ABSTRACT

Title of Document: IMPACTS OF CLIMATE CHANGE VARIABLES ON MOSQUITO COMPETITION AND POPULATION PERFORMANCE.

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Directed By: Assistant Professor, Paul Leisnham, and Environmental Science and Technology

Rising CO_2 concentrations and the resulting shifts in hydrology can have direct and indirect impacts on organisms and communities. The system studied was aquatic container habitats, where mosquito larvae often compete for food resources. I hypothesized that elevated atmospheric CO_2 concentrations (Chapter 2) and extreme precipitation regimes (Chapter 3) would alter leaf chemistry and competition between two locally competing mosquito species, *Aedes albopictus* and *Aedes triseriatus* in laboratory microcosm experiments. In Chapter 2, tannin concentration was higher in leaves grown under elevated CO_2 conditions than ambient, but competition was not affected. A two-fold increase was observed in leaf biomass in the elevated CO_2 chamber, and increasing leaf litter to a container system could increase toxicity to mosquito larvae. In Chapter 3, simulated drought conditions decreased leaf decay rate and increased tannin concentrations compared to continuously wet and wet-dry leaves, and amplified the competitive effects of *Ae. albopictus* on *Ae. triseriatus*.

IMPACTS OF CLIMATE CHANGE VARIABLES ON MOSQUITO COMPETITION
AND POPULATION PERFORMACE

by

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Master of Science
2011

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Acknowledgements

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Chapter 1: General Introduction

The mean concentration of atmospheric CO_2 has increased from 280 to 385 ppm since the 18th century due to accelerated fossil-fuel burning and industrial processes (IPCC 2007). The Intergovernmental Panel on Climate Change (IPCC) projects that atmospheric CO_2 concentrations may approach 970 ppm by 2100 (IPCC 2007). Recorded emissions from 2000-2005 exceeded the worst case scenario predicted by the IPCC (A1FI) and indicated that in 2100, CO_2 concentrations may even exceed 1000 ppm (Raupach et al. 2007). Elevated CO_2 concentrations have also been associated with land use change. Distinct CO_2 gradients have been observed along rural to urban transects with maximum CO_2 concentrations in urban environments, supporting the idea that urbanization is increasing atmospheric CO_2 concentrations (Ziska et al. 2003; Pataki et al. 2007). As a result of the steady and rapid increase in atmospheric CO_2, the hydrological cycle and global climate are in flux. The predicted change in global and regional weather patterns is another focus of the IPCC. In the IPCC 4th Assessment Report, it was noted that eastern North America observed significant increases in precipitation between 1900 and 2005. Extreme weather patterns have increased in frequency, and they are projected to continue to increase in frequency and strength due to the changing climate (IPCC 2007). The proportion of total rainfall that has fallen in heavy precipitation events has also increased (IPCC 2007). Heat waves, leading to localized droughts, have become more numerous. While there is uncertainty about the exact weather that regions will experience, it is evident that weather events are becoming more extreme. The mid-Atlantic region of the eastern United States may experience more numerous and more intense droughts and/or floods (IPCC 2007). Increases in atmospheric CO_2 (Hamilton et

al. 2001) and altered weather patterns can have direct and indirect effects on organisms and ecological communities.

Increasing CO_2 concentrations can have substantial impacts on vegetation, with numerous studies showing increased photosynthetic rates and altered chemistry in elevated CO_2 conditions (Lee et al. 2011; see reviews by Körner 2006, Taub and Wang 2008). An increase in photosynthetic rates, and the corresponding increase in total biomass to an ecosystem, could relax competitive effects of herbivores and detritivores in resource-limited systems. Elevated CO_2 has been shown to change foliar chemistry by increasing C:N ratios (Huttunen et al. 2009; Tuchman et al. 2003; Lindroth 2010; Kampichler et al. 2008) which decreases the nutritional content of the leaves, ultimately resulting in a lower uptake efficiency for herbivores (Bezemer and Jones 1998). As CO_2 levels increase, the carbon-based secondary metabolites within a leaf also increase (Huttunen et al. 2009; Tuchman et al. 2003; Peñuelas and Estiarte 1998) which reduces leaf digestibility and increases toxins leached into aquatic environments.

Resource-limited aquatic systems, such as natural (e.g., treeholes) or human-made (e.g., discarded tires, buckets) water holding containers, are affected by increased atmospheric CO_2 concentrations and regional climate patterns. Mosquito larvae are commonly the dominant detritivores colonizing containers, and the majority of the food resources are derived from allochthonous inputs of organic detritus consisting mainly of leaf litter (Carpenter 1983). Mosquito larvae subsist on the microorganisms associated with the decomposition of the leaf litter into fine particulate organic matter (Merritt et al. 1992). In conditions of elevated CO_2, submerged leaf litter has been shown to support fewer bacteria than leaf litter grown in ambient conditions (Tuchman et al. 2003). Leaves

of trees grown at elevated CO_2 levels were observed to have slower decay rates and leach increased concentrations of secondary metabolites, such as tannins (Huttunen et al. 2009; Tuchman et al. 2003; Peñuelas and Estiarte 1998). Past studies have shown that at high enough concentrations, leached tannic acid, which is a type of tannin, becomes toxic to mosquito larvae. Tannic acid can lengthen the larval development stages and increase mortality (Sota 1993; Mercer 1993).

The hydrological scenario that leaf litter is exposed to can also affect the quantity and quality of leaf material (Aspbury and Juliano 1998). In water holding tires and tree holes, resource drying has been shown to reduce the quality of resource (Aspbury and Juliano 1998). Resource drying, compared to constantly wet conditions, reduced resource quality in the Aspbury and Juliano 1998 study. It has been proposed that flash flood conditions could affect macroinvertebrates by decreasing the available allochthonous detritus and by removing aquatic larvae (Fisher et al. 1982), both of which can influence competition among macroinvertebrates. A weather regime that suppresses the development of one particular species may provide opportunities for proliferation and spread of another species.

Currently, Maryland experiences a rainfall event about 110 days out of a year (Western Regional Climate Center 2005). The region is classified as a temperate climate (Fang and Liang 2005), and there is a cyclical wetting and drying pattern. Precipitation patterns are important when studying aquatic communities because the localized precipitation is likely to affect the distribution of pest and vector insects, such as mosquitoes (Alto and Juliano 2001). For certain mosquito species, there exists a close relationship between rainfall and the fluctuation of abundance (Ho et al. 1971). Multiple

Aedes mosquito species oviposit their eggs along the insides of artificial or natural containers (Sota and Mogi 1992) and egg hatching requires a rainfall event and submersion (Novak and Shroyer 1978; Khatchikian et al 2008). The weather patterns will also dictate the duration that the aquatic habitat will persist. The timing and duration of droughts can affect the survivorship of mosquito larvae in treeholes (Bradshaw and Holzapfel 1988). Drought-resistant eggs are an advantageous life history trait present in species of mosquitoes such as *Aedes triseriatus* (Say), *Aedes albopictus* (Skuse), and *Aedes aegypti* (Linnaeus). (Bradshaw and Holzapfel 1988; Sota and Mogi 1992).

Two mosquitoes commonly found throughout the eastern United States are the Asian tiger mosquito *Ae. albopictus* and the eastern treehole mosquito *Ae. triseriatus* (Yee 2008). *Aedes albopictus* invaded the continental United States in the mid-1980s (Juliano and Lounibos 2005). Since then, it has spread throughout the southeastern United States and become one of the most common human-biting mosquitoes in its new range (Juliano and Lounibos 2005). *Aedes albopictus* often co-occurs and competes with the native congener *Ae. triseriatus* in container systems. These mosquito species are medically important: *Ae. albopictus* is a competent vector for West Nile virus, dengue, eastern equine encephalitis, and LaCrosse encephalitis and *Ae. triseriatus* is a vector for LaCrosse encephalitis virus (Gerhardt et al. 2001; Ibañez-Berñal et al. 1997; Turell et al. 2005; Juliano and Lounibos 2005; Joy and Hildreth-Whitehair 2000). While the symptoms and the deaths associated with these viruses are often reported in the media, the economic and social implications of the viruses tend to go unnoticed. Utz et al. 2003 estimated the cost for the lifelong medical care of a person with persistent neurological sequelae due to LaCrosse encephalitis to be between $48,000 and $3,000,000.

Despite the fact that past research consistently shows that *Ae. triseriatus* is competitively inferior to *Ae. albopictus* (Teng and Apperson 2000)*, Ae. triseriatus* has not been displaced in many areas and even remains the dominant mosquito in forested habitats (Juliano and Lounibos 2005). It is possible that superior tolerance of *Ae. triseriatus* to tannin compounds compared to *Ae. albopictus* may be fostering the persistence of *Ae. triseriatus*, but this has not been tested. Since the population level outcome of competition for resources by mosquitoes can be driven by the quantity and quality of the food supply, factors affecting the food resources need to be fully understood. The outcome of larval competition can have profound impacts on the epidemiology of diseases by altering the distribution and abundance of each mosquito species.

My thesis and the experiments that were performed examine the effects of elevated CO_2 (Chapter 2) and altered hydrology (Chapter 3) and the subsequent changes to leaf production and chemistry on the competition between *Ae. albopictus* and *Ae. triseriatus*. The mechanisms by which leaf production and chemistry could affect competition are directly through altered microbial production (resource abundance) and indirectly though the toxicity of increased tannin concentration. Microbial production is influenced by leaf litter C:N, decay rate, and the release of nutrients or toxic compounds. The purpose of this study was to evaluate the abiotic and the biotic factors that could influence mosquito spread and invasion as a result of climate change.

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Chapter 2: Effects of Tannins Leached from Leaf Detritus on Mosquito Competition and Population Performance: Implications of Increased Atmospheric CO$_2$

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Impacts of Climate-Change on Mosquito Competition and Population Performance

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Abstract

The invasive mosquito *Aedes albopictus* breeds in water-holding containers where it often competes for food as larvae with the native *Aedes triseriatus*. Container food mainly consists of allochthonous detritus inputs of leaf litter and associated microbes. CO_2-enrichment can impact plants, by increasing growth rates and altering leaf chemistry. Consistent throughout the literature, carbon-based secondary or structural compounds, specifically tannins, increase in many plants when they are exposed to elevated CO_2 levels. Such changes can affect communities of leaf-feeding insects by altering the nutrition or toxicity of leaves. I tested the hypothesis that litter with elevated tannins affects competition among *Ae. albopictus* and *Ae. triseriatus*. To produce leaf litter with altered tannin concentrations, I grew white oak trees (*Quercus alba*) under ambient CO_2 concentrations (~388-ppm) and elevated CO_2 concentrations representing worst-case, but increasingly likely, conditions predicted by IPCC scenarios (~1034-ppm). Resulting leaf litter had 58.00 ± 4.0 mg/L vs. 96.50 ± 5.0 mg/L (66% increase) of tannins leached into water (over 4-25 days). I observed a two-fold increase in leaf biomass under elevated CO_2 conditions. Competition among *Ae. albopictus* and *Ae. triseriatus* was tested in laboratory microcosms provisioned with one of the two litter types and with differing species-density combinations. In a separate set of microcosms, single-species cohorts of either *Ae. albopictus* or *Ae. triseriatus* were provided with increasing amounts of one of the two litter types or concentrations of commercial tannic acid to test potential toxicity effects of leaf-derived tannins. I found no differences in mosquito competition between litter types, with *Ae. albopictus* consistently superior to *Ae. triseriatus*. Single-species microcosms provisioned with elevated leaf litter had slower male development

time than microcosms with ambient (lower-tannin) litter. *Aedes albopictus* and *Ae. triseriatus* also experienced similarly higher mortality and slower development with increasing commercial tannic acid concentrations. These results suggest that possible future CO_2 conditions may suppress mosquito production by increasing the toxicity of leaf litter, but that it would not affect population-level competition between *Ae. albopictus* and *Ae. triseriatus.*

Keyword index: tannic acid, competition, *Aedes*, elevated CO_2

Introduction

The mean concentration of atmospheric CO_2 has increased from 280 to 385 ppm since the 18th century due to accelerated fossil-fuel burning and industrial processes (IPCC 2007). The Intergovernmental Panel on Climate Change (IPCC) projects that atmospheric CO_2 concentrations may approach 970 ppm by 2100 (IPCC 2007). Recorded emissions from 2000-2005 exceeded the worst case scenario of the IPCC (A1FI) and indicated that in 2100, CO_2 concentrations may even exceed 1000 ppm (Raupach et al. 2007). Elevated CO_2 concentrations have also been associated with land use change. Distinct CO_2 gradients have been observed along rural to urban transects, supporting the idea that urbanization is increasing atmospheric CO_2 concentrations (Ziska et al. 2003). Average atmospheric CO_2 concentrations, measured along a transect in 2001, ranged from 389 ppm on an organic farm in rural Maryland to 511 ppm in the urban downtown of Baltimore, MD (Ziska et al. 2003). An observational study performed in Tokyo documented concentrations of carbon dioxide to be around 1400 ppm near a busy

highway (Tomoda et al. 1997). These increases in atmospheric CO_2 can have direct and indirect effects on organisms and ecological communities (Hamilton et al. 2001).

Increasing CO_2 concentrations can have substantial impacts on vegetation, with numerous studies showing increased photosynthetic rates and altered chemistry in elevated CO_2 conditions (Lee et al. 2011; see reviews by Körner 2006, Taub and Wang 2008). An increase in photosynthetic rates, and the corresponding increase in total biomass to an ecosystem, could relax competitive effects of herbivores in resource-limited systems. Elevated CO_2 has also been shown to change foliar chemistry by increasing C:N ratios (Huttunen et al. 2009; Tuchman et al. 2003; Lindroth 2010; Kampichler et al. 2008) which decreases the nutritional content of the leaves, ultimately resulting in a lower uptake efficiency for herbivores (Bezemer and Jones 1998). As CO_2 levels increase, the carbon-based secondary metabolites within a leaf also increase (Huttunen et al. 2009; Tuchman et al. 2003; Peñuelas and Estiarte 1998). Secondary metabolites, compounds not directly used in the plant's growth or reproduction, can be classified based on their composition as terpenes, phenolics, or alkaloids (Hagerman 2002). A group of secondary metabolites important to both terrestrial and aquatic communities is tannins which are carbon-based. Some tannins reduce digestibility by precipitating proteins while others act like toxins (Hagerman et al. 1992; Murdiati et al. 1992). Soluble tannins are found in many species of plants including *Quercus alba* L. Numerous studies have documented the effects of tannins on herbivory of mammals and insects in terrestrial systems (Hagerman et al. 1992; Mehansho et al 1987; Martin et al. 1985). Leaf material is the energetic basis for many aquatic habitats (Walker et al. 1992)

and fewer studies have investigated how altered C:N ratios and tannins of leaf materials affect aquatic communities and detritivores.

Resource-limited aquatic systems, such as natural or human-made containers, are affected by increased atmospheric CO_2 concentrations. Container-dwelling mosquito larvae commonly inhabit these enclosures, and the majority of the food resources in containers are derived from allochthonous inputs of organic detritus consisting mainly of leaf litter (Carpenter 1983). Mosquito larvae subsist on the microorganisms associated with the decomposition of the leaf litter into fine particulate organic matter (Merritt et al. 1992). Leaf litter grown in elevated CO_2 has been shown to support fewer bacteria when submerged than leaf litter grown in ambient conditions (Tuchman et al. 2003). Alternatively, the increase of total leaf litter to a container due to elevated CO_2 conditions is expected to relax competition between mosquito larvae due to the greater abundance of microbial colonies (Griswold and Lounibos 2005). Leaves of trees grown at elevated CO_2 levels were observed to have slower decay rates and increased tannins in the leachate (Huttunen et al. 2009; Tuchman et al. 2003; Peñuelas and Estiarte 1998), which can also have adverse effects on microbial colonies and mosquito larvae. At high enough concentrations, leached tannic acid has been shown to lengthen the mosquito larval development stages and increase mortality (Sota 1993; Mercer 1993; Tuchman et al. 2003; Alto et al. 2005). The intraspecific and interspecific competition for resources by mosquitoes is affected by the quantity and quality of the food supply (Murrell and Juliano 2008). These past studies have only examined single species, and have neither tested the effects of altered leaves on interspecific competition nor separated the effect of nutrition versus secondary metabolites (Mercer 1993; Strand et al. 1999).

Two mosquitoes commonly found throughout the eastern United States are the Asian tiger mosquito *Ae. albopictus* (Skuse) and the eastern treehole mosquito *Aedes triseriatus* (Say) (Yee 2008). *Aedes albopictus* invaded the continental United States in the mid-1980s (Juliano and Lounibos 2005). Since then, it has spread throughout the southeastern United States and become one of the most common human-biting mosquitoes in its new range (Juliano and Lounibos 2005). *Ae. albopictus* uses natural (e.g., treeholes, plant axils) and human-made (e.g., buckets, used tires) containers that collect water as sites for larval development, where it often co-occurs and competes with the native congener *Ae. triseriatus*. These mosquito species are medically important: *Ae. albopictus* is a competent vector for West Nile virus, dengue, eastern equine encephalitis, and LaCrosse encephalitis and *Ae. triseriatus* is a vector for LaCrosse encephalitis virus (Leisnham et al. 2008; Juliano and Lounibos 2005; Joy and Hildreth-Whitehair 2000). Despite the fact that past research consistently shows that *Ae. triseriatus* is competitively inferior to *Ae. albopictus, Ae. triseriatus* has not been displaced in many areas and even remains the dominant mosquito in forested habitats (Juliano and Lounibos 2005). It is possible that superior tolerance of *Ae. triseriatus* to tannins compared with *Ae. albopictus* may be fostering the persistence of *Ae. triseriatus*, but this has not previously been tested.

Changes to leaf production and chemistry from elevated atmospheric CO_2 may affect the invasion of *Ae. albopictus* and the outcome of competition with *Ae. triseriatus* via a number of mechanisms. Higher leaf production from trees grown under elevated CO_2 is expected to increase food inputs into containers and relax inter-specific competition thus fostering species coexistence. However, decreasing quality of litter (i.e., higher C:N ratio) could counteract the increase of leaf inputs and intensify competition.

Carbon-based tannin concentrations may also specifically affect the population level outcome of competition, especially if there is extremely high leaf production, by suppressing microbial growth or through direct toxicity to mosquito larvae.

The objective of our study was to determine if leaf amount and chemistry, altered through elevated CO_2 growing conditions, would affect competition between *Ae. albopictus* and *Ae. triseriatus.*

Methods and Materials

Tree growth, leaf production, and leaf chemistry

To obtain leaf litter for mosquito larval experiments, 30 three-year old white oak (*Quercus alba*) trees were grown in two environmental growth chambers (Model #M40, Environmental Growth Chambers, Ohio) for 6 months (from April to September 2009) at the University of Maryland, College Park. Each tree was potted in 6-gallon black plastic pots with potting soil. These pots were considered large for the size of the trees and were used to minimize any effects of potting on tree growth. White oak trees were chosen as the nutritional food source for the mosquitoes in these experiments for two reasons: 1) the effects of elevated CO_2 concentrations on white oak tree growth have been previously studied (Norby and O'Neill 1988); 2) White oak is a common tree to Maryland (Brown and Brown 1972) and often falls into mosquito-breeding containers and is a common food resource (personal observations). Prior to the growing season in March 2009, the height and stem diameter of each tree was measured. Fifteen randomly selected trees were placed in one growth chamber at 385 ppm CO_2 while the remaining fifteen trees were placed the second growth chamber at 1000 ppm CO_2. Mean measured CO_2

concentrations were 388 ± 1 ppm and 1034 ± 6 ppm for 20 point measures over the growing duration in each chamber, respectively. Both chambers were set at a photoperiod of 14:10 h (light: dark) and 26:14°C diurnal temperature to mimic summer conditions. Five mL of commercial fertilizer (Miracle-Gro; 12-4-8, N-P-K, The Scotts Company LLC, Ohio) were dissolved in water and applied weekly to each tree over the duration of the growing season. Each tree received a total of 2.6 grams of nitrogen over the treatment period (0.1 g of N per week over 26 weeks), which is a rate of 157 kg N ha-1 yr-1 to mimic elevated N deposition and storage commonly found in anthropogenic environments (van Heerwaarden et al. 2005, Wallace et al. 2007, Huttunen et al. 2009). Chamber CO_2 assignments and trees were switched after 3 months to help minimize any unmeasured chamber effect. During the growing season, five of the trees grown under elevated CO_2 concentrations had to be eliminated from the study because they were unhealthy or dead. These trees were exhibiting characteristics of being root-bound or iron deficient. By eliminating these trees, we retained only trees that appeared to be uninhibited by potting and mimic field conditions.

After five months (in August 2009), the height and stem diameter of each tree was remeasured and the total number of leaves on three randomly selected trees in each CO_2 treatment was counted. Ten fresh leaves were randomly selected from each tree, measured for leaf area using a LI-3100C Area Meter (LI-COR Biosciences, Nebraska), dried (>48 hrs at 40°C) and weighed. To estimate total leaf biomass for each tree, the tree's mean leaf area was multiplied by the average number of leaves per tree in that treatment.

Tree senescence was induced by gradually decreasing the temperature and day length of both chambers in a stepwise fashion until all trees were fully senesced, which took eight weeks. Leaves were collected from each chamber, pooled, and dried (>48 hrs at 40°C) until analysis. A LECO CHN-2000 instrument (Leco Corporation, Michigan) was used to analyze the leaf chemistry for percent C and N of the pooled dry litter from all the trees and totaling 15 ambient and 10 elevated samples.

Because I lacked the facilities to replicate CO_2 across multiple chambers, I am forced to assume that interchamber variation (other than CO_2) is negligible to use error to test for the main effect of CO_2. This assumption is often made in analyses of treatment effects (e.g., temperature, CO_2) in chamber experiments on vegetation and insects (e.g., Rueda et al. 1990, Teng and Apperson 2000, Alto and Juliano 2001a,b, van Heerwaarden et al. 2005). However, to test for interchamber differences and to determine if any differences in trees and litter are likely due to only CO_2, I measured CO_2 concentration, light intensity ($\mu mol/m^2 s$), relative humidity (percent), and temperature (°C) within each environmental chamber 15 times before and after trees and chamber assignments were switched (block). I used MANOVA to test the effects of chamber, block, date nested in block, and chamber-block interaction on 1) CO_2, 2) relative humidity, 3) temperature, and 4) light using proc GLM (SAS Institute 2004). Contributions of each response variable to significant effects were accessed using standardized canonical coefficients (SCCs) (Scheiner 2001). MANOVA showed a significant chamber effect (Pillai's Trace $F_{4,54} =$ 1.00, P <0.0001) due overwhelmingly to differences in CO_2 (SCC=21.1), while relative humidity (SCC=0.18), light (SCC=-0.28), and temperature (SCC=0.04) had little effects. Date nested in block (Pillai's Trace $F_{4,54} = 0.04$, P = 0.728), Block (Pillai's Trace $F_{4,54} =$

0.04, P = 0.708), and chamber x Block interaction (Pillai's Trace $F_{4,54} = 0.12$, P = 0.125) were not significant. These results indicate that the assumption of negligible effects of other chamber variables is reasonable and that any differences in tree growth, leaf production, morphology, and chemistry of trees and leaves between chambers are highly likely due to CO_2 only.

Mosquito Competition Experiment

Mosquito competition trials were run using 10 density combinations of *Ae. albopictus* and *Ae. triseriatus* to create a response surface design. Microcosms, consisting of 400-mL cups filled with 360 mL of deionized (DI) water were provisioned with 0.7 g of either ambient or elevated CO_2 leaf litter (each of which included leaf blades and one leaf petiole) and inoculated with 100 µL of pond water obtained from a pond on the University of Maryland's campus in College Park, MD. This addition of pond water was intended to help establish the microbial communities within the microcosms. All microcosms were placed in a Percival I-36VL growth chamber (Percival, Perry, IA) set at 14:10 (L:D) photoperiod and 24°C to mimic summer conditions. After four days, one of ten density combinations of newly hatched (<24 hrs) *Ae. albopictus* and *Ae. triseriatus* larvae (*Ae. albopictus: Ae. triseriatus*: 10:0, 20:0, 40:0, 10:10, 10:30, 20:20, 30:10, 0:40, 0:20, 0:10) were added to each microcosm. Larvae were sourced from F_2-F_3 generation eggs of laboratory colonies hatched in a 0.3 g/L nutrient (lactalbumin) mixture. Four replicates of each litter-density combination were established to give 80 total microcosms. The microcosms were rotated daily to control for any chamber effects. On days 14, 28, and 42 of the experiment, the same amount and detritus type as the initial quantity was supplied to each microcosm. This addition of food supply

for the larvae imitated the periodic addition of leaf litter and nutrients to containers in natural systems. Pupae were removed from the microcosms daily and placed in separate vials until they eclosed as adults. Eclosed adults were sexed, speciated, dried, weighed, and had their wing lengths measured.

Twenty-four additional microcosms without mosquitoes (two treatments and twelve replicates) were established. The microcosms were provisioned with 0.7 g of either ambient (A) or elevated (E) CO_2 leaf litter (which included leaf blades and one leaf petiole) and inoculated with 100 µL of pond water. Three microcosms were randomly selected from each litter type (A and E and totaling 6 cups) on days 0, 4, 7, and 21 and destructively sampled. From each sampled microcosm, tannin concentration was measured from a water sample using the High Range Test instructions of a Hach calorimeter and tannin-lignin TA-3 Test Kit (Hach Company, Colorado). Bacterial productivity was measured from a second water sample using a heterotrophic plate count method (SM9215C) at a 1 to 10 dilution. Detritus decay rate was calculated by filtering all the coarse detritus with a 2-mm sieve, drying it, and weighing it.

Litter Toxicity Experiment

To test the potential toxicity effects of elevated and ambient litter on the two *Aedes* species, amounts of leaf litter in microcosms were experimentally manipulated in multiples of two as a result of our biomass calculations between the two chambers. Sixteen treatment combinations were setup with four replicates of each treatment (64 microcosms). Treatments consisted of microcosms provisioned with 0.7, 1.4, 2.8, or 5.6 grams of leaf litter (including leaf blades and 1, 2, 3, or 4 leaf petioles respectively) grown at either ambient or elevated CO_2 concentrations and cohorts of ten newly hatched

(< 24 hrs) *Ae. albopictus* or *Ae. triseriatus* larvae. Microcosms were inoculated with pond water, rotated daily, and censured for mosquito pupae in exactly the same manner as the mosquito competition experiment. Additionally, 0.7 g of the same litter type was supplied to each microcosm on days 14, 28, and 42 of the experiment. On day 10, the pH and the dissolved oxygen was measured in each of the microcosms using a YSI Environmental pH 100 meter and a YSI Environmental Dissolved Oxygen 55 meter (YSI, Inc., Ohio).

Tannic Acid Experiment

Commercial tannic acid (LOT 085769, Fisher Scientific, New Jersey) in powder form was used test the dose-response relationship of tannic acid on both *Aedes* species under resource rich conditions. Tannin powder was mixed into microcosms holding 360 ml of a 0.2 g/L Lactalbumin solution (LOT NO. 4292F, MP Biomedicals, LLC, Ohio) to create tannic acid concentrations of 0, 50, 100, 500, and 1000 mg/L. *Aedes albopictus* and *Ae. triseriatus* eggs (F_2-F_3 generation) were hatched simultaneously using a 0.3 g/L nutrient (lactalbumin) mixture. Ten newly hatched (< 24 hrs) *Ae. albopictus* larvae were added to four replicate microcosms of each concentration (20 total microcosms). The remaining 20 microcosms were each filled with 10 *Ae. triseriatus* larvae. Microcosms were inoculated with pond water, rotated daily, and censured for mosquitoes in exactly the same manner as the mosquito competition and toxicity experiments.

The chambers in which all experiments were conducted were at ambient CO_2 conditions. The change in pH was measured of 4 replicate microcosms provisioned with 0.7 g ambient leaf litter and without mosquitoes in each of the ambient and elevated CO_2 chambers over 8 weeks. Repeated measures ANOVAs were used to test effects of CO_2 (between subjects) on pH, with microcosms as the subjects and weeks as the within

subjects factor. There was no difference in pH between microcosms in the different CO_2 environments ($F_{1,6} = 0.04$, $P = 0.8453$) which is consistent with the findings of Alto et al. 2005.

Data Management

Proportion of survivorship to adulthood (♀ & ♂), mean time to eclosion (♀ & ♂), and mean body size (♀ & ♂) (dry mass and wing length) were recorded for each species from each microcosm. These demographic variables were used to estimate the finite rate of population change for each species (lambda, λ'), which is a composite index of population performance (Livdahl and Sugihara 1984):

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

Where N_0 is the initial number of females (assumed to be 50% per microcosm), x is the mean time to eclosion (measured in days), A_x is the mean number of females eclosing on x, and w_x is the mean body size on x. The function $f(w_x)$ is different for each mosquito species and describes the fecundity and female size relationship. D is the mean days it takes for an adult mosquito to mate, bloodfeed, and oviposit.

Statistical Analyses

Tree growth, leaf production, and leaf chemistry

One-way ANOVAs (Analysis of Variance) were used to test effects of CO_2 treatment on tree morphology (leaf area, leaf dry mass, change in tree steam diameter, change in tree height, numbers of leaves per tree) and leaf chemistry (total carbon, total

nitrogen, C:N), and to test effects of CO_2, day, and CO_2 X day interaction on the quality of microcosm habitats (tannin concentration, decay rate, and bacterial abundance).

Mosquito Competition Experiment

A second set of ANOVAs were used to test effects of litter type (ambient or elevated), densities of each species, and their interactions on proportion of survival to adulthood, mean time to eclosion (♀ & ♂), mean body size (♀ & ♂), and lambda of both species. A negative relationship between response variables with *Aedes* densities represents an effect of competition, and a significant interaction between litter type and either density indicates that litter type moderates the effect of competition. ANOVAs were used on the data from the additional set of microcosms without mosquitoes to test the effects of litter type (ambient or elevated), day, and litter type X day interaction on tannin concentration, pH, detritus decay rate, and bacterial productivity.

Litter Toxicity Experiment

A third set of ANOVAs were used to test effects of litter type (ambient or elevated), litter amount (0.7 g, 1.4 g, 2.8 g, or 5.6 g), mosquito species (*Ae. albopictus or Ae. triseriatus*), and their interactions on proportion of survival to adulthood, mean time to eclosion (♀ & ♂), mean body size (♀ & ♂), and lambda. A negative relationship between response variables with litter amount represents an effect of litter toxicity, and a significant interaction between litter amount with either species indicates that litter amount affects the fitness parameters of the individual species.

Tannic Acid Experiment

A fourth set of ANOVAs were used to determine the significance of the main effects of tannin concentration (0, 50, 100, 500, and 1000 mg/L) and the mosquito species

(*Ae. albopictus or Ae. triseriatus*), and their interactions. A significant interaction between tannic acid concentration and species would indicate that increasing concentrations of tannic acid affect the species differently.

Experimental results were considered to be statistically significant when $p < 0.05$. ANOVA tests performed utilized the SAS program package (SAS Institute 2004) or Microsoft Excel 2007 (Microsoft Corporation 2007). To meet the assumptions of normality, we $\log_{10}$ transformed *Ae. triseriatus* lambda data and arcsine square-root transformed survival data for the Mosquito Competition Experiment. Lambda data was $\log_{10}$ transformed and survival data was arcsine square-root transformed for the Toxicity Experiment analyses, and survival data was arcsine square-root transformed for the Tannic Acid experiment. Mosquito density, litter amount, and tannin concentration were all treated as continuous variables in analyses.

Results

Tree growth, leaf production, and leaf chemistry

Trees grown in elevated atmospheric CO_2 concentration exhibited higher growth rates (shown through change in height and change in stem diameter) and larger leaves (with respect to leaf area and leaf dry mass) compared to trees grown in ambient atmospheric CO_2 concentrations, but there was no difference in numbers of leaves per tree between the two CO_2 treatments (Table 1).

Total carbon was higher in elevated CO_2 leaf litter than ambient CO_2 leaf litter but the increase constituted less than 1% of leaf material (Table 2). Total nitrogen and C:N did not differ between the two detritus types (Table 2). Tannin concentration was

significantly higher in leachate of elevated CO_2 leaf litter than microcosms provisioned with ambient litter each day it was measured (Table 3, Fig 1a). Bacterial abundance, measured in colony forming units, was different among the litter types (Table 3) with ambient litter leachate having higher bacteria counts (Fig 1b). The decay rate between the two leaf litter types differed significantly, with the elevated litter having a faster decay rate (Table 3, Fig 1c). The decay rate of the detritus was affected by day (Table 3).

Mosquito Competition Experiment

Aedes albopictus had a consistently higher lambda than *Ae. triseriatus* across all densities and both litter types, indicating that it was the superior competitor for food (Fig 2 a,b). Interspecific and intraspecific densities negatively affected body size and extended development time of *Ae. triseriatus* males and females (Table 4). Intraspecific density negatively affected body size and development time of *Ae. albopictus* males and females, while interspecific density negatively affected body size of both *Ae. albopictus* sexes (Table 4). Leaf litter type modified intraspecific competition on the development time of *Ae. albopictus* females, with females provisioned with elevated litter having longer development time than females provisioned with ambient litter (Table 4, Fig 2g). Effects of competition on other fitness parameters of *Ae. albopictus* and *Ae. triseriatus* were consistent between detritus types (Table 4, Fig 2).

Litter Toxicity Experiment

Results of ANOVAs testing the effects of leaf litter amount and detritus type on the lambda and fitness parameters of *Ae. albopictus* and *Ae. triseriatus* showed no significant two or three way interactions. Lambda, survival, and development time of females were affected by the main effects of litter amount and species, but not by detritus type (Table 5). Lambda and survival declined (Fig 3 a,b) and female development time

was prolonged as litter amount increased (Fig 3d). Mass of male and female mosquitoes was not affected by either litter amount, species, or detritus type (Table 5). Development time of males was affected by species and detritus type (Table 5). Males of both species that were provisioned with elevated litter (higher tannins) had longer development time (Table 5). No *Ae. triseriatus* survived the 5.6 g litter amount (Fig 3). *Aedes albopictus* had significantly higher lambda than *Ae. triseriatus* across all litter amounts and types (Fig 3a).

There was a difference in the pH of leachate in microcosms provisioned with elevated and ambient litter types ($F_{1,57} = 4.32$, P= 0.0422), with microcosms provisioned with ambient litter having a higher pH than microcosms provisioned with elevated litter (Fig 4). Secondly, the pH differed significantly between litter amounts ($F_{1,57} = 11.22$, P= 0.0014) and 5.6 grams of leaf litter resulted in the lowest pH values (Fig 4). Litter amount significantly affected the dissolved oxygen (mg/L) in the microcosms ($F_{1,57} = 32.26$, P < 0.001); however, detritus type did not affect the DO concentration (Fig 5).

Tannic Acid Experiment

ANOVAs testing the effects of commercial tannic acid on the lambda and fitness parameters of *Ae. albopictus* and *Ae. triseriatus* showed no significant concentration by species interactions. Lambda was negatively affected by tannic acid concentration and higher for *Ae. albopictus* than *Ae. triseriatus.*(Table 6, Fig 6a). No *Ae. albopictus* or *Ae. triseriatus* survived the 500 or 1000 mg/L concentrations (Fig 6). In addition, *Aedes albopictus* had consistently higher survival than *Ae. triseriatus* averaged over all tannic acid concentrations (Table 6). There was no effect of tannic acid concentration on the

mass of female or male mosquitoes or on the development time of females (Table 6, Fig 6 c,d). Increasing tannic acid concentration prolonged male development time (Table 6).

<u>Discussion</u>

Our experiment found that trees grown in elevated atmospheric CO_2 produced over twice the amount of leaf litter than the ambient chamber. Leaf chemistry was altered due to elevated or ambient CO_2 growing conditions, with elevated litter leaching higher tannin concentrations, possessing a faster decay rate, and resulting in fewer bacteria than ambient litter. Litter type, when controlling for amount, did not affect the per capita rate of population change of *Ae. albopictus* or *Ae. triseriatus* or the outcome of competition between these species with *Ae. albopictus* consistently superior than *Ae triseriatus*. As expected, intra- and interspecific competition affected the mass of mosquitoes and time to development. Tannic acid concentrations above 100 mg/L were completely fatal to both species of mosquitoes. The increase in elevated CO_2 leaf litter, and additional food resources, indicates that competition between larval mosquitoes may be relaxed in future scenarios of high atmospheric CO_2 concentrations. However, if containers collect large amounts of leaf litter due to the increase in plant growth rates, then high levels of tannins will accumulate and could suppress development of both species.

Our finding that competition was not affected by litter type is supported by previous studies (Alto et al. 2005; Strand et al. 1999). *Aedes albopictus* had a consistently higher lambda than *Ae. triseriatus* across all densities and both litter types, indicating that it was the superior competitor for food (Fig 2 a,b). The leaf litter chemistry indicates why there was no difference in the competition outcome when utilizing our two litter types.

Our litter types contained similar C:N ratios, and the bacterial abundance varied widely but was higher in ambient litter. We had applied a high nitrogen regime to the trees during the growing season, potentially mimicking anthropogenic conditions, and causing N to be in abundance and not a limiting factor. Therefore, the C:N was not altered as we had expected it would be and the high N in the leaf litter may have fostered relatively high microbial growth. As a result of this resource abundance, mosquito competition was not stressed. Although we saw an effect of density on female mass and time to development (Table 4), we did not see an altered per capita rate of population change among our density levels, which may have been due to low competition. While there is no clear consensus, carbon and nitrogen storages are expected to change as a result of climate change. Interestingly, this could further alter the C:N ratios within plants.

As opposed to Alto et al. 2005, we found the decay rates of our two litter types to differ, with elevated litter having a faster decay rate (Fig 1c). We propose that the slight increase in carbon that was measured in the elevated leaf litter (Table 2) was likely soluble carbon in the form of secondary metabolites. An increase in structural carbon would have caused the elevated litter to have a slower decay rate. As the elevated litter decayed, it released more tannins which could have been toxic to the bacteria, causing elevated litter to be colonized by fewer bacteria. Even though the elevated litter contained statistically fewer colony forming units, we believe there was still an excess of food resources for the mosquito larvae.

A key difference in our experimental litter was in the tannin concentrations measured in the leachate (Table 3, Fig 1a). Under our lab conditions, the elevated leaf litter with higher tannin concentrations of ~40 mg/L was not enough to affect the

population-level outcome of competition between *Ae. albopictus* and *Ae. Triseriatus* (Table 4). This result is supported by the wide range of tannins commonly observed in natural containers. In one study, where treehole water chemistry was measured in Japan, tannin-lignin concentrations were found to vary greatly, from 2.5-109.2 mg/L (Sota 1993). Mercer and Anderson 1994 observed a wide range in tannic acid concentrations in treeholes, from a mean of 59 ± 14 mg/L to 138 ± 36 mg/L. Our trees that were grown under elevated CO_2 conditions did not leach (at the 0.7 gram amount) tannin concentrations sufficiently different from ambient litter to alter the performance of *Aedes* or to cause mortality. From our Mosquito Competition Experiment, it can be postulated that the invasion of *Ae. albopictus* will not be altered due to the increasing levels of atmospheric CO_2. The litter type used in this experiment was white oak since it is a tree commonly found in Maryland (Brown and Brown 1972). Different effects may be observed if alternative types of leaf litter, such as birch or maple, are utilized. Birch and maple leaves have been shown to possess higher concentrations of condensed tannins than oaks (Koike et al. 2006), and these leaf types have the capacity to increase toxicity to aquatic macroinvertebrates.

Alto et al. 2005 concluded that there would be minimal effects on freshwater ecosystems through inputs of leaf litter that has been grown under elevated atmospheric CO_2. Their conclusion overlooks the evidence that elevated atmospheric CO_2 increases plant growth rates, which was the reason for our Litter Toxicity Experiment. Our tree biomass calculation revealed that the trees in the elevated CO_2 conditions produced over double the amount of biomass than the trees in the chamber with ambient CO_2 levels (Table 1). In natural systems, an increase in biomass would result in both an increase in

food supply for organisms and an increase in toxins leached from leaf material. Our results show that toxicity in microcosms was not altered by the ambient or elevated leaf litter, but was altered due to the amount of leaf litter. The IPCC projects atmospheric CO_2 to reach 1000 ppm in 2100, and this correlates to a doubling in biomass to 1.4 grams in experimental microcosms. The 1.4 gram amount of leaf litter did not leach tannins at a toxic concentration. Therefore, a realistic increase in leaf biomass due to an increase in CO_2 concentration would likely relax competition since the levels of tannins will not reach toxic levels by 2100. As litter amount increased in the microcosms, both the pH and DO in the microcosms decreased (Fig 4 & 5). The pH range was minimal, and the lower pH values that occurred (at the higher litter amounts) were near 6.5 and likely would not have caused a biological difference among *Aedes* species (Clements 2000). Under conditions of increasing toxicity and across all litter amounts, *Ae. albopictus* had superior population performance to *Ae. triseriatus*, which is consistent with past research on competition between these species (Fig 3a) (Teng and Apperson 2000). Notably, there was no survival of *Ae. triseriatus* in the 5.6 gram treatments of ambient and elevated leaf litter. Leachate analyzed from microcosms provisioned with 5.6 grams of elevated leaf litter revealed an average 762.22 ± 27.32 mg/L of tannins (unpublished data). The *Ae. triseriatus* larval mortality indicates that *Ae. triseriatus* is more susceptible than *Ae. albopictus* to the decreased DO or the increased tannins that accumulated in the microcosms.

The third experiment (Tannic Acid Experiment) focused on mosquito susceptibility to tannin levels that could accumulate in containers under elevated CO_2 conditions. It could be hypothesized that *Ae. triseriatus*, the eastern treehole mosquito,

and *Ae. albopictus* would possesses similar resistance to tannins that have leached from leaf litter since both species have evolved in forest ecosystems over time. More recently, *Ae. albopictus* has adapted to and proliferated in urban areas where CO_2 has been on the rise. This elevated CO_2 would affect the leaf litter, and therefore, increase the secondary metabolites that *Ae. albopictus* is exposed to, potentially increasing its resistance to tannins. With these hypotheses, it was difficult to make predictions regarding the relative susceptibility of *Ae. albopictus* to *Ae. triseriatus*. Our results reveal that single species populations of *Ae. albopictus* and *Ae. triseriatus* differ in their susceptibility to tannic acid, with *Ae. albopictus* exhibiting a consistently higher lambda (Fig 6a). This result is supported by Sota 1993 who found that a treehole species (*Ae. riversi*) experienced a higher mortality at higher levels of commercial tannic acid than *Ae. albopictus*. While Mercer (1993) observed survival to adulthood of the western treehole mosquito (*Ae. sierrensis*) at concentrations of 1.0 g/L, our results were similar to Sota (1993) in that they show that tannic acid was toxic to both species and resulted in total mortality of both species above 100 mg/L (Fig 6). According to our results, *Ae. albopictus* and *Ae. triseriatus* development and survival would be suppressed in containers when levels of tannic acid surpass 100 mg/L.

Mosquitoes have been shown to be affected by other forms of leachate in their larval phase, which has led to genetic adaptations to toxins. *Ae. albopictus* primarily breed in tire wells (Yee et al. 2010), and benzothiazole (BZT) is a major compound leached from automobile tires. BZT has been found to induce P450, a cytochrome that detoxifies insecticides and other toxic compounds such as tannins (Suwanchaichinda and Brattsten 2002). This can lead to mosquito tolerance and potentially to resistance.

Mosquitoes have extremely short life-cycles and can go from an egg to an adult in 7-10 days. This life history trait could enhance tannin resistance if the mosquitoes are able to adapt to the changing toxicity in the container breeding habitats. Further research, including a generational study, is needed to establish whether mosquitoes will adapt over time. CO_2 is increasing in a linear manner (IPCC 2007); therefore, organisms that are affected by increasing CO_2 may develop resistance in a similarly linear fashion. Based on the findings of this experiment, the relative distributions between these *Aedes* mosquitoes, as dictated by competition, are unlikely to change due to the expected increases in phytotannins with various stressors such as climate change and urbanization. However, the results indicate that development of individual populations may be suppressed, potentially affecting abundance and invasion success to some degree, due to increasing container-water toxicity.

Table 1. Morphological characteristics of *Quercus alba* trees grown under ambient (388 ± 1 ppm) or elevated (1034 ± 6 ppm) CO_2 concentrations. Numbers reported are averages plus or minus SE

Dependent variables	Ambient CO_2	Elevated CO_2	*df*	F	P value
Leaf area (cm^2)	44.05 ± 2.10	59.58 ± 5.81	1, 23	8.39	**0.0081**
Leaf dry mass (g)	0.28 ± 0.02	0.49 ± 0.06	1, 23	15.80	**0.0006**
Number of leaves per tree	298.67 ± 70.20	365.00 ± 49.70	1, 4	0.59	0.4836
Change in height (cm)	14.65 ± 3.05	44.84 ± 7.59	1, 28	13.63	**0.001**
Change in stem diameter (cm)	0.13 ± 0.02	0.37 ± 0.07	1, 28	10.06	**0.0037**

Table 2. Chemistry from dry leaf material of *Quercus alba* leaf litter grown under ambient (388 ± 1 ppm) or elevated (1034 ± 6 ppm) CO$_2$ concentrations. Numbers reported are averages ± SE.

Leaf Chemistry Variables	Ambient CO$_2$ (388 ppm)	Elevated CO$_2$ (1034 ppm)	P value
Total Carbon (%)	46.58 ± 0.161	47.29 ± 0.256	**0.0218**
Total Nitrogen (%)	1.20 ± 0.077	1.29 ± 0.135	0.5383
C:N	40.57 ± 2.14	39.56 ± 3.40	0.7920

Table 3. ANOVA results for tannin concentration (mg/L), bacterial abundance (cfu), and decay rate (percent/day) measured in microcosm leachate at four time periods: 0, 4, 7, and 21 days. Five days prior to the first sampling point, microcosms were provisioned with 0.7 g of *Quercus alba* leaf litter grown under ambient (388 ± 1 ppm) or elevated (1034 ± 6 ppm) CO_2 concentrations.

Source	df	Tannin Concentration		Bacterial Abundance		Decay Rate	
		F value	P value	F value	P value	F value	P value
Litter type	1	70.68	**<.0001**	4.89	**0.0419**	38.17	**<.0001**
Day	3	6.73	**0.0038**	2.95	0.0642	189.42	**<.0001**
Litter type X Day	3	2.05	0.1470	0.07	0.9758	4.39	**0.0727**
Error	16						

Table 4. Results of ANOVAs testing the effects of mosquito densities and detritus type on the population performance and fitness parameters of *Ae. albopictus* and *Ae. triseriatus*. Detritus type was *Quercus alba* leaf litter grown under ambient (388 ± 1 ppm) or elevated (1034 ± 6) CO_2 concentrations.

Dependent variables	*Aedes albopictus* density	*Aedes triseriatus* density	Detritus type	*Aedes albopictus* X Detritus	*Aedes triseriatus* X Detritus
Aedes albopictus					
lambda	ns	ns	ns	ns	ns
Survival	ns	ns	ns	ns	ns
Mass ♀	***	**	ns	ns	ns
Mass ♂	**	*	ns	ns	ns
Development Time ♀	***	ns	**	*	ns
Development Time ♂	***	ns	ns	ns	ns
Aedes triseriatus					
lambda	ns	ns	ns	ns	ns
Survival	ns	ns	ns	ns	ns
Mass ♀	ns	ns	ns	ns	ns
Mass ♂	**	*	ns	ns	ns
Development Time ♀	***	*	ns	ns	ns
Development Time ♂	***	*	ns	ns	ns

ns = not significant (P > 0.05)

* = P < 0.05

** = P < 0.01

*** = P < 0.001

Table 5. Results of ANOVAs testing effects of leaf litter amount on the population performance and fitness parameters of *Ae. albopictus* and *Ae. triseriatus*. Detritus type was *Quercus alba* leaf litter grown under ambient (388 ± 1 ppm) or elevated (1034 ± 6 ppm) CO_2 concentrations. No two or three way interactions were significant and are not shown.

Dependent variables	Litter amount	Species	Detritus type
lambda	**	*	ns
Survival	***	***	ns
Mass ♀	ns	ns	ns
Mass ♂	ns	ns	ns
Development Time ♀	*	**	ns
Development Time ♂	ns	**	*

ns = not significant ($P > 0.05$)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

Table 6. Results of ANOVAs testing effects of commercial tannic acid (concentration 0, 50, and 100 mg/L) on the population performance and fitness parameters of *Ae. albopictus* and *Ae. triseriatus*. Concentrations 500 and 1000 mg/L were removed from analyses due to zero survival from those microcosms.

Dependent variables	Concentration	Species	Concentration X Species
lambda	*	***	ns
Survival	ns	**	ns
Mass ♀	ns	ns	ns
Mass ♂	ns	ns	ns
Development Time ♀	ns	ns	ns
Development Time ♂	*	ns	ns

ns = not significant (P > 0.05)

* = P < 0.05

** = P < 0.01

*** = P < 0.001

Figure 1. Five days prior to the first sampling point, microcosms were provisioned with 0.7 g of *Quercus alba* leaf litter grown under ambient (388 ± 1 ppm) or elevated (1034 ± 6 ppm) CO_2 concentrations. **a)** Tannin concentration (mg/L) and **b)** bacterial abundance (cfu) were measured in microcosm leachate at four time periods: days 0, 4, 7, and 21 of the experiment. **c)** Decay rate (percent/day) was measured by destructively sampling at five time periods: the initial date of leaf addition (day -5) and days 0, 4, 7, and 21 of the experiment. Means $\pm$ SE

Figure 2. The effects of conspecific densities, heterospecific densities, and ambient or elevated grown CO_2 leaf litter on the finite rate of increase (lambda), female survivorship, female mass, and female days to eclosion of *Aedes albopictus* (**a, c, e, g**) and *Aedes triseriatus* (**b, d, f, h**). Values reported for female survivorship are back-transformed means for each detritus type.

Figure 3. The effects of varying amounts (0.7, 1.4, 2.8, and 5.6 grams) of *Quercus alba* leaf litter grown in ambient (388 ± 1 ppm) or elevated (1034 ± 6 ppm) CO_2 concentrations on **a)** the finite rate of increase (lambda), **b)** female survivorship, **c)** female mass, and **d)** female days to eclosion of *Aedes albopictus* and *Aedes triseriatus*. Values reported for female survivorship are back-transformed means for each detritus type. Means $\pm$ SE

Figure 4. pH of microcosm leachate provisioned with varying amounts (0.7, 1.4, 2.8, and 5.6 grams) of *Quercus alba* leaf litter grown in ambient (388 ± 1 ppm) or elevated (1034 ± 6 ppm) CO_2 concentrations. Mean pH $\pm$ SE

Figure 5. Dissolved oxygen concentration of microcosm leachate provisioned with varying amounts (0.7, 1.4, 2.8, and 5.6 grams) of *Quercus alba* leaf litter grown in ambient (388 ± 1 ppm) or elevated (1034 ± 6 ppm) CO_2 concentrations. Mean DO $\pm$ SE

**Figure 6.** The effects of increasing tannic acid concentrations (0, 50, 100, 500, and 1000 mg/L) on **a)** the finite rate of increase (lambda), **b)** female survivorship, **c)** female mass, and **d)** female days to eclosion of *Aedes albopictus* and *Aedes triseriatus*. Values reported for female survivorship are back-transformed means for each detritus type. Means $\pm$ SE
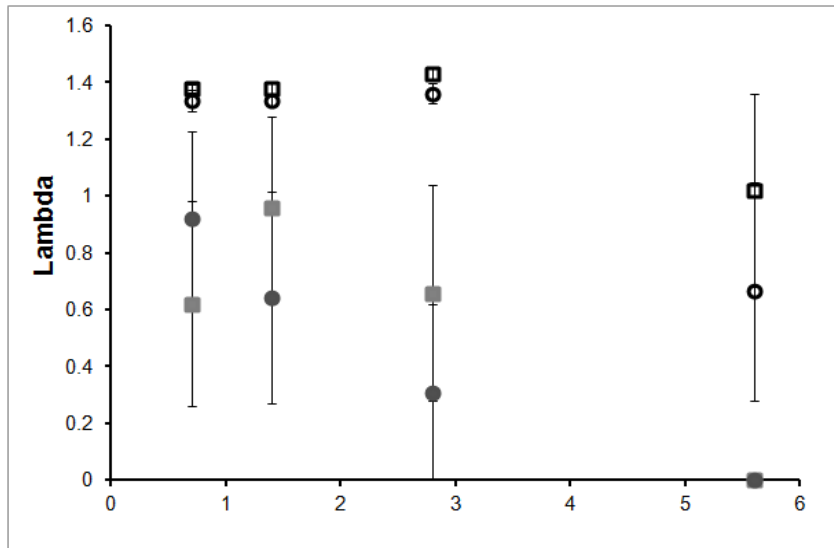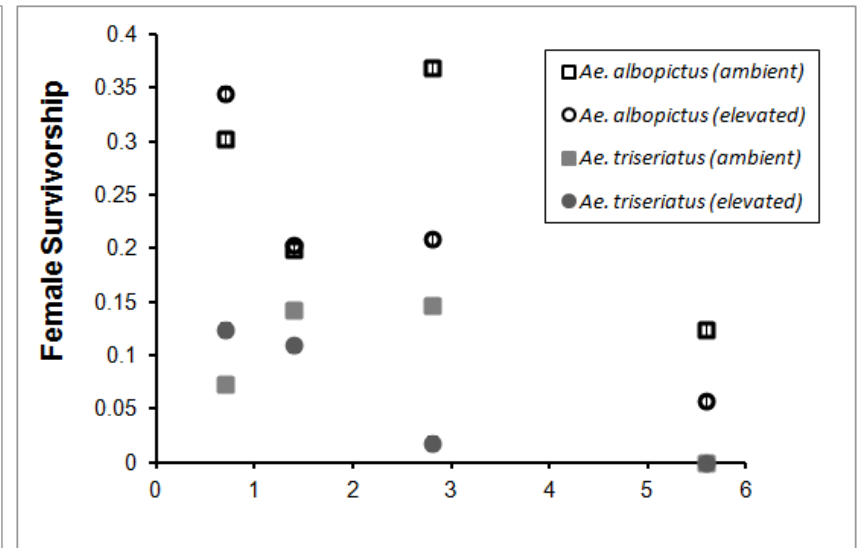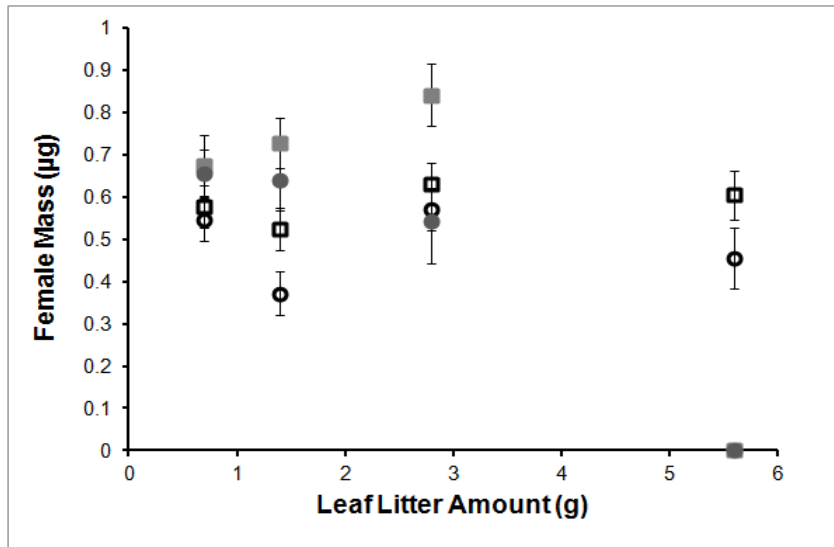
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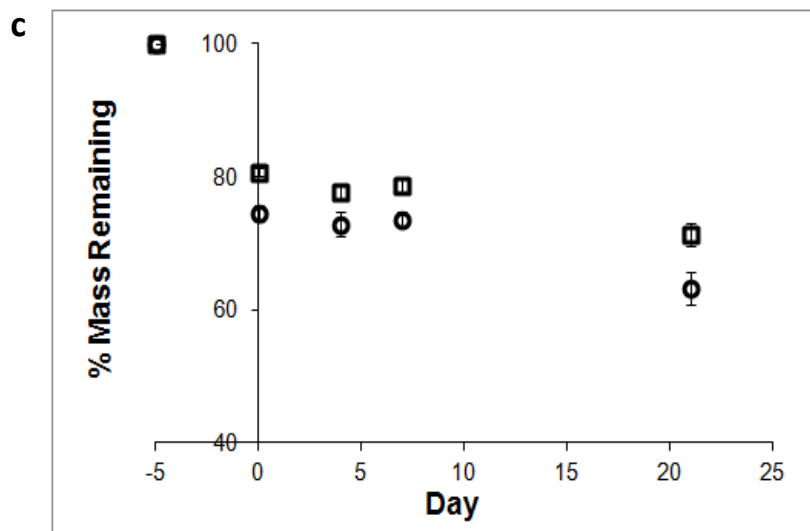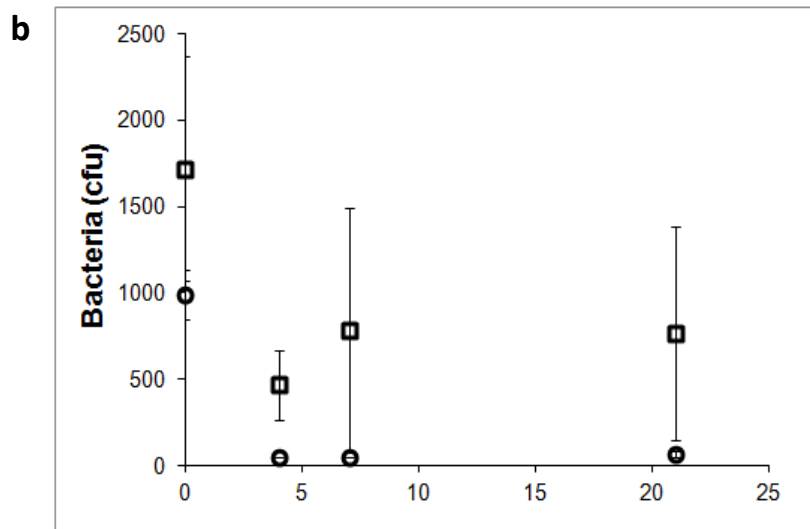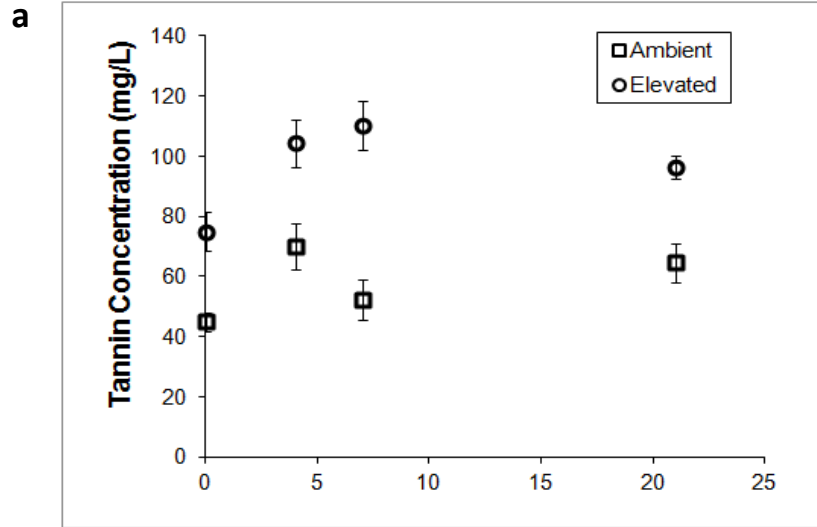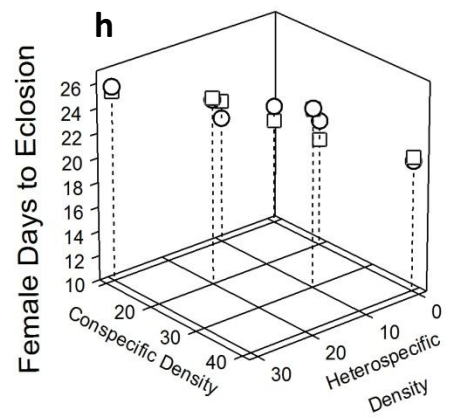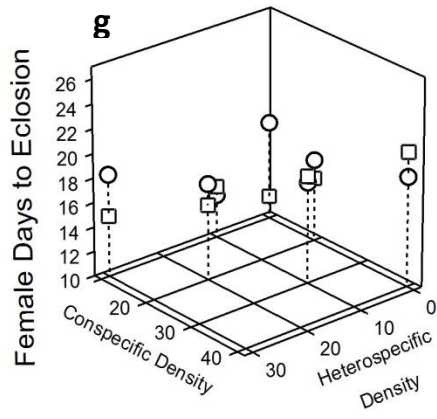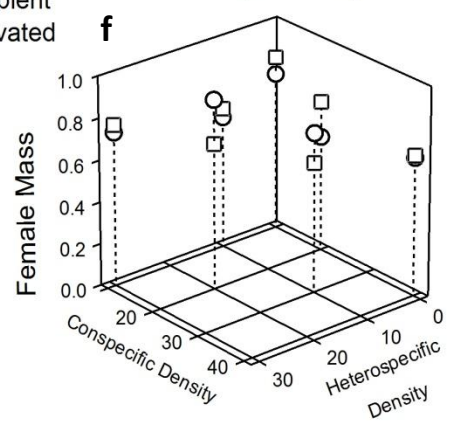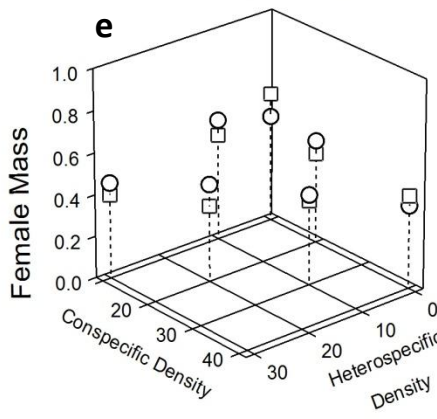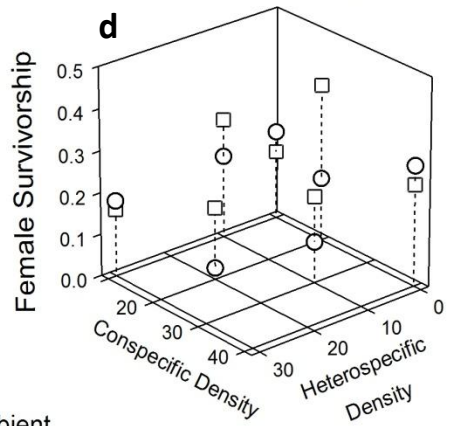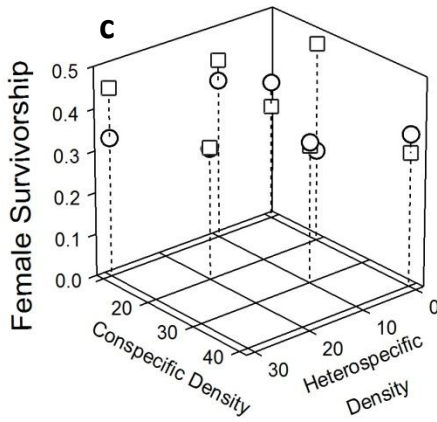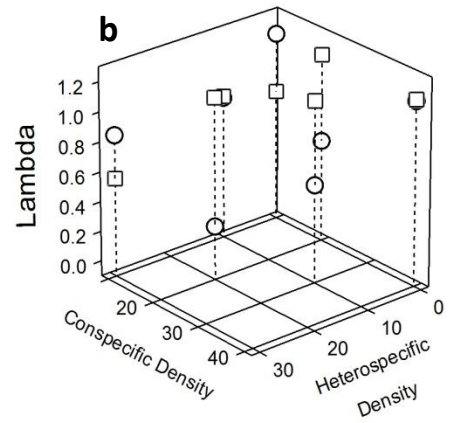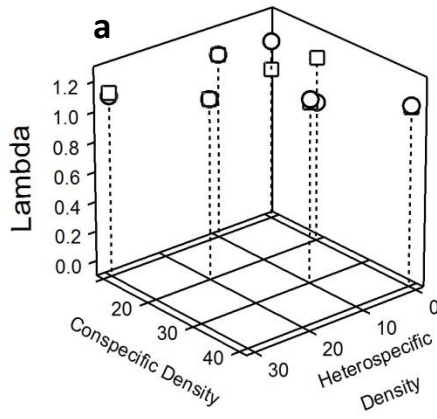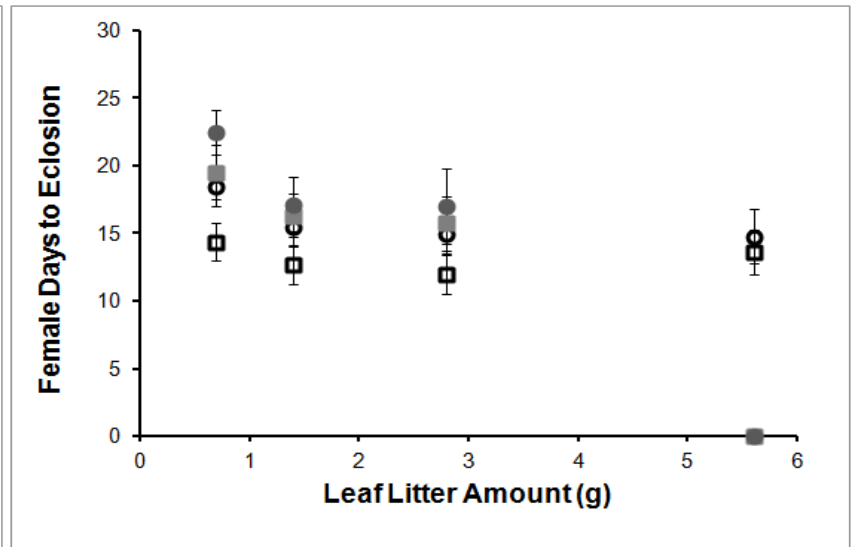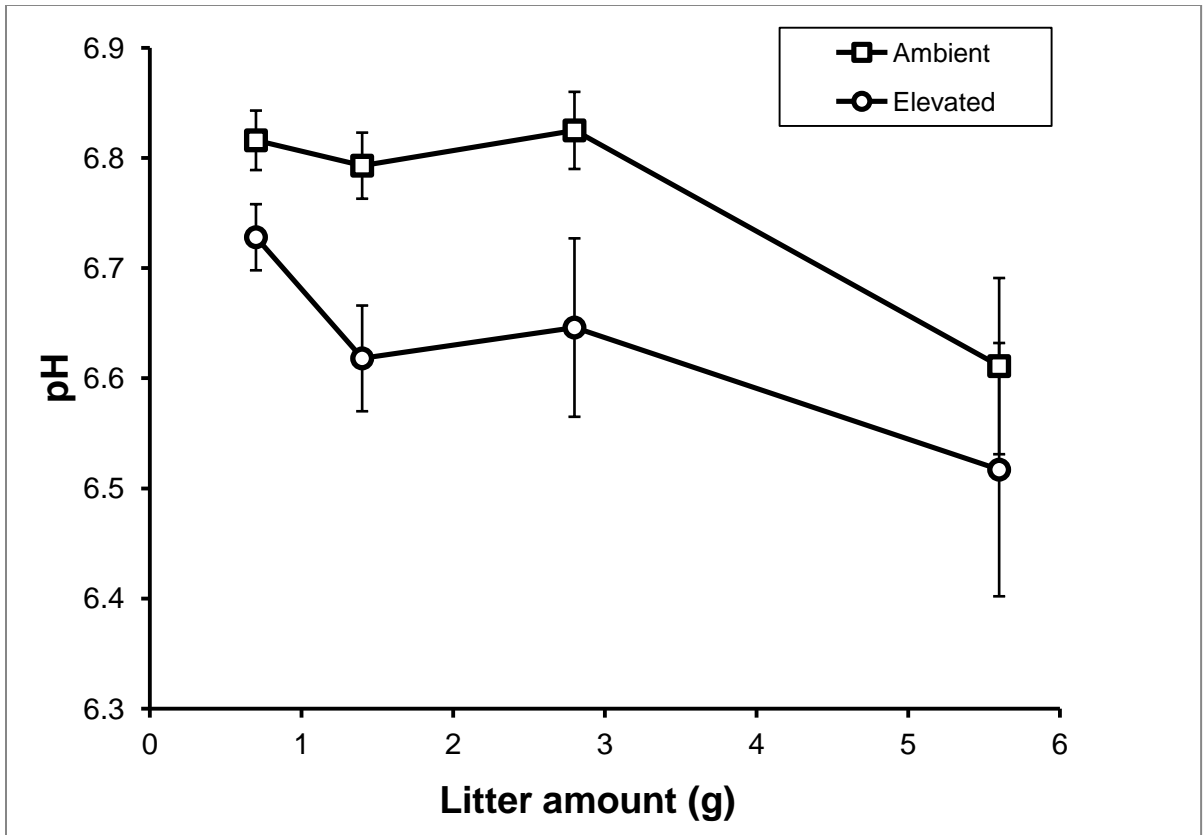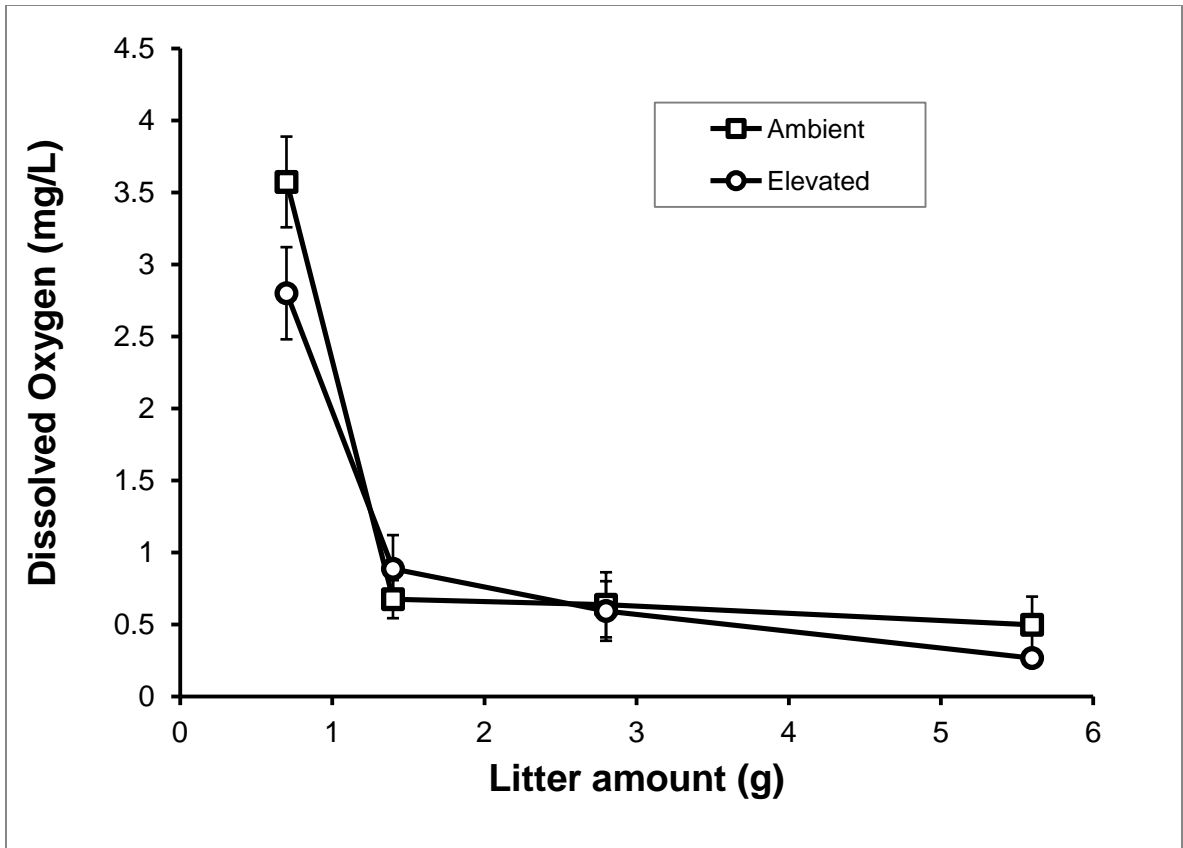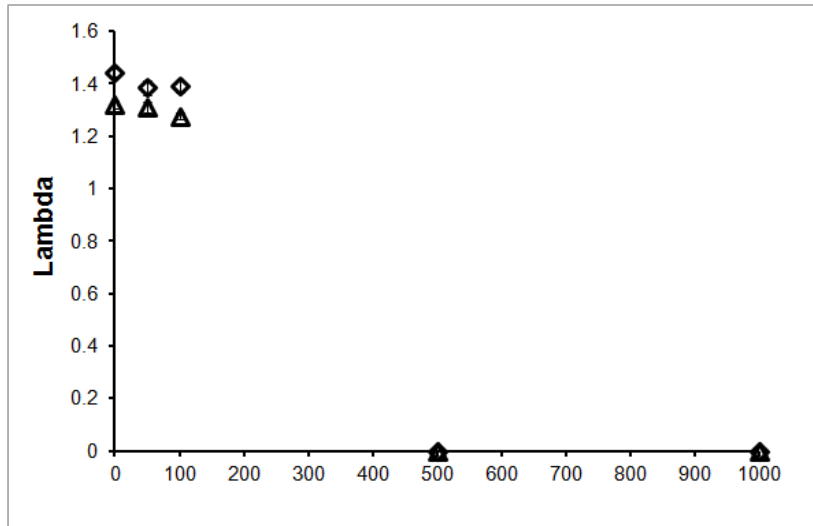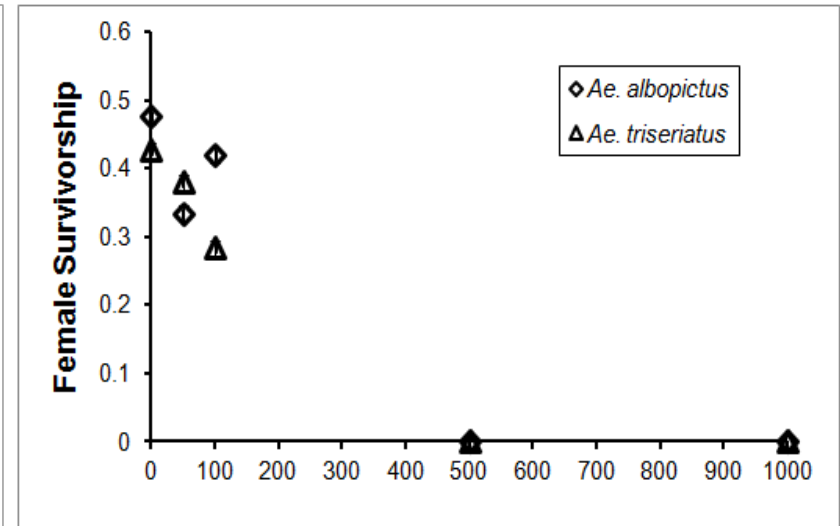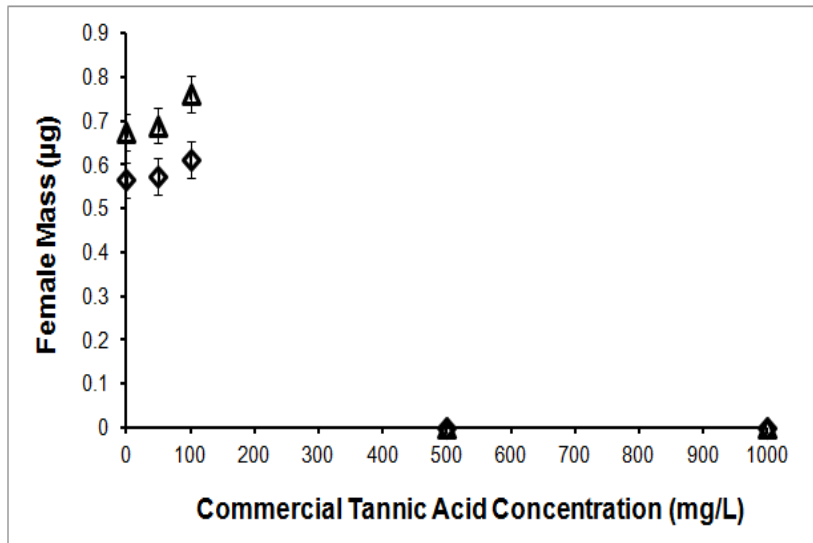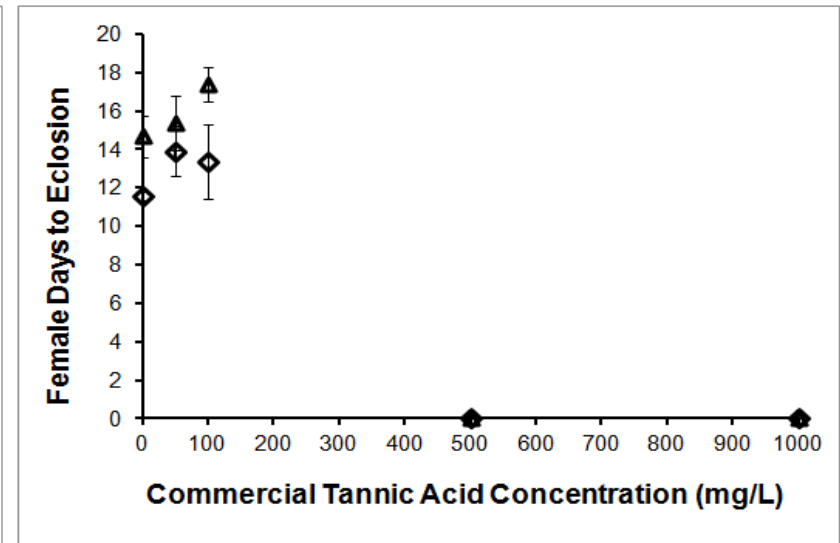
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Chapter 3: Effects of Hydrological Regimes and Leaf Litter on Mosquito Competition and Population Performance

Cover Page

Impacts of Hydrological Regimes on Mosquito Competition and Population Performance

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Running Head: Smith et al. Impacts of Hydrological Scenarios on Mosquito Populations

Key Words: litter quality, competition, *Aedes*, hydrology

Word Count: 6,752

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Abstract

The quantity and quality of leaf litter, the energetic basis for container habitats, can be affected by the hydrological regime it is exposed to. The invasive mosquito *Aedes albopictus* breeds in water-holding containers where it competes for food as larvae with the native *Aedes triseriatus*. We tested the hypothesis that hydrological regimes would differentially affect the competition between *Ae. albopictus* and *Ae. triseriatus* through changes in leaf resources and associated microbes and tannins. Senesced white oak litter was treated and prepared to mimic the three hydrological extremes that are projected to be a result of climate change in the mid-Atlantic region of the United States. The three hydrological regimes were dry (D), constant flooding (F), or wet then dry cycles (WD). Interspecific competition of *Ae. albopictus* on *Ae. triseriatus* was mediated by detritus type, with microcosms provisioned with D leaf litter supporting fewer resources and exhibiting stronger competitive effects. *Aedes triseriatus* was negatively affected by the D leaf treatment and experienced the longest female development time and a lambda value indicating that the population was in decline. The dry treatment leaves had the slowest decay rate and leached the highest concentration of tannins. The wet-dry leaves from our experiment contained more total bacteria than the other leaf treatments both 9 and 13 days after re-flooding. *Aedes triseriatus* females experienced the fastest development time and the populations increased when exposed to the WD leaf treatment. Our results show that the success of *Ae. triseriatus* populations is dependent on the quality of leaf litter which can vary with hydrological regime. *Aedes albopictus* was not negatively impacted by, and had lambda values all greater than 1.0, in all of the

treatments. Drought conditions may negatively impact *Ae. triseriatus* larval development, and could allow for the spread and proliferation of *Ae. albopictus*.

Keyword index: litter quality, competition, *Aedes*, hydrology, climate change

Introduction

The predicted change in global and regional weather patterns is a focus of the Intergovernmental Panel on Climate Change (IPCC). In the IPCC 4th Assessment Report, it was noted that eastern North America observed significant increases in precipitation between 1900 and 2005. Extreme weather patterns have increased in frequency, and they are projected to continue to increase in frequency and strength due to the changing climate (IPCC 2007). The proportion of total rainfall that has fallen in heavy precipitation events has also increased (IPCC 2007). Heat waves, leading to localized droughts, have become more numerous. While there is uncertainty about the exact weather that regions will experience, it is evident that weather events are becoming more extreme. The mid-Atlantic region of the eastern United States is expected to experience more numerous and more intense droughts and/or floods (IPCC). An increase in droughts and/or floods would be an increase in disturbance events to aquatic systems in a region. These discrete events can structure communities by affecting resources and biological interactions (Schneider and Frost 1996).

Currently, Maryland experiences a rainfall event about 110 days out of a year (Western Regional Climate Center 2005). The region is classified as a temperate climate (Fang and Liang 2005), and there is a cyclical wetting and drying pattern. Precipitation

patterns are important when studying aquatic communities because the localized

precipitation is likely to affect the distribution of pest insects, such as mosquitoes (Alto

and Juliano 2001). There is evidence that hydrology affects the non-competing egg and

adult life stages of mosquito development. For certain mosquito species, there exists a

close relationship between rainfall and the fluctuation of adult abundance (Ho et al.

1971). The timing of rainfall events is important when looking at mosquito eggs.

Multiple *Aedes* mosquito species oviposit their eggs along the insides of artificial or

natural containers (Sota and Mogi 1992); egg hatching requires a rainfall event and

submersion (Novak and Shroyer 1978; Khatchikian et al 2008). Drought-resistant eggs

are an advantageous life history trait present in species of mosquitoes such as *Aedes*

triseriatus (Say), *Aedes albopictus* (Skuse), and *Aedes aegypti* (Linnaeus) (Bradshaw and

Holzapfel 1988; Sota and Mogi 1992). Climactic extremes can cause stress to aquatic

organisms (Parsons 1995), and while *Ae. albopictus* and *Ae. aegypti* are known to have

drought resistant eggs, Costanzo et al. 2005 found that interspecific competition

negatively affected *Ae. albopictus* in their dry treatment due to the higher egg mortality in

Ae. albopictus compared to *Ae. aegypti*.

Natural and human-made containers (e.g. tree holes and buckets) form important

aquatic ecosystems, and localized weather patterns dictate the duration that these aquatic

habitats persist. The timing and duration of droughts can affect the survivorship of

mosquito larvae in treeholes (Bradshaw and Holzapfel 1988). Habitat drying, a source of

larval mortality, alters the water level and the solute concentrations in container habitats

(Juliano and Stoffregen 1994). Habitat drying can also reduce food resources, predators,

and competitors in temporary habitats (Mokany and Mokany 2006). Mosquitoes can

often perceive volumetric changes in their habitat (Mokany and Mokany 2006), and they can modify their pupation timing to avoid death in the event of habitat drying (Juliano and Stoffregen 1994). For *Aedes triseriatus* females, decreasing water volume induced earlier pupation and at smaller sizes than in containers with constant volume (Juliano and Stoffregen 1994). For *Ae. albopictus*, a drying habitat produced larger sized adults than in wetter, fluctuating habitats (Alto and Juliano 2001). Tannins, soluble compounds that leach from leaf litter (Mercer 1993) and are toxic to mosquito larvae at high concentrations (Sota 1993), and ammonium, excreted by mosquito larvae (Walker et al. 1991), accumulate in container habitats and become more concentrated as water evaporates. Accumulated water-borne toxins, such as tannins and ammonium, could be reduced in conditions of heavy rainfall when containers flood and/or flush. Bacterial abundance and ammonium concentration has been shown to be reduced in tree holes when there was a flushing event (Walker et al. 1991). Further, it has been proposed that flash flood conditions could affect macroinvertebrates by decreasing the available allochthonous detritus and by removing aquatic larvae (Fisher et al. 1982), both of which can influence competition.

The energetic basis for aquatic container systems is primarily allochthonous organic matter, including leaf litter (Walker et al. 1991). Our study focuses on larval mosquitoes, which is the phase of mosquito development where the organisms compete for food resources. The hydrological scenario that leaf litter is exposed to could affect the quantity and quality of leaf material (Aspbury and Juliano 1998). The quality of organic material is determined by the nitrogen content and toughness of the leaf litter (Feeny 1970), and in aquatic systems, litter quality is also determined by the corresponding

amount of bacteria and fungi populations that have colonized the detritus (Cummins and

Klug 1979). In habitats including tires and tree holes, resource drying, opposed to

constantly wet leaf material, has been shown to reduce the resource quality (Aspbury and

Juliano 1998). In tree holes, the resources that had been previously dried negatively

affected the population performance of *Ae. triseriatus* (Aspbury and Juliano 1998). In

tires, resource drying increased development time and decreased dry mass of *Ae.*

triseriatus (Aspbury and Juliano 1998). A precipitation regime that suppresses the

development of one particular species may provide opportunities for proliferation and

spread of another species. Additionally, the more negatively affected species could

become locally displaced. The distribution of medically important species could affect

the epidemiology of a region.

Two commonly competing mosquitoes found in the Maryland area are *Aedes*

albopictus (Skuse) and *Aedes triseriatus* (Say). The Asian tiger mosquito, *Ae. albopictus,*

invaded the continental United States in the mid-1980s (Juliano and Lounibos 2005).

Since then, it has spread throughout the southeastern United States and become one of the

most common human-biting mosquitoes in its new range (Juliano and Lounibos 2005).

Ae. albopictus uses natural (e.g., treeholes, plant axils) and human-made (e.g., buckets,

used tires) containers that collect water as sites for larval development, where it often co-

occurs with the native congener *Ae. triseriatus*. These mosquito species are medically

important because *Ae. albopictus* is a competent vector for West Nile virus, dengue,

eastern equine encephalitis, and LaCrosse encephalitis and *Ae. triseriatus* is a vector for

LaCrosse encephalitis virus (Leisnham et al. 2008; Juliano and Lounibos 2005; Joy and

Hildreth-Whitehair 2000). Despite the fact that past research consistently shows that *Ae.*

triseriatus is competitively inferior to *Ae. albopictus, Ae. triseriatus* has not been displaced in many areas and even remains the dominant mosquito in forested habitats (Juliano and Lounibos 2005). It is possible that superior tolerance of *Ae. triseriatus* to tannic compounds to *Ae. albopictus* may be fostering the persistence of *Ae. triseriatus*, but this has not been tested. The intraspecific and interspecific competition for resources by mosquitoes is affected by the quantity and quality of the food supply.

Past studies have looked at how habitat drying affects larval development and adult production of either *Ae. albopictus* or *Ae. triseriatus* (Alto and Juliano 2001; Aspbury and Juliano 1998; Juliano and Stoffregen 1994; Costanzo et al. 2005;). Alto and Juliano (2001) studied *Ae. albopictus* in a drying regime and two fluctuating regimes. Their study did not examine competition or lambda. Juliano and Stoffregen (1994) examined the effects of habitat drying on *Ae. triseriatus* time to development and mass, but they did not examine competition or lambda, and they did not identify the solutes that were in the leachate. Aspbury and Juliano (1998) studied how resource drying versus constantly wet leaf material altered the quality of the leaf resources. They only looked at *Ae. triseriatus* and did not test any leaf litter characteristics (Aspbury and Juliano 1998). Costanzo et al. (2005) examined interspecific competition between *Ae. albopictus* and *Ae. aegypti* in wetter fluctuating conditions and dry conditions, but they did not look at leaf litter changes and their results focused on egg mortality. Few studies have examined how competition may vary across hydrological gradients (Juliano 2009), but no other studies have looked at how competition may vary across hydrological gradients in container systems. The objective of our study was to determine if the leaf quality, altered prior to the addition of mosquito larvae via three different hydrological regimes, would affect the

competition dynamics between *Ae. albopictus* and *Ae. triseriatus.* Our study emulates

real-world scenarios regarding climate change and larval competition, and takes leaf litter

changes into account. We examine multiple mosquito parameters and utilize a composite

index of population change, lambda. This study focuses on both the abiotic and the biotic

factors that could influence mosquito spread and invasion as a result of climate change.

Methods and Materials

Pre-competition Treatment

Leaf litter was treated and prepared to mimic the three broadly different

hydrological regimes projected by the IPCC to increase due to climate change in the mid-

Atlantic region of the United States. The three precipitation regimes were abbreviated

with "D" for severe drought, "F" for constant flooding, or "WD" for wet then dry cycles.

Senesced dried white oak (*Quercus alba* L.) tree leaves (collected in Illinois in the fall of

2007) were cut into approximately 1 in^2 pieces. A microcosm was a 400-mL clear plastic

cup. In each microcosm, 0.5 grams of leaves (which included leaf blades and one leaf

petiole) were added. To prepare the F microcosms, the microcosms were filled with 140

mL of distilled water. These microcosms were monitored and were filled weekly to keep

them at 140 mL. The WD microcosms were filled with 140 mL of distilled water. The

water was allowed to evaporate completely from these microcosms, resulting in dry

leaves. The D microcosms consisted of the dry leaf litter; they were not filled with water.

All of the microcosms were placed in an environmental chamber set at 14:10 (L:D)

photoperiod and 26°C. They were rotated weekly to reduce the possibility of chamber

effects.

Microcosms were checked daily to determine when no moisture remained in the WD microcosms and the litter was dry. After 43 days, the WD microcosms had dried completely and 250 mL of distilled water were then added to each of the D and WD microcosms. We added 250 mL to the F microcosms (already containing 140 mL of water) and 140 mL were poured out to mimic how a rain event would cause full containers to overflow in a flooded climate. The microcosms were inoculated with 100 µL of pond water that was obtained from a pond on the University of Maryland's campus in College Park, MD. This was intended to establish the microbial communities within the microcosms. Five days later, mosquitoes were added to all the microcosms, which contained 250 mL.

Mosquito Competition Experiment

Competition trials were run between 10 density combinations of *Ae. albopictus* and *Ae. triseriatus* to create a response surface design. Thirty different treatments were setup with three replicates of each (90 microcosms). Each microcosm was either the wet-dry, dry, or flooded leaf litter (already prepared) and one of the 10 mosquito density combinations (*Ae. albopictus: Ae. triseriatus*: 10:0, 20:0, 40:0, 10:10, 10:30, 20:20, 30:10, 0:40, 0:20, 0:10). *Aedes albopictus* and *Ae. triseriatus* eggs (F_2-F_3 generation) were hatched simultaneously using a 0.3 g/L nutrient mixture. Twenty-four hours later, the larvae were added to the prepared microcosms at the appropriate density combination (day 0 of the experiment). The 90 microcosms were placed in an environmental chamber set at 14:10 (L:D) photoperiod and 26°C to mimic summer conditions in Maryland. The microcosms were rotated every day to decrease the possibility of chamber effects. On day 26, 0.5 grams of dry leaves (including leaf blades and one petiole) were added to mimic

the natural input of leaf material into containers that occurs in natural ecosystems. The leaves added as supplement food were not treated and were the same leaves as the originals. While prior mosquito competition experiments have added supplementary food sources every two weeks (Murrell and Juliano 2008), this addition of leaf material was deferred as long as possible to ensure the possibility of a treatment effect.

Pupae were removed from the microcosms daily and placed in separate vials until they eclosed as adults. After the pupae eclosed, the sex, the proportion of survivorship to adulthood ($\female$), mean days to eclosion ($\female$ & $\male$), and mean body size ($\female$ & $\male$) (dry mass and wing length) were recorded for each species from each microcosm. These demographic variables were used to estimate the finite rate of population change for each species (lambda, λ'), which is a composite index of population performance (Livdahl and Sugihara 1984):

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

Where N_0 is the initial number of females (assumed to be 50% per microcosm), x is the mean time to eclosion (measured in days), A_x is the mean number of females eclosing on x, and w_x is the mean body size on x. The function $f(w_x)$ is different for each mosquito species and describes the fecundity and female size relationship. D is the mean days it takes for an adult mosquito to mate, bloodfeed, and oviposit. Because only the adult

female mosquitoes are pests and disease vectors, their fitness parameters (measured as mass, time to development, and survival) are the most important.

An additional 36 microcosms with no mosquitoes (three treatments and twelve replicates) were established. The leaves were prepared in the exact same manner as earlier described to obtain the WD, D, and F leaves; this took 45 days. Of the 36 microcosms, all of which contained 0.5 grams of leaf litter and one petiole, 12 contained WD leaves, 12 contained D leaves, and 12 microcosms contained F leaves. The microcosms were placed in the same environmental chamber set at 14:10 (L:D) photoperiod and 26°C. Six microcosms were randomly selected from each treatment type (WD, F, D and totaling 18 microcosms) on days 9 and 13 and were destructively sampled. A sample of water was removed from the 18 microcosms and the tannic acid concentration was measured using the High Range Test instructions of a Hach calorimeter and tannin-lignin TA-3 Test Kit (Hach Company, Colorado). The decay rate of the detritus in the microcosms was calculated by removing the coarse detritus with a 2-mm sieve, drying and weighing it. Bacterial productivity was also measured using a Heterotrophic Plate Count method (SM9215C) and a 1 to 10 dilution; this quantified microbial growth in the microcosms.

Statistical Analyses

Two-way ANOVAs (Analysis of Variance) were used to test effects of detritus type (either F, D, or WD) and day (9 and 13) on the quality of microcosm habitats (tannin concentration, decay rate, and bacterial abundance).

A second set of ANOVAs were used to test effects of detritus type (either F, D, or WD), densities of both species (continuous variables), and their interactions on

proportion of survival to adulthood, mean time to eclosion (♀ & ♂), mean body size (♀ & ♂), and lambda of both species. A negative relationship between response variables with *Aedes* densities represents an effect of competition, and a significant interaction between litter type and either density indicates that litter type affects the outcome of competition. Post-hoc pairwise mean comparison tests (Tukey-Kramer method) were performed on all significant main effect and interaction treatments. Error degrees of freedom (df) differed if no mosquitoes of a given sex emerged from the microcosms. Experimental results were considered to be statistically significant when $p \leq 0.05$ and marginally significant when $0.05 < p < 0.1$. All ANOVA tests performed utilized the SAS program package (SAS Institute 2004). To meet the assumptions of normality in SAS, we $\log_{10}$ transformed the bacterial abundance and arcsine square-root transformed survival.

<u>Results</u>

There was marginal significance regarding the effect of detritus type on bacterial abundance (measured in colony forming units) in the microcosm leachate with WD leaf litter colonized by the most colony forming units, followed by F litter, then D litter with the least. There was a highly significant effect of day on bacterial abundance, and a detritus by day interaction, with the rate of increase in bacterial abundance highest in WD litter, followed by F litter, then D litter with the least (Table 1) (Fig 1). Posthoc pairwise comparison tests detected a significant difference between WD and D slopes (P= 0.0162) Decay rate of the leaf litter was affected by detritus type, by day, and there was a day by detritus interaction (Table 2) (Fig 2). Posthoc pairwise mean comparisons showed that D litter decayed significantly slower than F and WD litter (P< 0.0001). Posthoc tests regarding the interaction showed that all slopes were significantly different from each

other on days 9 and 13 (P< 0.0001) (Fig 2). The tannin-lignin concentration was significantly different in the leachate of microcosms provisioned by the three detritus types and there was a marginally significant interaction of detritus type by day (Table 3) (Fig 3). The D leaf litter leached significantly more tannins than the F and WD litter (P< 0.0001), and the tannin concentration in the D microcosms increased from day 9 to day 13. The F and WD litter leached fewer tannins that were not significantly different from each other, and the tannin concentration in the F and WD microcosms decreased from day 9 to 13 (Fig 3).

The population performance (lambda) of *Aedes albopictus* was affected by detritus type and by the increasing densities of both *Ae. albopictus* and *Ae. triseriatus* larvae (Table 4) (Fig 4a). F litter resulted in the highest lambda, followed by D litter then WD litter; posthoc pairwise comparison tests detected no significant differences between leaf types (P> 0.10). Zero females of *Ae. triseriatus* emerged from any of the 20:20 D microcosms (Fig 4b, 5b, 5d). Lambda of *Ae. triseriatus* was affected by detritus type, with microcosms provisioned with WD litter resulting in the highest lambda (above 1.0), followed by F litter (~1.0), and then D litter (< 1.0). Posthoc pairwise comparison tests detected a significant difference between WD and D leaf types (P = 0.0098). Lambda of *Ae. triseriatus* was also affected by the increasing density of heterospecifics, and an interaction of *Ae. albopictus* density by detritus type (P= 0.0015) (Table 4). Similarly, pairwise comparison tests of the interaction revealed that microcosms provisioned with D leaf litter exhibited stronger competition than microcosms provisioned with WD leaf litter (P< 0.05). There was a marginally significant interaction of *Ae. triseriatus* density by detritus type on *Ae. triseriatus* lambda (Table 4). Detritus type marginally affected

both *Ae. albopictus* and *Ae. triseriatus* survival (Table 5). Increasing heterospecific and conspecific densities affected *Ae. albopictus* and *Ae. triseriatus* survival (Table 5).

Increasing densities of heterospecifics and conspecifics affected the mean days to eclosion (time to development) of both *Ae. albopictus* females and males (Table 6). There was an interaction of *Ae. albopictus* density by detritus type on the mean days to eclosion of *Ae. albopictus* males (Table 6), but pairwise comparison tests of the interaction revealed no differences between leaf types. Detritus type affected the mean days to eclosion for *Ae. triseriatus* females and males (Table 6). For *Ae. triseriatus* females, wet-dry leaves resulted in the fastest development time followed by flooded and dry, in that order. Posthoc pairwise comparison tests detected no significant differences between leaf types (P> 0.30). For *Ae. triseriatus* males, F leaves had the fastest development time followed by WD and D. Posthoc pairwise comparison tests detected no significant differences between leaf types (P> 0.13). Increasing densities of heterospecifics and conspecifics affected the mean days to eclosion of both *Ae. triseriatus* females and males, and there was an interaction of *Ae. triseriatus* density by detritus type on the mean days to eclosion of *Ae. triseriatus* males (Table 6). Pairwise comparison tests of the interaction revealed no differences between leaf types.

When analyzing the mean mass of the eclosed adults, only *Ae. albopictus* males were affected by the main effect of detritus type (Table 7). Microcosms provisioned with D leaf litter resulted in the largest mean mass, followed by WD leaf litter, and F leaf litter. Posthoc pairwise comparison tests detected no significant differences between leaf types (P> 0.35). Mean mass for *Ae. albopictus* males was also affected by increasing densities of heterospecifics and by an interaction of *Ae. albopictus* density by detritus

type (Table 7). Pairwise comparison tests of the interaction revealed no differences between leaf types. *Ae. albopictus* and *Ae. triseriatus* females were not affected by leaf type or the increasing densities of mosquito larvae (Table 7).

Discussion

Our experiment revealed that the hydrological regimes that leaf litter was exposed to affected the decay rate of the detritus and the tannin concentration in the leachate, while marginally affecting the bacterial abundance. There was a significant interaction of *Ae. albopictus* density by detritus type on *Ae. triseriatus* population performance. This indicates that interspecific competition of *Ae. albopictus* on *Ae. triseriatus* was altered by detritus type. Detritus type affected lambda of *Ae. albopictus* and *Ae. triseriatus*, with D litter causing a declining population of *Ae. triseriatus*. Intra- and interspecific competition affected the mean days to eclosion and mean survival of both species. Detritus type affected the development time of *Ae. triseriatus* females, with D litter resulting in the slowest development time. In future conditions of severe drought, *Ae. albopictus* densities and dry leaf litter will amplify the competitive effects on *Ae. triseriatus*, potentially increasing the range and/or abundance of *Ae. albopictus*.

The most important result observed was a significant interaction of *Ae. albopictus* density by leaf type on *Ae. triseriatus* population performance (P= 0.0015). This indicates that interspecific competition of *Ae. albopictus* on *Ae. triseriatus* was altered by detritus type. Tables 5-7 show that the influence of detritus on competitive effect was not driven by one primary fitness correlate. The D leaf litter increased the competitive effect of *Ae. albopictus* on *Ae. triseriatus* in microcosms. The WD and F litter resulted in the highest

Ae. triseriatus lambda values at all con- and heterospecific densities. Our litter quality analysis supports this result because dry litter released higher concentrations of tannins than flooded or wet then dry litter. Further, dry litter supported fewer bacterial colonies (food resources) which could be due to less nitrogen in the leaves, as indicated by the decay rate. Without testing the water chemistry, Juliano and Stoffregen 1994 proposed that increased solutes lead to increased microbial growth. However, we found that D litter leached the most tannins and was colonized by the fewest colony forming units. Detritus types varied in bacterial abundance on day 13 but not on day 9. This could be important regarding mosquito competition if the effect of competition is stronger with some species at certain instars. We saw that *Ae. albopictus* was largely not affected by detritus type. This may be explained if the majority of their competition occurs at the earlier instars, when the detritus types did not vary in bacterial abundance. Future studies should examine how particular resource types may affect bacterial abundance over time, differentially affecting *Ae. albopictus* versus *Ae. triseriatus* competition.

The population performance of *Aedes albopictus* was affected by leaf type (P= 0.0364) and by the densities of heterospecifics and conspecifics, however there were no interactions of intraspecific competition by leaf type or interspecific competition by leaf type. Microcosms provisioned with F litter resulted in the highest lambda, followed by D litter then WD litter; posthoc pairwise comparison tests detected no significant differences between leaf types (P> 0.10). All of the *Ae. albopictus* lambda values were above 1.0, indicating that all of the populations were increasing when exposed to D, WD, and F leaf litter (Fig 4a). While there was statistical significance regarding the effect of leaf type on *Ae. albopictus* lambda, the values were minutely different and likely would

not have caused a biological difference. Costanzo et al. 2005 found that *Ae. albopictus* was negatively affected through their dry treatment, but we could not reach the same conclusions since our experiment did not study multiple generations or the egg stage.

The mean lambda values for the treatments varied significantly for the *Ae. triseriatus* populations (P= 0.0389) and the WD treatment resulted in the highest lambda mean of above 1.0 indicating that the populations subjected to the WD leaves were increasing. The D leaf treatment had a mean lambda value less than 1.0 indicating an *Ae. triseriatus* population in decline. The mean of the F leaf treatment microcosms was very near 1.0, demonstrating that the population was stable. Posthoc pairwise comparison tests detected a significant difference between WD and D leaf types (P = 0.0098). The main effect of leaf type on *Ae. triseriatus* lambda is important because it shows that even in conditions without interspecific competition, *Ae. triseriatus* populations are still impacted by the D leaf litter. Our results show that in dry conditions leading to drought, *Ae. triseriatus* would be negatively affected and the populations would be in decline. The negative impact resulted directly from the leaf litter quality. A declining population in one species could create favorable conditions for populations of competitors. Comparing Figure 4a and Figure 4b shows that *Ae. albopictus* had consistently similar or higher lambda values than *Ae. triseriatus*, showing that it is the superior competitor for food resources.

We measured time to development because when mosquitoes experience prolonged development and remain at the larval stage for longer, they are subjected to increased risk of mortality through predation and habitat drying (Aspbury and Juliano 1998). There was a main effect of leaf type on *Ae. triseriatus* female development time

(F_2= 3.65, P= 0.0335), with wet-dry (WD) leaves having the fastest development time followed by flooded and dry, in that order. However, posthoc pairwise comparison tests detected no significant differences between leaf types (P> 0.30). Aspbury and Juliano (1998) found different results, with resource drying (similar to our WD treatment) increasing the days to eclosion of *Ae. triseriatus*. However, they treated their leaf litter then moved the litter to a tire or treehole while we treated the leaf litter and performed the experiments in the same microcosms. Performing the experiments in the same microcosms allowed for us to test if chemicals leached from the leaves affected the water chemistry and the mosquito larvae.

We found no effect of leaf type, intraspecific competition, or interspecific competition on female sizes (Table 7). This is an important result because the mass of the female mosquito is closely linked to the fecundity of the mosquito (Livdahl 1982), and a smaller female body size has been shown to increase the mosquito's potential to contract and transmit diseases (Ameneshewa and Service 1996).

A significant main effect of leaf type, but no interaction between leaf type and density, suggests a leaf type effect that is independent of competition. The mechanism behind the main effect of leaf type could be toxicity and/or bacterial abundance. In our experiment, the dry treatment leaves had the slowest decay rate and leached the most tannins (Table 1). *Aedes triseriatus* was negatively affected by the D leaf treatment because that treatment resulted in the longest female development time and a lambda value indicating that the population was in decline. This leaf type could have been more directly toxic to the larvae due to the high tannins (Figure 2). The amount of edible microorganisms, which is the main food source for mosquito larvae, is the other

important aspect of litter quality. The wet-dry leaves from our experiment contained more total bacteria than the other leaf types both 9 and 13 days after re-flooding (Figure 1). *Aedes triseriatus* females experienced the fastest development time and the population increased when exposed to the WD leaf treatment. Our results show that *Ae. triseriatus* populations are highly dependent on the quality of leaf litter. Research shows that *Ae. triseriatus* is not currently being displaced by *Ae. albopictus* in forest ecosystems (Juliano and Lounibos 2005). In natural systems, *Aedes* species require a wet or damp habitat to oviposit, so the leaf litter present in those containers has likely already experienced wet then dry cycles, or constantly wet conditions. Our results show that *Ae. triseriatus* populations were stable or increasing when exposed to F and WD leaf litter. Therefore, one reason that *Ae. triseriatus* is not currently being displaced could be due to the natural pretreatment of the container leaf litter. None of the three hydrological leaf treatments negatively impacted *Ae. albopictus*, and all the lambda values were greater than 1.0, indicating population increases. Drought conditions will negatively impact *Ae. triseriatus* larval development, and could allow for the spread and proliferation of *Ae. albopictus*.

The three hydrological regimes chosen for this experiment represented climate extremes and were based on the projections by the IPCC for the mid-Atlantic region of the United States. These are also general hydrological conditions that can occur in many locations. Hydrological disturbances, such as droughts and floods, have been shown to affect community structure (Schneider and Frost 1996) and disease risk (Chase and Knight 2003). These effects can occur through biotic interactions, including competition, and can dictate the ultimate range of mosquitoes and the diseases they transmit. However, results from previous studies examining the effects of changing hydrology on mosquitoes

still seem to be highly variable. *Ae. triseriatus* lambdas were negatively affected by resource drying, comparable to our study's WD treatment, in tree holes (Aspbury and Juliano 1998), while our study revealed *Ae. triseriatus* lambdas increasing and above 1.0 when exposed to the WD treatment. Deichmeister and Telang (2011) found that West Nile Virus proliferates in low precipitation or drought conditions. Another study found that drought conditions negatively affected the egg stage of *Ae. albopictus*, a main transmitter for WNV (Costanzo et al. 2005). *Ae. albopictus* increased while *Ae. aegypyi* decreased in vase occupancy in sampled Florida cemeteries as seasonal precipitation increased (Lounibos et al. 2010). Further laboratory studies should emulate real-world scenarios by examining all three possible hydrological scenarios and over multiple mosquito generations.

Table 1: ANOVA results for $\log_{10}$ transformed bacterial abundance (cfu) measured from microcosm leachate at two time periods: 9 and 13 days after flooding

Source	df	F value	P value
Detritus	2	3.12	0.0589
Day	1	84.95	**<.0001**
Detritus X day	2	6.39	**0.0049**
Error	30		

Table 2: AVOVA results for the decay rate (percent/day) of detritus at two time periods: 9 and 13 days after flooding

Source	df	F value	P value
Detritus	2	14.57	**<.0001**
Day	1	15.22	**<.0001**
Detritus X day	2	2.05	**0.0062**
Error	12		

Table 3: AVOVA results for the tannin-lignin concentration (mg/L) measured from microcosm leachate at two time periods: 9 and 13 days after flooding

Source	df	F value	P value
Detritus	2	22.42	**<.0001**
Day	1	1.08	0.3073
Detritus X day	2	3.24	0.0534
Error	30		

Table 4: ANOVA results for the per capita rate of population change (lambda) for *Ae. albopictus* and *Ae. triseriatus*

Source	df	*Ae. albopictus*		*Ae. triseriatus*	
		F value	P value	F value	P value
Detritus	2	3.53	**0.0364**	3.45	**0.0389**
albopictus	1	159.31	**<.0001**	4.42	**0.0403**
triseriatus	1	75.21	**<.0001**	0.16	0.6881
albopictus X detritus	2	1.68	0.1961	7.32	**0.0015**
triseriatus X detritus	2	2.08	0.1349	2.88	0.0647
Error	54				

Table 5: ANOVA results for mean *Ae. albopictus* and *Ae. triseriatus* female survival (arcsine-square root transformed)

		Ae. albopictus			*Ae. triseriatus*	
Source	df	F value	P value	df	F value	P value
Detritus	2	2.62	0.0815	2	2.61	0.0825
albopictus	1	125.45	**<.0001**	1	31.18	**<.0001**
triseriatus	1	32.59	**<.0001**	1	50.15	**<.0001**
albopictus X detritus	2	0.96	0.3880	2	1.48	0.2363
triseriatus X detritus	2	1.18	0.3148	2	1.89	0.1612
Error	56			54		

Table 6: ANOVA results for mean days to eclosion for each species and sex

Sex	Source	Ae. albopictus			Ae. triseriatus		
		df	F value	P value	df	F value	P value
Female	Detritus	2	1.69	0.1933	2	3.65	**0.0335**
	albopictus	1	128.25	**<.0001**	1	62.32	**<.0001**
	triseriatus	1	55.18	**<.0001**	1	63.47	**<.0001**
	albopictus X detritus	2	0.76	0.4738	2	0.77	0.4703
	triseriatus X detritus	2	0.15	0.8600	2	2.55	0.0891
	Error	54			47		
Male	Detritus	2	0.09	0.9179	2	5.48	**0.0071**
	albopictus	1	66.99	**<.0001**	1	136.73	**<.0001**
	triseriatus	1	19.37	**<.0001**	1	83.75	**<.0001**
	albopictus X detritus	2	3.25	**0.0462**	2	2.57	0.0870
	triseriatus X detritus	2	0.97	0.3848	2	4.58	**0.0150**
	Error	56			49		

Table 7: ANOVA results for mean mass for each species and sex

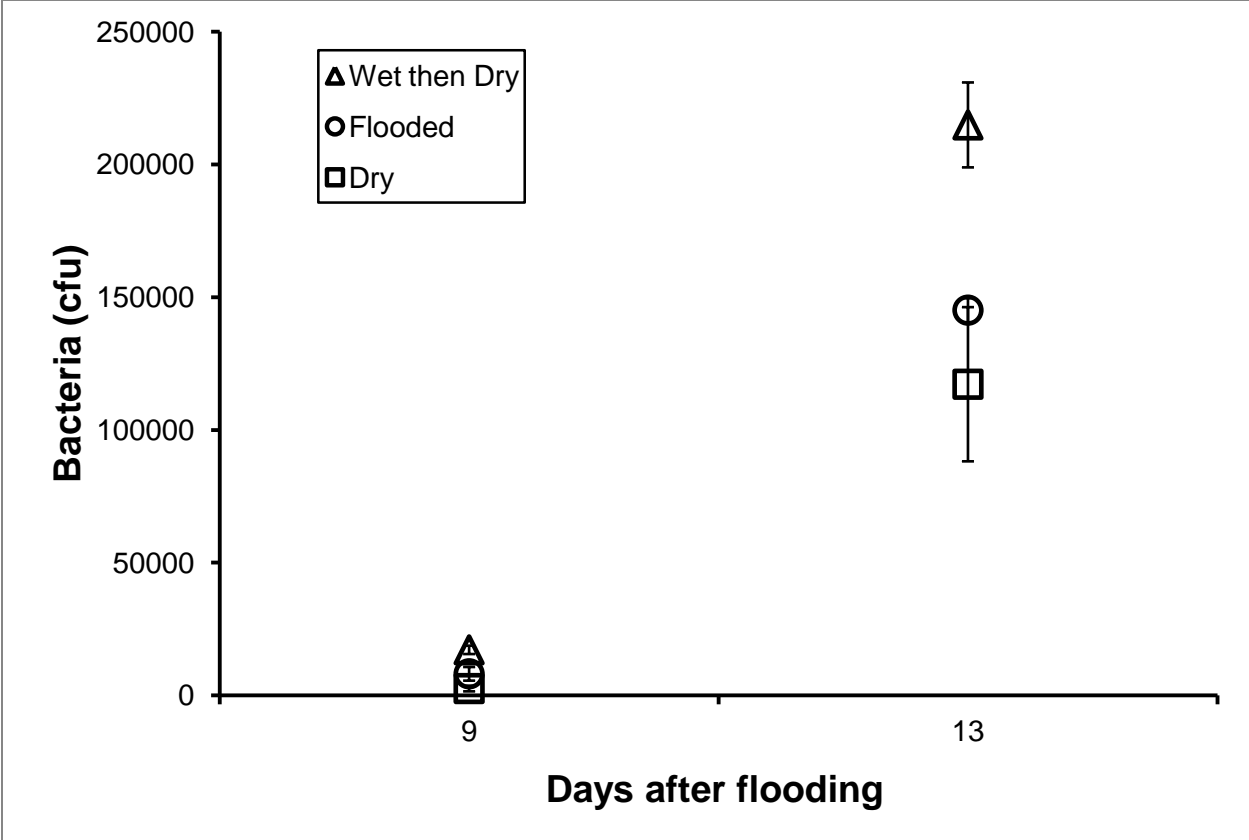| Sex	Source	Ae. albopictus			Ae. triseriatus		
		df	F value	P value	df	F value	P value
Female	Detritus	2	1.26	0.2911	2	0.18	0.8335
	albopictus	1	0.20	0.6549	1	0.34	0.5643
	triseriatus	1	2.45	0.1235	1	0.05	0.8165
	albopictus X detritus	2	0.60	0.5509	2	1.49	0.2369
	triseriatus X detritus	2	0.90	0.4117	2	0.02	0.9845
	Error	54			47		
Male	Detritus	2	3.24	**0.0466**	2	1.03	0.3656
	albopictus	1	3.65	0.0612	1	2.40	0.1281
	triseriatus	1	7.29	**0.0092**	1	0.00	0.9585
	albopictus X detritus	2	4.63	**0.0138**	2	0.42	0.6601
	triseriatus X detritus	2	2.62	0.0821	2	0.29	0.7462
	Error	56			49		

Figure 1. *Quercus alba* leaf litter (0.5 grams) was subjected to three hydrological regimes: dry (D), constantly flooded (F), and wet then dry (WD). Points shown are original bacterial abundance data measured in colony forming units (graphed with SE) on days 9 and 13 after the flooding event.

Figure 2. *Quercus alba* leaf litter (0.5 grams) was subjected to three hydrological regimes: dry (D), constantly flooded (F), and wet then dry (WD). Data points shown are decay rate measured in grams/day (graphed with SE) on days 9 and 13 after the flooding event.

Figure 3. *Quercus alba* leaf litter (0.5 grams) was subjected to three hydrological regimes: dry (D), constantly flooded (F), and wet then dry (WD). Data points shown are tannin concentration measured in mg/L (graphed with SE) on days 9 and 13 after the flooding event.

Figure 4. Mean lambda and mean proportion survival, according to leaf type (either dry "D", flooded "F", or wet then dry "WD"), for *Ae. albopictus* (a, c) and *Ae. triseriatus* (b, d) at each conspecific and heterospecific density. The lambda axis are scaled differently between the two species to highlight the leaf type differences. For proportion survival, the values reported are back-transformed means for each detritus type.

Figure 5. Females mass and female days to eclosion, according to leaf type (either dry "D", flooded "F", or wet then dry "WD"), for *Ae. albopictus* (a, c) and *Ae. triseriatus* (b, d) at each conspecific and heterospecific density.
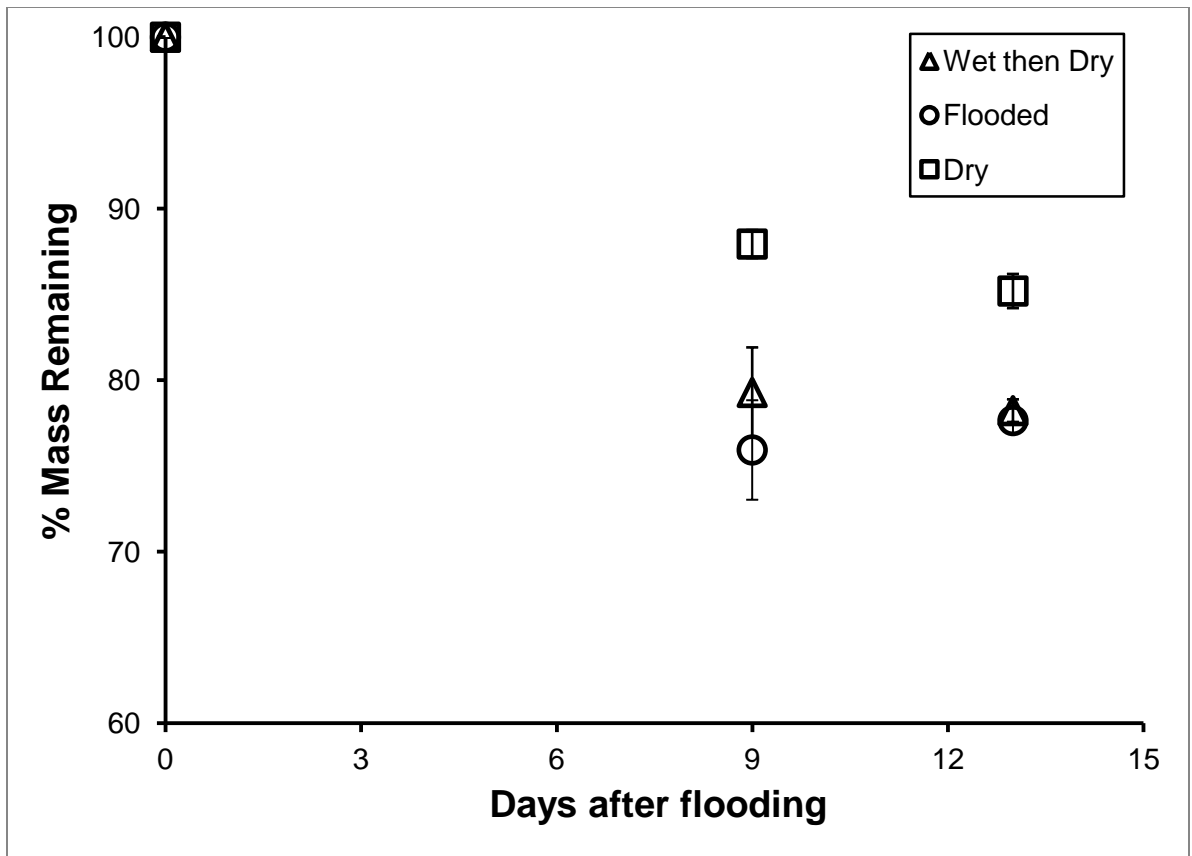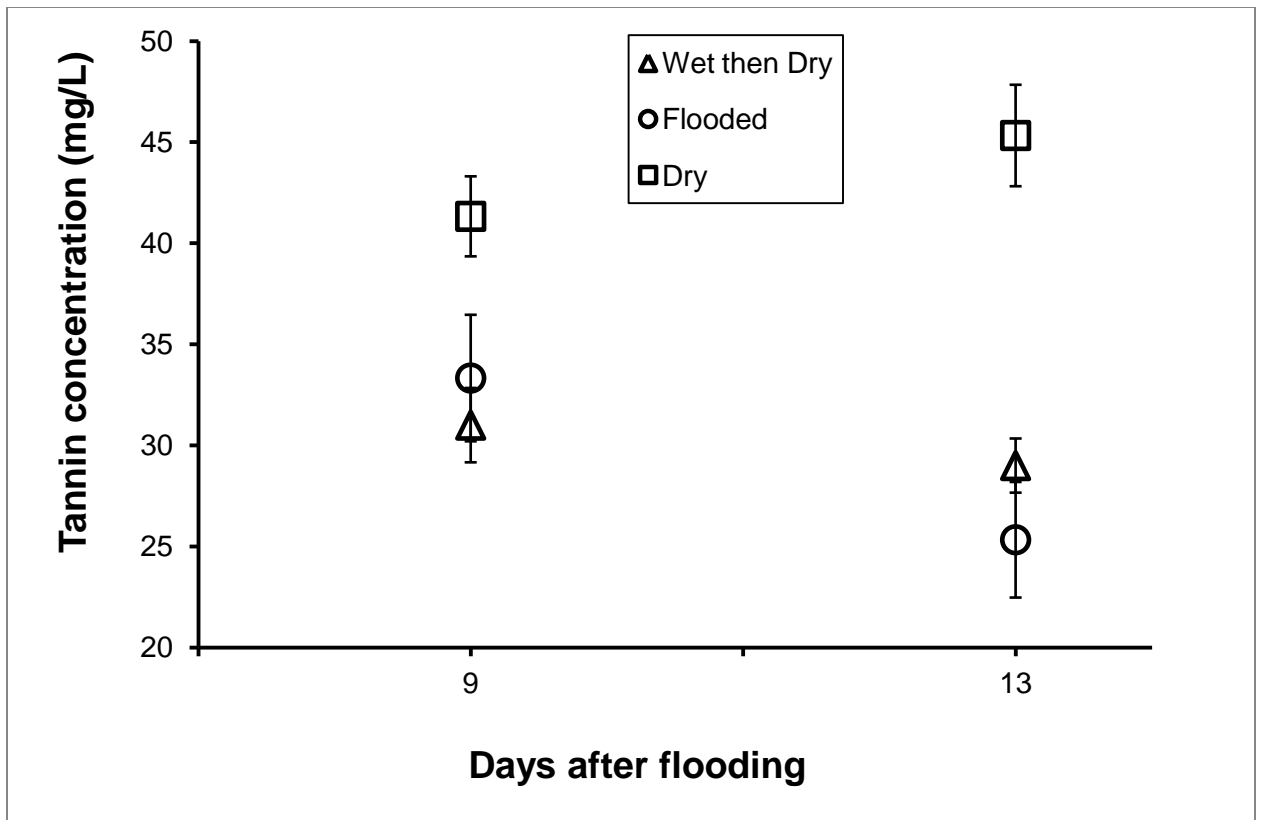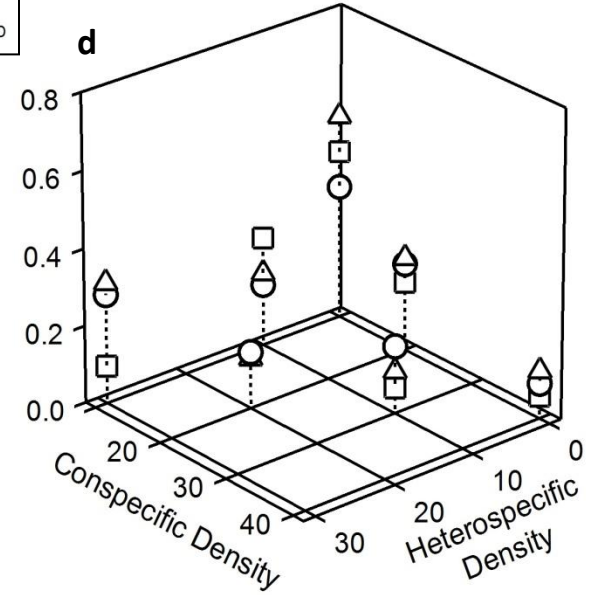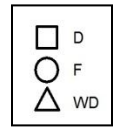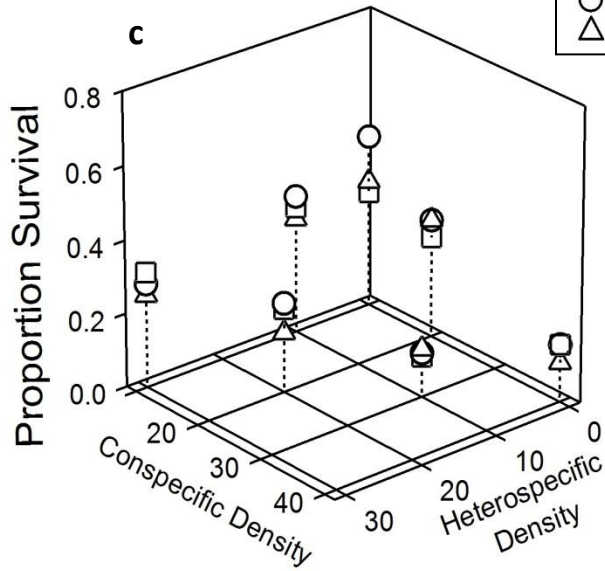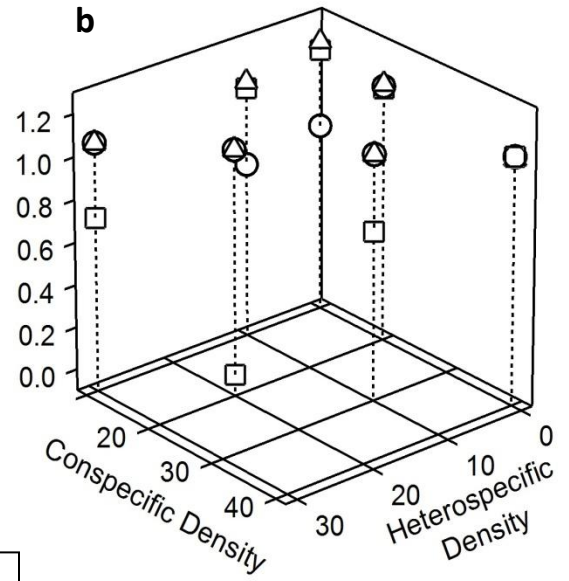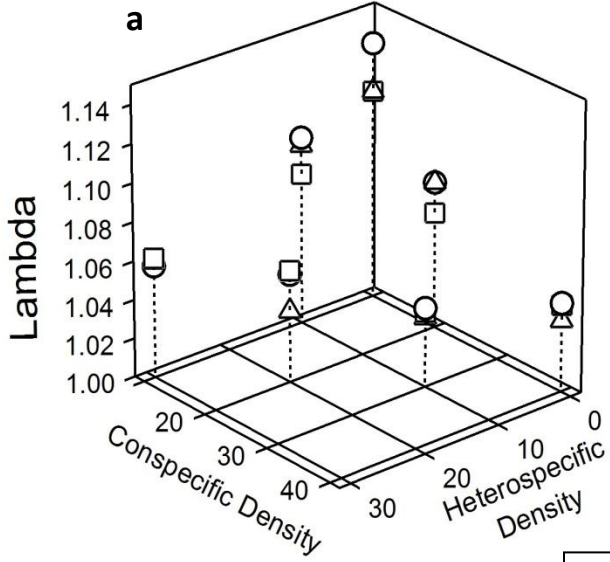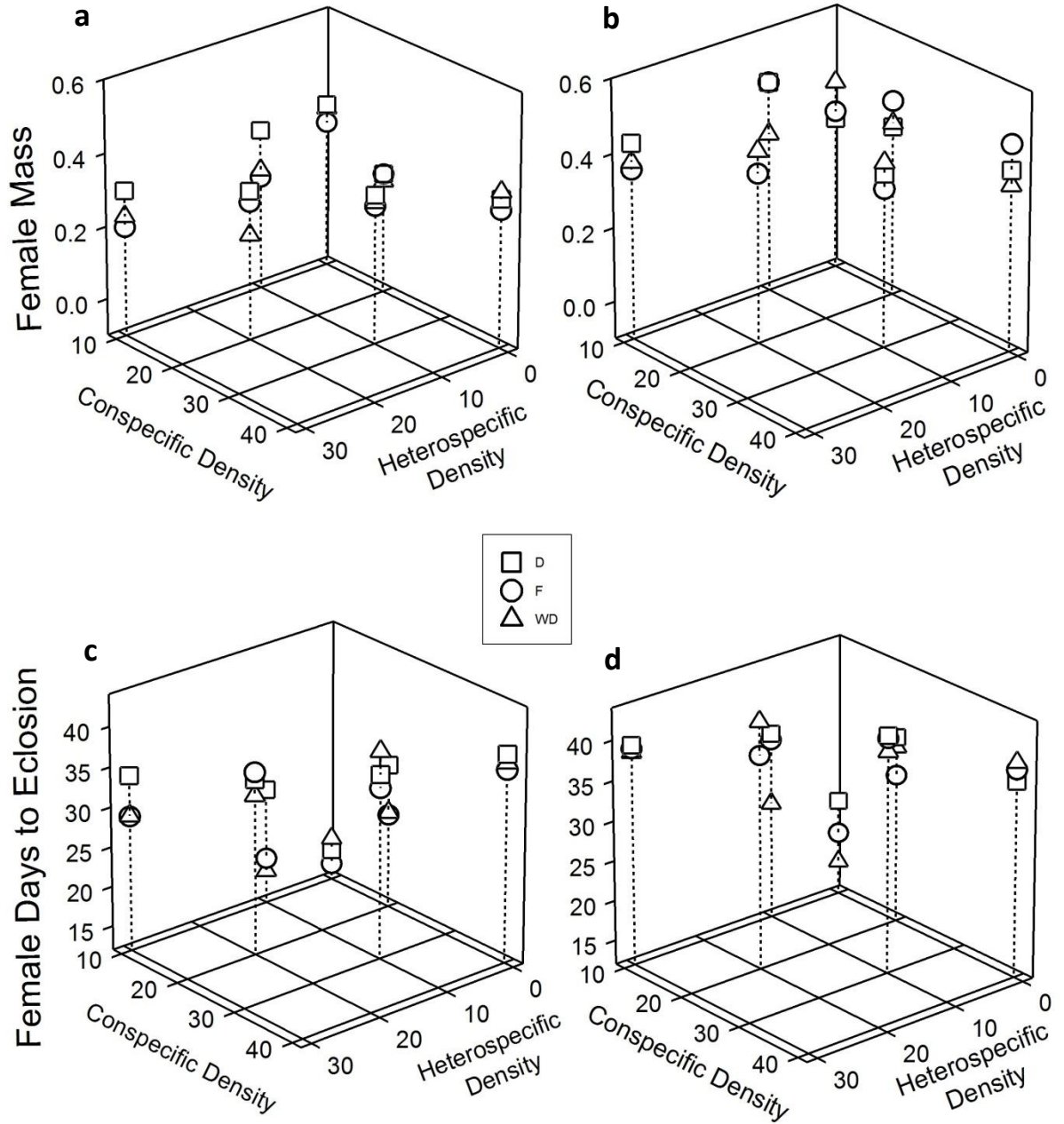
Ae. albopictus   Ae. triseriatus

Ae. albopictus   Ae. triseriatus

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Chapter 4: General Discussion

In this thesis, I tested the hypothesis that elevated atmospheric CO_2 concentrations and extreme precipitation regimes would alter leaf chemistry and competition between two locally competing mosquito species, *Ae. albopictus* and *Ae. triseriatus*.

In Chapter 2, the CO_2 Mosquito Competition Experiment revealed that litter type (either grown in ambient or elevated CO_2) did not affect the outcome of competition between *Ae. albopictus* and *Ae. triseriatus*. As expected, intra- and interspecific competition affected the mass of mosquitoes and time to development. Trees grown in elevated atmospheric CO_2 produced over twice the amount of leaf litter than the ambient chamber. Tannic acid concentrations above 100 mg/L were completely fatal to both species of mosquitoes. The increase in elevated CO_2 leaf litter, and additional food resources, indicates that competition between larval mosquitoes may be relaxed in future scenarios of high atmospheric CO_2 concentrations. However, if containers collect large amounts of leaf litter due to the increase in plant growth rates, then high levels of tannins will accumulate and could suppress mosquito development.

The finding that competition was not affected by litter type is supported by previous studies (Alto et al. 2005; Strand et al. 1999). *Aedes albopictus* had a consistently higher lambda than *Ae. triseriatus* across all densities and both litter types, indicating that it was the superior competitor for food. The leaf litter chemistry reveals why there was no difference in the competition outcome when utilizing two litter types. The decay rates of the elevated and ambient litter were similar, which is supported by Alto et al. 2005. My litter types contained similar C:N ratios, and the bacterial abundance was not different. During the growing season of the trees, I had applied a high nitrogen regime to the plants

causing N to be in abundance and not a limiting factor. Therefore, the C:N was not altered as I had expected it would be and there was ample nitrogen for bacterial proliferation. As a result of this resource abundance, mosquito competition was not stressed. From the Mosquito Competition Experiment, it can be postulated that the invasion of *Ae. albopictus* will not be altered due to the increasing levels of atmospheric CO_2.

The tree biomass calculation revealed that the trees in the elevated CO_2 conditions produced over double the amount of biomass than the trees in the chamber with ambient CO_2 levels. In natural systems, an increase in biomass would result in both an increase in food supply for organisms and an increase in toxins leached from leaf material. My results show that neither ambient nor elevated grown leaf litter had direct effects on toxicity. As litter amount increased in the microcosms, both the pH and DO in the microcosms decreased. The pH range was minimal, and the lower pH values that occurred (at the higher litter amounts) were near 6.5 and likely would not have caused a biological difference among *Aedes* species (Clements 2000). Under conditions of increasing toxicity and across all litter amounts, *Ae. albopictus* had superior population performance to *Ae. triseriatus*, which is consistent with past research on competition between these species (Teng and Apperson 2000). Notably, there was no survival of *Ae. triseriatus* in the 5.6 gram treatments of ambient and elevated leaf litter. Leachate analyzed from microcosms provisioned with 5.6 grams of elevated leaf litter revealed an average 762.22 ± 27.32 mg/L of tannins (unpublished data). The *Ae. triseriatus* larval mortality indicates that *Ae. triseriatus* is more susceptible than *Ae. albopictus* to the decreased DO or the increased tannins that accumulated in the microcosms.

The third experiment (Tannic Acid Experiment) focused on mosquito susceptibility to tannin levels that could accumulate in containers under elevated CO_2 conditions. My results reveal that single species populations of *Ae. albopictus* and *Ae. triseriatus* differ in their susceptibility to tannic acid, with *Ae. albopictus* exhibiting a consistently higher lambda. According to my results, *Ae. albopictus* and *Ae. triseriatus* development and survival would be suppressed in containers when levels of tannic acid surpass 100 mg/L.

In Chapter 3, the experiment revealed that the hydrological regimes that leaf litter was exposed to affected the decay rate of the detritus and the tannin concentration in the leachate, while marginally affecting the bacterial abundance. There was a significant interaction of *Ae. albopictus* density by detritus type on *Ae. triseriatus* population performance. This indicates that interspecific competition of *Ae. albopictus* on *Ae. triseriatus* was altered by detritus type. Detritus type affected lambda of *Ae. albopictus* and *Ae. triseriatus*, with D litter causing a declining population of *Ae. triseriatus*. Intra- and interspecific competition affected the mean days to eclosion and mean survival of both species. Detritus type affected the development time of *Ae. triseriatus* females, with D litter resulting in the slowest development time. In future conditions of severe drought, *Ae. albopictus* densities and dry leaf litter will amplify the competitive effects on *Ae. triseriatus*, potentially increasing the range and/or abundance of *Ae. albopictus*.

The most important result observed was a significant interaction of *Ae. albopictus* density by leaf type on *Ae. triseriatus* population performance. This indicates that interspecific competition of *Ae. albopictus* on *Ae. triseriatus* was altered by detritus type. The influence of detritus on competitive effect was not driven by one primary fitness

correlate. The D leaf litter increased the competitive effect of *Ae. albopictus* on *Ae. triseriatus* in microcosms. The litter quality analysis supports this result because dry litter released higher concentrations of tannins than flooded or wet then dry litter. Further, dry litter supported fewer bacterial colonies (food resources) which could be due to less nitrogen in the leaves, as indicated by the decay rate.

The population performance of *Aedes albopictus* was affected by leaf type. However, all of the *Ae. albopictus* lambda values were above 1.0, indicating that all of the populations were increasing when exposed to D, WD, and F leaf litter. The mean lambda values for the treatments varied significantly for the *Ae. triseriatus* populations and the WD treatment resulted in the highest lambda mean of above 1.0 indicating that the populations subjected to the WD leaves were increasing. The D leaf treatment had a mean lambda value less than 1.0 indicating an *Ae. triseriatus* population in decline. The mean of the F leaf treatment microcosms was very near 1.0, demonstrating that the population was stable. The main effect of leaf type on *Ae. triseriatus* lambda is important because it shows that even in conditions without interspecific competition, *Ae. triseriatus* populations are still impacted by the D leaf litter. My results show that in dry conditions leading to drought, *Ae. triseriatus* would be negatively affected and the populations would be in decline. The negative impact resulted directly from the leaf litter quality. A declining population in one species could create favorable conditions for populations of competitors. *Ae. albopictus* had consistently similar or higher lambda values than *Ae. triseriatus*, showing that it is the superior competitor for food resources.

There was a main effect of leaf type on *Ae. triseriatus* female development time, with wet-dry (WD) leaves having the fastest development time followed by flooded and

dry, in that order. A significant main effect of leaf type, but no interaction between leaf type and density, suggests a leaf type effect that is independent of competition. The mechanism behind the main effect of leaf type could be toxicity and/or bacterial abundance. In this experiment, the dry treatment leaves had the slowest decay rate and leached the most tannins. *Aedes triseriatus* was negatively affected by the D leaf treatment because that treatment resulted in the longest female development time and a lambda value indicating that the population was in decline. This leaf type could have been more directly toxic to the larvae due to the high tannins. The amount of edible microorganisms, which is the main food source for mosquito larvae, is the other important aspect of litter quality. The wet-dry leaves from the experiment contained more total bacteria than the other leaf types both 9 and 13 days after re-flooding.

It is medically and scientifically important to predict where disease vectors will be as a result of the changing climate and hydrology. Based on the findings of the CO_2 experiments, the relative distributions between these *Aedes* mosquitoes, as dictated by competition, are unlikely to change due to the expected increases in phytotannins with various stressors such as climate change and urbanization. However, the results indicate that development of individual populations may be suppressed, potentially affecting abundance and invasion success to some degree, due to increasing container-water toxicity. Based on the hydrology study, *Aedes triseriatus* females experienced the fastest development time and the population increased when exposed to the WD leaf treatment. The results show that *Ae. triseriatus* populations are highly dependent on the quality of leaf litter. None of the three hydrological leaf treatments negatively impacted *Ae. albopictus*, and all the lambda values were greater than 1.0, indicating population

increases. Drought conditions will negatively impact *Ae. triseriatus* larval development, and could allow for the spread and proliferation of *Ae. albopictus*. More studies on a local scale are necessary to predict the future spread of *Ae. albopictus* in relation to its resident competitors.

Appendix A: Biomass Tannin Data

Table 1: AVOVA results for the tannin-lignin concentration (mg/L) measured from microcosm leachate with varying litter amounts (0.7, 1.4, 2.8, 5.6 g) at three time periods: 4, 7, and 21 days after flooding. Day 0 was removed from the analysis.

Source	df	F value	P value
Litter amount	3	121.37	**<.0001**
Day	1	0.00	0.9887
Litter amount X day	3	1.11	0.3607
Error	28		
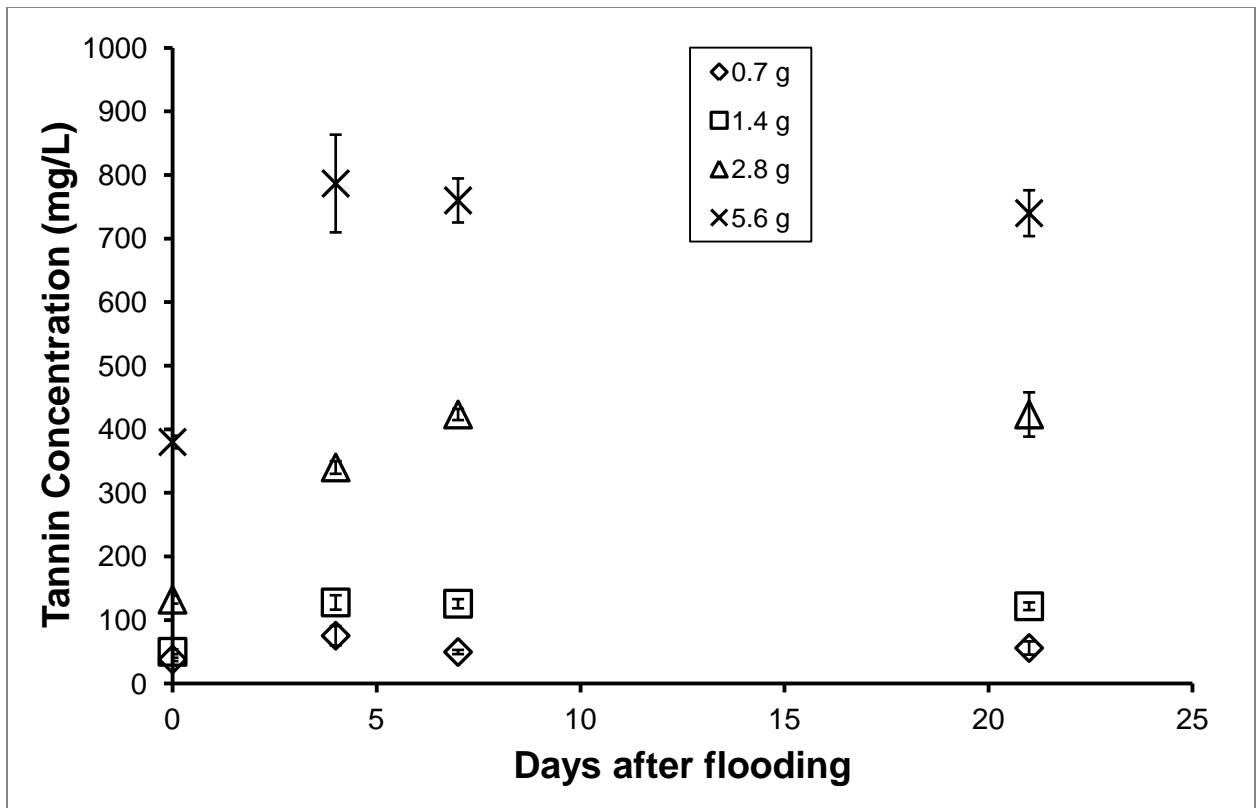
Figure 1. Tannin-lignin concentration (mg/L) of microcosm leachate provisioned with varying amounts (0.7, 1.4, 2.8, and 5.6 grams) of *Quercus alba* leaf litter grown in elevated (1034 ± 5.76 ppm) CO_2 concentrations and measured at four time periods: 0, 4, 7, and 21 days after flooding. Mean concentration $\pm$ SE

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