13}C. Carbon is mainly produced by primary producers and thus can be studied to

determine the plant base of the food chain in the studied environment, or to see if plants are the primary food source of a consumer. The isotopic ratios of nitrogen can be used to determine the trophic level of a consumer. Nitrogen (e.g., ^{15}N) isotopes are primarily retained by consumers, and thus are excreted at a very low ratio compared to the amount consumed. Therefore, ^{15}N isotopes are passed from consumer to consumer up the food chain meaning the higher the ^{15}N isotope level the higher the organism's trophic level (Fig 4.)(Post 2002).

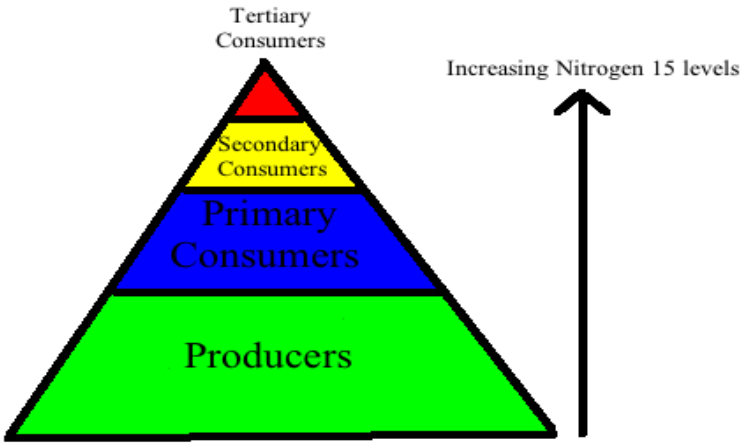

Figure 4: Diagram of how nitrogen 15 travels up the food chain, enabling one to determine the trophic level of an organism.

Methodology

Research perspective

This study was conducted during the 2013-2014 academic year and was concerned with determining the competitive outcome of three species of container mosquitoes: *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus*. I expected *Ae. albopictus* to have higher survivorship than the other species in beakers with limited resources (e.g., leaves only). I also expected *Ae. albopictus* to have the highest survivorship in beakers where interspecific competition was occurring.

Research design

Collection of Eggs

The mosquitoes used in this research were of the species *Aedes albopictus, Aedes aegypti*, and *Culex quinquefasciatus*. Eggs of *Ae. albopictus* were obtained from lab colonies that originated from larvae collected in abandoned tires in the Hattiesburg area, whereas *Ae. aegypti* eggs were obtained from cemetery vases in New Orleans, Louisiana. Egg rafts of *Cx. quinquefasciatus* were collected from abandoned tires in the Hattiesburg area, but a lab colony was not established prior to use. All first instar larvae used in the experiment were hatched on site.

Hatch and Treatments

The eggs were hatched in a solution of 0.33g Nutrient Broth (Difco™, BD, Sparks, MD, USA) and 750 ml of water that was purified through reverse osmosis (RO). Upon hatching, larvae were rinsed to remove all remnants of the nutrient broth. The larvae were then placed in 250 ml tripour beakers containing various ratios of animal (Freeze-dried crickets (*Acheta domestica*) and leaf (senescent red maple (*Acer rubrum*) detritus. The

leaves were collected from the Lake Thoreau Center, Hattiesburg, MS, U.S.A.(31° 19′ 37.63′ ′N, 89° 17′ 25.22′ ′W).

There were four amounts of detritus used in this experiment: 2:0, 1:1, 2:10 and 0:10 animal:leaf, with one unit of detritus equaling 0.10 g. These amounts were based on a prior experiment testing intraspecific competition among these same species (Ezeakacha et al. unpublished data). The detritus treatment levels were crossed with five larval densities: 0:20. 20:0, 20:20, 0:40, 40:0 (species A and B) with all two species combinations (i.e., *Ae. albopictus*: *Ae. aegypti*, *Ae. albopictus*: *Cx. quinquefasciatus*, *Cx. quinquefasciatus*: *Ae. aegypti*). Detritus and density combinations were replicated three times for a total of 108 experimental beakers (Fig 5).

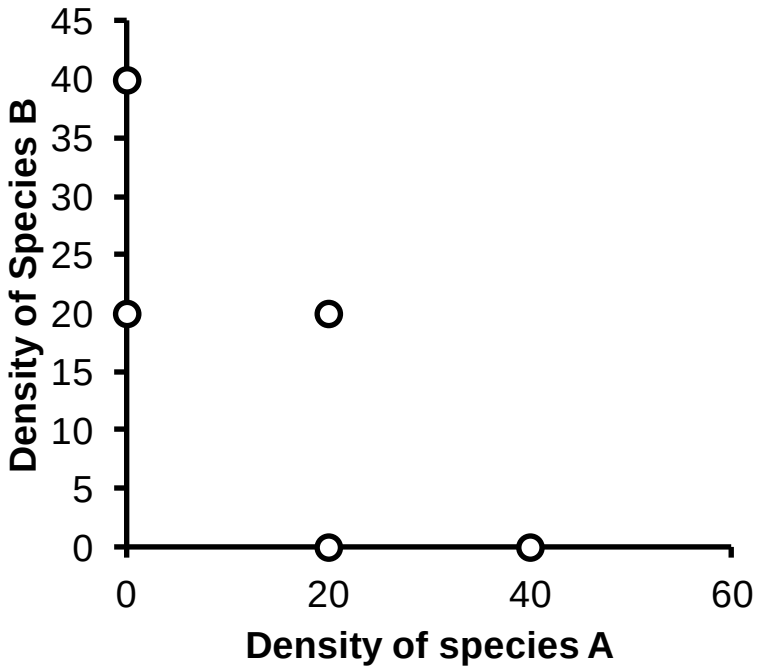

Figure 5. Shows experimental design in relation to larval ratios. Note beakers contained either one or two species, never all three.

The beakers were prepared 48 hrs prior to larval addition. Each beaker contained detritus and 199 ml of RO water and 1 ml of homogenized tire inoculum collected from

field tires in the Hattiesburg area to allow for microorganism growth. Throughout the experiment RO water was added to maintain the 200 ml water level. Beakers were placed into an environmental chamber (Percival Scientific, Inc., Perry, IA, USA) set to 20°C on a 12h:12h light:dark cycle (Ezeakacha et al. unpublished data, Winters and Yee, 2012). Trays were rotated daily in a clockwise motion. The experiment ran for a total of 57 days during which time all but 45 of the 3,600 larvae which started in the experiment either eclosed or died. As a result of contamination a number *Cx. quinquefasciatus* beakers had to be removed from the experiment. These treatments were replicated and re-run after the initial experiment concluded.

Collection of Pupae and Identification

Beakers were checked daily for pupae and when present they were removed and placed in individual shell vials until they eclosed. Adults were identified to sex and species, freeze-killed and placed in an oven set to 50 ^{0}C for 48 hrs. Once dry, the mass of each mosquito was measured using a XP2U ultramicrobalance (Mettler Toledo Inc., Columbia, Ohio). The data collected for each treatment included male and female development time, dry mass, and survivorship rate of larvae to adult for each species.

Analysis

Analysis of variance (ANOVA) was used to determine differences among detritus types, competition densities, and their interaction for survivorship for *Aedes albopictus* and *Aedes aegypti*. Because I did not use the high intraspecific density of *Culex quinquefasciatus* (i.e., 40 larvae), a standard two-way (ANOVA) would not contain all possible combinations of density and detritus and would thus would be unbalanaced. Therefore, I used a one-way (ANOVA) in which I combined detritus ratios and larval

densities into one treatment. This approach gave me a total of 15 treatment combinations. For all test follow-up Tukey-Kramer HSD post-hoc test for multiple comparisons were used to determine the differences in the survivorship rates between species for any significant effects. A log transformation was conducted on the data for *Culex quinquefasciatus* prior to analysis to meet assumptions of ANOVA. A x^2 transformation was applied to both *Ae. albopictus* and *Ae. aegypti* to meet assumptions of ANOVA.

Results

Survival

For *Aedes albopictus*, there were no significant differences across detritus treatment levels, across larval densities, or their interaction (Table 1). Mean survivorship of *Ae. albopictus* was high (mean ± SE, 80.2% ± 1.98%) regardless of detritus ratio or larval density.

There were no significant differences among the larval combinations or the interaction between larval densities and detritus ratios, however there was a significant difference in the survivorship of *Ae. aegypti* across detritus treatments (Table 1). Post-hoc tests indicated that the 0:10 had the lowest survival compared to the detritus ratios that contained high animal detritus (2:10, 2:0) with the 1:1 mixed treatment showing intermediate survival (Fig 6).

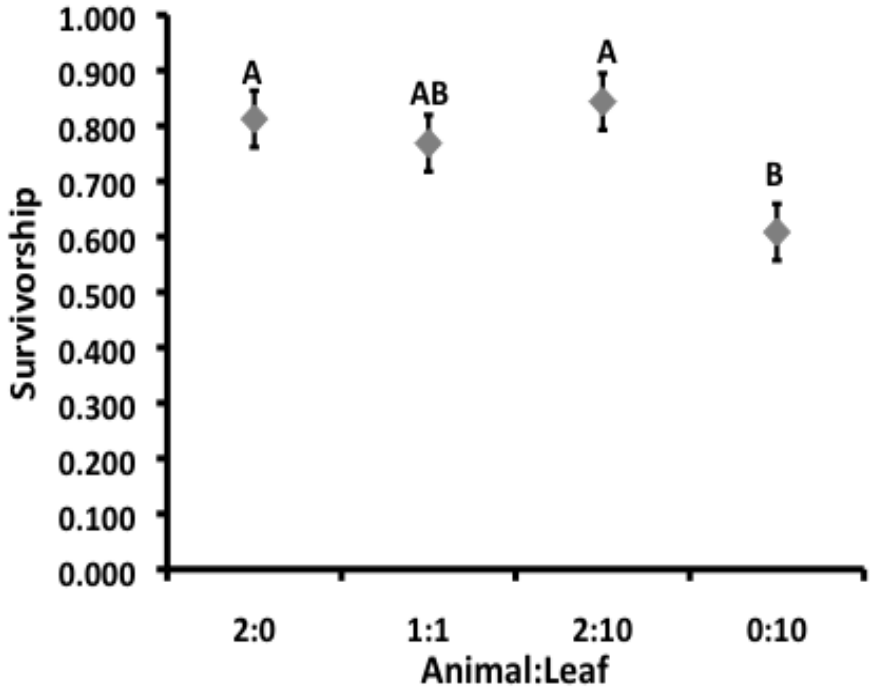

Figure 6: Survivorship (mean ± SE) of *Aedes aegypti* across detritus treatment levels. Letters A and B indicate relationship between means.

Survivorship differed significantly with detritus ratio, larval density, and their interaction for *Culex quinquefasciatus* (Table 1). Post-hoc tests indicated that *Cx. quinquefasciatu*s survival was significantly higher in treatments that contained high animal detritus (2:0, 2:10) compared to the leaf only 0:10 (Fig 7). The test also indicated that in most cases survival was higher in the low larval intraspecific density 0:20 when compared to the interspecific larva density 20:20 treatment levels regardless of the other species. A notable exception being that survivorship for *Culex* was at its highest in the *Aedes albopictus* high animal detritus ratio (20:20 larva, 0:10 detritus).

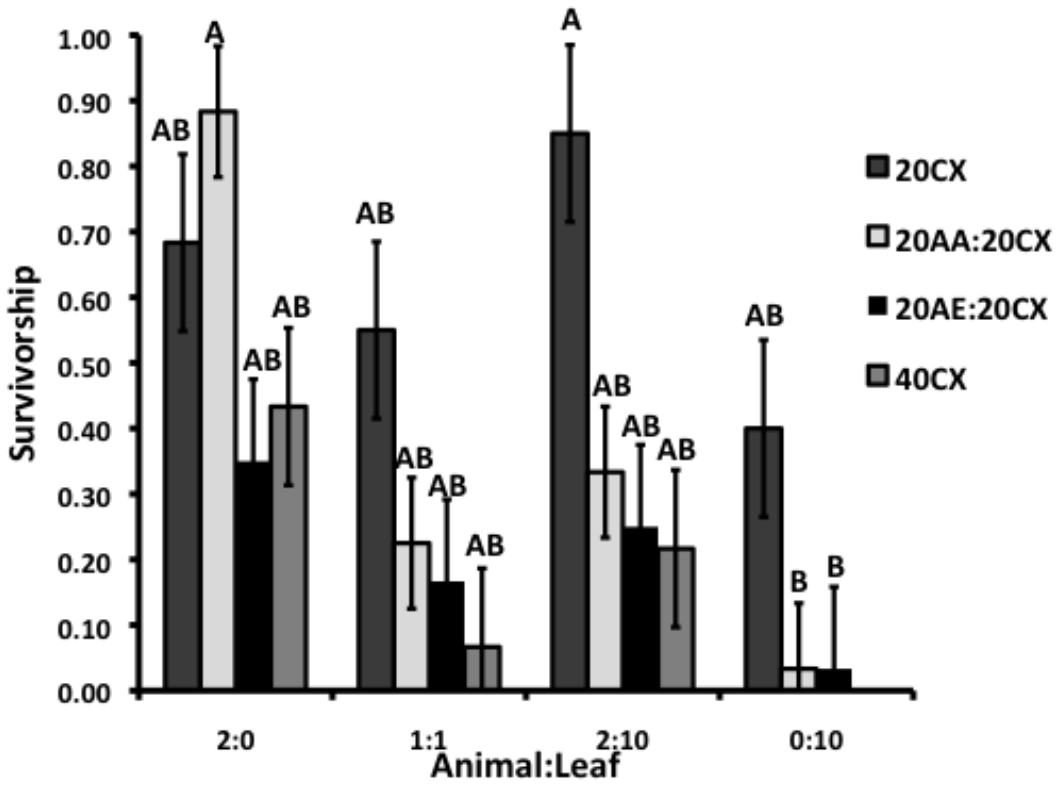

Figure 7: Survivorship (mean ± SE) of *Culex. quinquefasciatus* (CX) across all detritus and larva densities. No data was available for the (0:40, 0:10) larva to detritus treatment for *Culex quinquefasciatu*s. (AA) *Aedes albopictus*, (AE) *Aedes aegypti*. Letters A and B indicate relationship between means.

Table 1. Results of two-way ANOVA for *Aedes albopictus, Aedes aegypti* and *Culex quinquefasciatus*. There are no individual values for detritus and larva ratio for *Culex quinquefasciatus*. Significant effects are listed in bold.

Factor	DF	F	P-value
Aedes albopictus			
Detritus ratio (D)	3	0.557	0.6472
Larval ratio (L)	3	0.949	0.4286
D x L	9	0.1313	0.2690
Aedes aegypti			
D	3	5.151	**0.0051**

Table 1 continued

Factor	DF	F	P-value
L	3	2.115	0.1177
D x L	9	1.213	0.3212
Culex quinquefasciatus			
D	3	2.321	0.132
L	3	2.416	0.122
D x L	14	3.385	**0.0027**

Discussion

The purpose of this study focused on determining the competitive outcome of three species of container mosquitoes under varying detritus types and larval densities. It is well known that *Ae. albopictus* is generally a superior competitor in container environments (Juliano 1998, 2010). As expected the data collected in this experiment supports that idea as *Ae. albopictus* mean survivorship was unaffected by *Cx. quinquefasciatu*s or *Ae. aegypti*, and also did not vary with the type of detritus used. *Aedes aegypti* also showed no decrease in survival due to intra- or interspecific interactions, and was only affected by differences among detritus ratios. However, *Culex quinquefasciatus* did show a decrease in survivorship due to changes in both detritus and larval density.

What is not positively known is why *Ae. albopictus* was the superior competitor. However, the idea that they may be superior based on their need for fewer nutrients to develop has been raised (Winters and Yee 2012). My results show that *Ae. aegypti* and

Cx. quinquefasciatus survivorship was significantly higher in beakers that contained animal detritus compared to beakers that had leaf detritus only. However, *Ae. albopictus* showed no significant difference in survivorship regardless of the type of detritus present. The lack of any significant change in mean survivorship across all treatments supports the idea that *Ae. albopictus* has a competitive advantage that other species don't have when it comes to surviving in low nutrient enviroments. The fact that the other species showed significant differences between low quality leaf only and higher quality mixed detritus suggest that they are significantly limited by the quality of detritus present. Similar studies have also shown results that suggest that the quality of detritus affects survivorship. Yee and Juliano (2006) found that a related species *Ochlerotatus*(*Aedes*) *triseriatus* survivorship was significantly higher when reared in animal detritus compared to plant-only situations . Studies have also shown that competition between *Ae. aegypti* and *Ae. albopictus* intensifies as the amount of animal detritus available decreases (Daugherty et. al 2000). Thus, my data supports the idea presented by Winters and Yee (2012), that *Ae. albopictus's* competitive advantage may be explained by their need for fewer nutrients, nutrients that were limiting in some of the detritus ratios used (e.g., leaf-only).

Though analysis shows no significant change in survivorship based on competitive density for *Ae. aegypti* it should be noted that *Ae. aegypti's* mean survivorship when paired with *Ae. albopictus* in leaf detritus only was 37.5%. Though this level of survivorship analytically is not significant, it does represent *Ae. aegypti's* lowest survivorship in any treatment. This data is supported by other research that has found that *Ae. albopictus* outcompetes *Ae. aegypti* under some resource environments, especially

when reared under plant detritus only (e.g., Daugherty et al. 2000, Juliano 1998, Juliano et al. 2004). However, I did not see total exclusion of *Ae. aegypti* by *Ae. albopictus* as reported by Daugherty et al. (2000). This may be do to the fact that though the 0:10 leaf detritus only treatment represented the lowest nutrient level it represents more nutrients than were available in the treatments that led to the exclusion of *Ae. aegypti* in that experiment. Therefore, my data suggest the idea that *Ae. albopictus's* competitive advantage increases with increased difficulty of survival. Analysis of population growth rates, which are often used to assess competitive outcomes (e.g., Daugherty et al. 2000, Juliano 1998) may be more meaningful to understand the outcome of these interactions.

Survivorship of *Culex quinquefasciatus* showed significant differences based both on the detritus and larval treatment present. Specifically, survivorship in the 0:20 larval density treatment was often higher than survivorship in either the 20:20 or 0:40 , especially when leaves were type of detritus used (Fig.7). One exception to this trend was the 20:20 larval density level when *Ae. albopictus* was present in the animal detritus only (2:0) treatment, where survival was near 90% (Fig. 7). Overall survivorship for this species was very low compared to the survivorship of the *Aedes* genus. Which was to be expected as a similar study conducted by Winters and Yee found that *Culex restuan's* mean survivorship to be significantly lower then that of *Ae. albopictus* across some of the same detritus treatments used in this study (2012).

Culex quinquefasciatus showed the lowest overall survivorship (0.368 ± 0.05), whereas *Aedes aegypti* was higher (0.758 ± 0.029), and *Aedes albopictus* had the highest (0.802 ± 0.020). *Culex quinquefasciatus* survivorship showed that the most significant differences occurred between treatments that contained animal detritus, and treatments

that were leaf only. These findings are supported by the results of similar studies. Winters and Yee (2012) found that *Culex restuans* showed a much a lower survival rate in leaf detritus only compared to leaf and animal detritus mixes. They also found that the nitrogen requirement for *Culex restuans* was higher than that of *Ae. albopictus*. This latter point supports the results found in this study based on the fact that animal detritus is a more significant source of nitrogen than is leaf detritus (Winters and Yee 2012). Thus, the survival of *Culex* should be higher in animal detritus based on their need for more nitrogen. This idea also helps to explain why *Aedes albopictus* were less affected by the potentially low nitrogen content found in the leaf only treatments. It has been hypothesized that the reason for the difference in nitrogen and carbon content between *Aedes* and *Culex* is due to the difference in their feeding types (Winters and Yee 2012). Specifically *Ae. albopictus* is mainly a browser, and spends most of its time feeding in the middle or near the bottom of beakers directly on the detritus. In contrast, *Culex*, including *Cx. pipiens,*are filter feeders that spend most of the time feeding in the water column near the surface and often consume detritus indirectly though consumption of microbes that are found in the water column (Yee et. al. 2004). The different feeding types may also explain why survival for *Culex* was unusually high in the 2:0 detritus treatment when paired with *Aedes albopictus*. It could be that the *Ae. albopictus* boosted *Culex* growth by adding more particles of animal detritus (found on the bottom of the beaker) to the water column as they broke down the animal detritus. The breakdown of animal detritus has been shown to be accelerated by the direct feeding of *Aedes albopictus* (Yee et al. 2007). However, *Ae. aegypti* feed in the same manner as *Ae. albopictus* and no such increase in survival was shown for *Cx. quinquefasciatus* when

raised with *Ae. aegypti* in the 2:0 detritus treatment. Thus, it is difficult to make any conclusions without further testing.

Sources for Error

Not enough *Cx. quinquefasciatus* larvae were collected initially to complete all treatment levels, therefore the leaf detritus only 0:40 larvae treatment was excluded for that species. Future studies should include this treatment to allow for comparison between *Cx. quinquefasciatus* larval survivorship in high density inter and intraspecific competition in low nitrogen environments.

Culex quinquefasciatus larvae used in the experiment came from egg rafts collected in the field. Do to the inability to identify *Culex* larvae at the first instar the identity of the larvae could not be confirmed until after the experiment started. It was determined that many of the larvae were not *Cx. quinquefasciatus*. The contaminated beakers were removed from the experiment. A second experiment was conducted to replace many of the removed beakers.

In addition, not enough senescent red maple leaves were originally collected to fill all treatments. A second batch had to be collected at a later date. The leaves were collected from the same location. This is not believed to lead to any statistical differences as the leaves from both batches were dried in the same fashion and the batches were homogenized.

Due to time constraints the results for stable isotope analysis and development times are not yet available. It is expected that they will provide a more detailed account of the exact effects competition and differing detritus ratios have on each species. However, the

lack of the data at this point is not a significant source of error as the overall effects of competition and detritus type can be determined from the mean survival of the species.

Conclusion

The data in this experiment has suggested that *Ae. albopictus* is the superior competitor under most detritus and density combination circumstances. This experiment has offered some insight into how this species is able to out compete *Ae. aegypti* and *Cx. quinquefasciatus*. Though we are far from understanding the exact mechanism for their competitive ability we are well on our way. The data in this report agrees with the recently introduced idea that *Ae. albopictus* is the superior competitor based on its apparent need of very little nutrition. Though, we accept that knowledge is still very limited on the subject we hope to continue to expand what is known through further study.

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