

ABSTRACT

Title of Dissertation: SPATIO-TEMPORAL MECHANISMS OF
URBAN MOSQUITO COEXISTENCE IN
BALTIMORE, MD

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Understanding the interactions governing species distributions and community structure are of fundamental ecological importance. Mosquitoes that utilize container habitats at their larval stage usually engage in strong competition and competitive exclusion is expected; however, numerous container-utilizing mosquito species co-occur in the same individual container habitats and regionally coexist. I investigated spatial and temporal mechanisms governing the distributions and abundances of the competitively superior invasive *Aedes albopictus* and resident *Culex* spp. mosquitoes in four neighborhoods with varying socioeconomic status in Baltimore, Maryland. Specifically, I investigated if the findings from both field surveys and field and laboratory experiments were consistent with four spatial and temporal hypotheses for species coexistence that act at different scales: spatial partitioning among neighborhoods and blocks, seasonal condition-specific competition, aggregation among individual container habitats, and priority colonization effects within

individual containers. I found modest but important evidence for all hypotheses that could each facilitate *Culex* spp. coexistence with *Ae. albopictus*. I found clear neighborhood effects, with low SES neighborhoods supporting higher abundances of mosquitoes than high SES neighborhoods overall, but with the highest abundances of *Ae. albopictus* in low SES neighborhoods and *Culex* spp. being more variable among neighborhoods. *Culex* spp. abundances were higher in the early summer compared to mid-summer peaks in abundance for *Ae. albopictus*. Laboratory competition trials showed increased aggregation of *Ae. albopictus* had a slight positive effect on *Culex* spp. population performance, and aggregation conditions sufficient for coexistence among experimentally placed ovitraps and negative associations of *Aedes* and *Culex* genera in resident containers in the field. Lastly, I found that priority colonization of a container leads to stronger population performance for both species, and that resource availability seems to affect *Culex* spp. more than competition. The results of my dissertation have revealed the role of several ecological mechanisms that may facilitate the regional coexistence of *Culex* spp. with *Ae. albopictus* and is among the first bodies of work to do so. Due to their roles in the transmission of human pathogens, future examination of other spatial and temporal mechanisms of coexistence between *Ae. albopictus* and resident *Culex* spp. is warranted.

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IN BALTIMORE, MD

by

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Chapter 1: General Introduction

Mosquitoes and public health

Mosquitoes (Diptera: Culicidae) are a family of insects that have important ecologic, economic, and public health impacts worldwide. Mosquitoes are ubiquitous vectors of human and veterinary pathogens, as well as nuisance pests, making them of interest to scientists, health officials, and the public (Gubler 2002, Lounibos 2002, Juliano and Lounibos 2005). Additionally, invasive mosquitoes may introduce novel pathogens into an environment or alter the natural transmission cycles of domestic pathogens (McMichael et al. 2000). Mosquito-borne diseases induce staggering global burdens. In 2017, there were an estimated 219 million cases of malaria with approximately half of the world's population living at risk of contracting the disease (WHO 2018). Dengue virus is currently considered the most important mosquito-borne viral disease by the World Health Organization (WHO) with an estimated 50 to 100 million new infections annually, although this burden is likely underestimated (WHO 2012), especially when considering the economic and social impacts associated with the disease (Gubler 2012, Shepard et al. 2016).

Although many mosquito-borne diseases originate in tropical and subtropical climates, temperate climates are not excluded from significant vector-borne disease transmission. In the United States the invasive West Nile virus (WNV), and the domestic arthropod-borne viruses (arboviruses) Eastern equine encephalitis virus (EEEV) and LaCrosse virus (LACV), can cause severe morbidity, mortality, and economic loss on an annual basis. Following the introduction of WNV in 1999, the

cumulative cost of the epidemic through 2012 was approximately \$778 million (Staples et al. 2014), with an estimated annual burden of \$56 million for hospitalized patients (Barrett 2014). Since its initial introduction to the United States, WNV has spread to all of the lower 48 states and caused two nationwide epidemics in 2003 and 2012 (CDC 2013), and an estimated 7 million human infections as of 2016 (Ronca et al. 2019). Utz et al. conducted a study investigating the economic burden of LACv infection, finding that direct and indirect costs of infection averaged \$33,000 per patient (Utz et al. 2003), while the estimated lifetime cost of a single infection of EEEv is estimated at over \$1.5 million per patient (Villari et al. 1995).

Global environmental and climate change may also expand the range and varieties of vectors found globally allowing for introductions of novel viruses into susceptible populations and more frequent and/or continuous disease transmission (Chaves et al. 2011, Leisnham and Juliano 2012, Rochlin et al. 2013, Parham et al. 2015). Recent introductions of chikungunya virus (CHIKv) to the Caribbean and the Zika virus to the Americas have caused alarm in the scientific community because the two vector mosquitoes implicated in transmission, *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), are commonly found in urban environments in the United States (Leparc-Goffart et al. 2014, Powers 2015, Fauci and Morens 2016, Hahn et al. 2016, Hahn et al. 2017). Since the introduction of the Zika virus to the Americas in 2015 (Hayes 2009), there have been local outbreaks of Zika in the continental United States with more than 200 people locally acquiring the virus in Florida in 2016 and 10 people locally acquiring the virus in Texas that same year (Hahn et al. 2017, Martin et al. 2019). These outbreaks highlight the fact that in the presence of the appropriate

vectors, under the right conditions, both endemic and seasonal transmission of novel arboviruses can occur (Ruiz-Moreno et al. 2012, Fauci and Morens 2016).

In addition to spreading numerous pathogens, many vector mosquitoes such as *Ae. albopictus*, are voracious biting pests that can affect the daily activity and quality of life of human populations (Ratigan 1999). The presence of nuisance mosquitoes may even alter daily behaviors (Dowling et al. 2013a). For example, one study found that children spent less time outside in the presence of *Ae. albopictus* than children who lived in neighborhoods where mosquito abatement occurred (Worobey et al. 2013). Similarly, a study by Halasa et al. showed that mosquito infestations forced residents inside and decreased their perceived quality of life (Halasa et al. 2014). When coupled with the fact that mosquitoes are the source of an immense global disease burden, these studies highlight the broad range of impacts that mosquitoes can have on both physical and mental human health.

Urban mosquitoes in the northeastern United States

The Asian tiger mosquito, *Ae. albopictus*, is the most common mosquito species in urban areas in the northeastern United States. It is also one of the most important invasive species worldwide. Originally a native of Asian forests, it has spread extensively in the last three decades, largely through the international used tire trade and via other artificial containers (Benedict et al. 2007). Following its introduction in to the United States in the 1980s, *Ae. albopictus* quickly spread throughout the country, becoming the most abundant and pestiferous mosquito in a number of regions of the country mainly due to its ability to utilize a wide range of container habitats (Moore and Mitchell 1997, Gratz 2004, Juliano and Lounibos

2005). The invasion and spread of *Aedes albopictus* is well documented due to its potential to spread numerous arboviruses and its negative impacts on resident mosquitoes, often resulting in local extinctions of resident species (Moore and Mitchell 1997, Lounibos 2002, Sardelis et al. 2002, Gratz 2004, Juliano and Lounibos 2005, Turell et al. 2005, Fader 2016).

Aedes albopictus is commonly found in sites with both the northern house mosquito, *Culex pipiens* (L.) and the white dotted mosquito, *Culex restuans* (Theobald) in the northeastern United States, extending from Massachusetts to the District of Columbia and Virginia (Darsie Jr 2002, Darsie Jr and Ward 2005). *Culex pipiens* was introduced to North America from Europe approximately 400 years ago and is now firmly established as a resident in the northeastern United States (Ross 1964, Vinogradova 2000, Lounibos 2002). *Culex pipiens* is also implicated as primary vector of enzootic and epidemic WNV in the eastern United States (Turell et al. 2001, Hamer et al. 2008, Farajollahi et al. 2011). *Culex restuans* is a resident mosquito to North America and occupies a similar ecological niche to *Culex pipiens* (Reiskind and Wilson 2008). *Culex restuans* exhibits similar ecological traits to *Cx. pipiens*, showing near ecological equivalence (Reiskind and Wilson 2008); however, studies have shown seasonal segregation which identifies *Cx. restuans* as dominant in the early summer and *Cx. pipiens* dominating the mid-to-late summer season in the Northeast (Madder et al. 1980, Lampman and Novak 1996). *Culex restuans* has also been implicated as an important contributor to urban WNV transmission (Kilpatrick et al. 2010, Johnson et al. 2015).

Most laboratory and field studies convincingly show that larval *Ae. albopictus* are superior competitors for resources compared to *Cx. pipiens* (Carrieri et al. 2003, Costanzo et al. 2005b, Costanzo et al. 2011) and nearly every other resident species they encounter in the Americas (Juliano 1998, Juliano 2009). This competitive superiority is the likely mechanism for displacement of some resident species in many locations in the field. The most well-studied impacts have been on *Ae. aegypti* in the southeastern part of the United States and *Aedes. triseriatus* (Say) in urban areas along the eastern seaboard (Juliano 1998, Teng and Apperson 2000, Juliano et al. 2004). Despite this frequently observed competitive advantage for *Ae. albopictus*, *Cx. pipiens*, and other *Culex* species manage to coexist with invading *Ae. albopictus* at most urban sites in the eastern United States (O'meara et al. 1995, Juliano et al. 2004, Juliano 2009).

Conditions that may foster mosquito coexistence can be found in urban northeastern population hubs. Adult mosquito abundances and distributions are strongly influenced by processes occurring during their immature aquatic (egg, larva, pupa) life stages (Washburn 1995, Juliano 2009). Mosquitoes that develop within container habitats are affected by both abiotic and biotic factors such as food resources, temperature, container size and type, competition, and predation (Hawley 1985, Teng and Apperson 2000, Harlan and Paradise 2006, Blaustein and Chase 2007, Lounibos et al. 2010, Costanzo et al. 2011, Dowling et al. 2013b). Container characteristics are also important for adult oviposition choice, with container size, container type, and nutrient input being associated with differential oviposition

preference (Harlan and Paradise 2006, Bartlett-Healy et al. 2012, Dowling et al. 2013b, Fader and Juliano 2014).

Container habitats do not exist in isolation and are influenced by the surrounding terrestrial environment in which they occur. Past studies have shown significant variation in mosquito communities among broad land uses (e.g., commercial, industrial, resident) in urban landscapes (Braks et al. 2003, Rey et al. 2006, Leisnham and Juliano 2009, Leisnham and Juliano 2012). Fewer studies have illustrated differences in mosquito communities within urban land uses. A handful of studies have, however, suggested that there may be significant and important social and environmental differences within urban landscapes that affect mosquito ecology, and that these differences most clearly occur in areas that vary in socio-economic status (SES), with lower SES areas being disproportionately affected by mosquito infestation (Dowling et al. 2013b, LaDeau et al. 2013, Becker et al. 2014, Little et al. 2017). In the northeastern United States, lower SES conditions in many cities are often defined by high numbers of abandoned buildings, vacant lots, decaying infrastructure, and unmanaged vegetation which are broadly considered disamenities (Reisen et al. 2008, LaDeau et al. 2013, Becker et al. 2014, Little et al. 2017). These disamenities may often represent increased trash, nutrient inputs, shade, and an abundance of vegetative overgrowth that may in turn be associated with favorable immature and adult mosquito habitats (Beier et al. 1983, Joshi et al. 2006, Brown et al. 2008, Harrigan et al. 2010, Dowling et al. 2013b, LaDeau et al. 2013). Further, mosquito container habitats can be affected by seasonal and monthly climatic variables such as precipitation and temperature (Bartlett-Healy et al. 2012, Rochlin et

al. 2013, Little et al. 2017). The northeastern United States experiences clear seasonality which can directly affect the survival of mosquito species, especially over the winter (NOAA 2016). Although the numerous abiotic and biotic gradients that occur in the urban Northeast can directly alter densities of *Ae. albopictus* and *Culex* species, it is perhaps of greatest ecological and public health interest to better understand how these gradients affect interactions between these two species, particularly at the larval level.

Importance of studying ecological mechanisms of coexistence

Understanding the interactions that govern distributions of species and the structure of communities are core ecological concepts. Theoretical and empirical work indicate that with one limiting factor in a constant environment, competition between species should result in competitive exclusion (Hardin 1960, Hutchinson 1961). Competitive exclusion is most likely to occur when the species exhibit strong competitive asymmetry (Lawton and Hassell 1981). However, competitive exclusion can be avoided thereby leading to coexistence of species via a number of mechanisms, including differential resource use (Tilman 1982), aggregation (Ives 1988b, a, 1991, Chesson 2000a), spatial partitioning (Amarasekare and Nisbet 2001, Amarasekare and Possingham 2001, Calcagno et al. 2006), priority effects (Alford and Wilbur 1985, Shorrocks and Bingley 1994, Blaustein and Margalit 1996), trade-offs between competitive ability and susceptibility to enemies (Blaustein and Chase 2007), and trade-offs between competitive ability and tolerances to environmental conditions (Chesson 2000b). These mechanisms of coexistence are not necessarily mutually exclusive, indicating that multiple drivers of coexistence may be at play in a

single environment. The mechanisms of coexistence are especially important when investigating invasive species, which may have both long and short-term impacts on resident species' diversity via a number of mechanisms including competition, predation, and parasitism (Williamson 1996). Understanding the mechanisms of coexistence, especially between invasive and resident mosquito species, is critical to considering future ecological, economic, and public health impacts related to competing species.

Spatial and temporal hypotheses of mosquito coexistence in temperate cities

Although multiple hypotheses may explain the coexistence of resident mosquito species with *Ae. albopictus*, few have thoroughly tested spatial and temporal mechanisms in seasonably variable urban environments (Rey et al. 2006, Leisnham and Juliano 2009, Leisnham et al. 2014). One hypothesis that may explain mosquito coexistence in such environments is spatial partitioning. Socioeconomic status often varies along neighborhood and block boundaries, and it is at this scale where one might expect spatial partitioning among mosquito species to be most strongly illustrated. Furthermore, because mosquito management often occurs at the neighborhood or block scales, examining species distributions at these scales is important to mosquito ecology and species coexistence (Makse et al. 1995, Cadenasso et al. 2007, Grimm et al. 2008). In neighborhoods characterized by below median household incomes and low housing value (low SES neighborhoods), city disinvestment often leads to high numbers of abandoned buildings and vacant lots that can potentially foster large amounts of trash and overgrown vegetation (Focks et al. 1981), both of which can support mosquito populations (Brown et al. 2008,

Dowling et al. 2013b, LaDeau et al. 2013, Becker et al. 2014). Artificial containers are the predominant type of larval habitat present in urban environments (Leisnham et al. 2005), and the type of container habitat present in an environment can vary with neighborhood SES (Joshi et al. 2006, Dowling et al. 2013b, Little et al. 2017). Lower SES neighborhoods that have experienced city disinvestment often have higher numbers of containers overall, as well as more disused or trash containers, while higher SES neighborhoods have fewer water-holding containers overall and a greater proportion of functional containers (Dowling et al. 2013b, LaDeau et al. 2013, Little et al. 2017). These differences in container habitat type, combined with block and neighborhood scale characteristics may provide the appropriate conditions for spatial partitioning of *Ae. albopictus* and *Culex* spp. that are sufficient to support local coexistence.

Aggregation is another form of spatial partitioning that could relax the effect of interspecific competition at the scale of individual container habitats. The aggregation hypothesis of species coexistence posits that the persistence of the inferior competitor is facilitated if the competing stage of the superior competitor is aggregated independently from the inferior competitor over spatially divided individual habitats. If the overlap of the competitors (degree of interspecific aggregation) is reduced in individual containers, the effects of interspecific competition are lessened, allowing for coexistence (Ives 1988a, b, Sevenster 1996). Aggregation of mosquitoes is most likely to arise due to attraction of competitors to different environmental cues at the landscape (e.g. neighborhood) or container (e.g.

disused versus functional) level, which is in turn a form of spatial partitioning (Ives 1988a, Chesson 2000a).

Seasonal condition-specific competition occurs when the outcome of competition is altered under different abiotic conditions. When there is temporal variation in abiotic environments, species may differentially react to these conditions and the expected competitive outcome can be altered (Chesson 2000b). The outcome of resource competition between species is determined by R^* , the level of resources necessary to produce zero net population growth (Tilman 1982). When discussing interspecific competition between mosquito species, larval mortality also affects R^* (Tilman 1982), and when species are competing for resources, abiotic conditions can affect mortality, and therefore the outcome of interspecific competitions, by differentially changing species' R^* s (Costanzo et al. 2005a, Costanzo et al. 2005b, Leisnham et al. 2014). For coexistence to occur under the effects of seasonal condition-specific competition, environments must fluctuate between conditions that favor the different species in the system. In the northeastern United States, the most common temporal changes in climatic conditions are seasonal. These seasons are driven primarily by temperature (e.g. winter to spring) in temperate latitudes. Mosquitoes are generally active in the northeastern United States from May through September with mosquito activity varying within this time period. Seasonal temperatures may differentially affect *Ae. albopictus* and *Culex* spp. abundances. *Culex* spp. overwinter almost exclusively as adults (Mitchell 1988), while *Ae. albopictus* overwinters in egg form (Hawley et al. 1989). These differences may allow for earlier adult *Culex* spp. activity and dispersal in the early summer compared

to *Ae. albopictus* providing a competitive advantage. Additionally, *Culex* spp. are more likely to be active at lower temperatures than *Ae. albopictus*, consistent with early summer conditions in the northeastern United States (Vinogradova 2000, Jackson and Paulson 2006, Kunkel et al. 2006, Brady et al. 2013, Brady et al. 2014), likely promoting earlier ovipositional activity of *Culex* spp. during the earliest part of the summer season and allowing *Culex* spp. to escape competitive exclusion.

Another form of temporal segregation that may foster coexistence is priority effects. Priority effects is the competition that occurs when one species colonizes a habitat before another species, and has been reported as an important factor in the development of community structure (Alford and Wilbur 1985, Shorrocks and Bingley 1994, Blaustein and Margalit 1996). The first colonist in an environment may have a competitive advantage over the second colonist, despite competitive superiority of the second species. In areas where *Ae. albopictus* is found to coexist with *Culex* species, the priority colonization of a container habitat by a *Culex* species may relax the competitive advantage of *Ae. albopictus*. Priority effects may also play an important role in coexistence at the beginning or end of the mosquito season, as the competitive advantage of *Ae. albopictus* may be relaxed during periods of cooler weather (Carrieri et al. 2003), leading to the increased probability of *Culex* spp. ovipositing in a container before *Ae. albopictus*.

Research goals

The overall goal of my research was to test these four spatial and temporal hypotheses that may explain the coexistence of *Culex* spp. mosquitoes with *Ae. albopictus* in temperate Baltimore City, Maryland. Each hypothesis has clear

predictions that were tested using field surveys and controlled laboratory experiments. My predictions for these hypotheses are as follows:

1. Spatial partitioning across neighborhoods predicts higher *Ae. albopictus* densities in lower SES neighborhoods with higher amount of trash compared to high SES neighborhoods, and higher *Culex* spp. abundances in high or medium SES neighborhoods. In Baltimore, Maryland, spatial partitioning among mosquito species is likely to be expressed at the scale of individual blocks and/or neighborhoods.
2. Seasonal condition-specific competition predicts strong temporal patterns of coexistence, with both genera present throughout the summer, but with higher *Culex* abundances early in the summer and higher *Ae. albopictus* abundances in the middle and late summer due to lower overwintering survival and slower ovipositional utilization of early summer habitats.
3. The aggregation hypothesis of coexistence among individual containers predicts *Ae. albopictus* and *Culex* spp. will be independently aggregated among individual ovipositional containers and independently in the field. *Culex pipiens* should show stronger population performance in containers with increasingly aggregated *Ae. albopictus* compared to microcosms with uniformly distributed *Ae. albopictus*.
4. The priority effects hypothesis of coexistence within individual containers predicts reduced or even reversed competitive impacts of *Ae. albopictus* on *Cx. pipiens* survival in the field. A theoretical test of the priority colonization hypothesis predicts greater population performance of *Cx. pipiens* in

experimental microcosms into which it is colonized first compared to microcosms in which it is colonized at the same time as *Ae. albopictus*.

In Chapter 2, I collect seasonal data on *Ae. albopictus* and resident *Culex* spp. at the block scale across four neighborhoods that vary in SES to assess if it is consistent with the spatial partitioning or seasonal condition-specific competition hypotheses of coexistence. In Chapter 3, I examine data on the distributions and abundances of *Ae. albopictus* and *Culex* spp. among individual container habitats in these same neighborhoods. I also report results from a manipulative laboratory experiment to test the role of the aggregation hypothesis of coexistence. Finally, in Chapter 4, I report the effects of colonization order within individual containers on *Ae. albopictus* and *Culex* spp. populations using both a laboratory and a field experiment to test the role of the priority effects hypothesis of coexistence. Chapters 2 through 4 are written as separate manuscripts for submission to peer-reviewed journals, with their own subsequently required sections.

Chapter 2: Spatial and temporal patterns of mosquito coexistence across socioeconomically diverse neighborhoods in Baltimore, MD

Introduction

Ecological theory predicts and empirical work has shown that in a resource limited environment interspecific competition should result in competitive exclusion. However, species that engage in strong competition can coexist via a number of mechanisms, including (but not limited to) spatial resource partitioning (Chesson 2000a), temporally varying condition-specific competition (Chesson 2000b), aggregation (Ives 1988a, b), and colonization-competition trade-offs (Amarasekare and Nisbet 2001, Calcagno et al. 2006). These mechanisms of coexistence are not mutually exclusive, meaning that multiple factors may support species coexistence in a single environment, making predictions of species distributions and abundances difficult. Additionally, different mechanisms of coexistences may be particularly important in understanding the impacts of biological invasions. Biological invasions by competitively superior species can affect the distributions of resident species, even causing extirpation in some areas, without resulting in their extinction over the entire range of introduction (Tilman 1982, Juliano and Lounibos 2005). Understanding the mechanisms of coexistence, especially between invasive and resident species, is critical considering future ecological, economic, and public health impacts related to competing species.

The Asian tiger mosquito, *Aedes albopictus* (Skuse), is the most common mosquito species in urban environments in the northeastern United States (Moore and Mitchell 1997, Gratz 2004, Juliano and Lounibos 2005). Native to Asia, it has invaded North and South America, Europe, and Africa during the last three decades via the international used tire trade and the exploitation of other artificial containers (Benedict et al. 2007). *Aedes albopictus* is adapted to extreme weather conditions, such as cold temperatures, by laying desiccation-resisting diapausing eggs that can survive colder winters and drought, which has contributed to its invasion success (Paupy et al. 2009, Waldock et al. 2013). Due to its extensive spread and public health importance, *Ae. albopictus* is one of the most studied container-inhabiting mosquitoes (Lounibos 2002), and provides researchers an opportunity to determine whether patterns of coexistence and interactions with native species in its current range are consistent with testable mechanisms of ecological coexistence. In northeastern urban environments, *Ae. albopictus* commonly competes with the northern house mosquito, *Culex pipiens* (L.), and a similar species, *Cx. restuans* (Theobald). These container-utilizing mosquitoes have the potential for vectoring a variety of arboviruses including West Nile virus, La Crosse encephalitis virus, dengue virus, and Chikungunya virus (Ibáñez-Bernal et al. 1997, Gerhardt et al. 2001, Ebel et al. 2005, Turell et al. 2005, Paupy et al. 2009, Kilpatrick et al. 2010), making their co-occurrence of public health importance.

The majority of laboratory and field studies show convincingly that larval *Ae. albopictus* are superior competitors for resources compared to *Cx. pipiens* and almost every other resident species it encounters in the Americas (Costanzo et al. 2005,

Juliano 2009, Juliano 2010). This competitive superiority is the likely mechanism for displacement of some resident species throughout their range. Despite this frequently observed competitive advantage in favor of *Ae. albopictus*, *Cx. pipiens* and other resident *Culex* species, including *Cx. restuans*, manage to coexist with the invader at most urban sites in the eastern United States (Costanzo et al. 2005, Juliano 2009). In this study I used three discrete field-based datasets to test two non-mutually exclusive spatio-temporal hypotheses about the persistence of resident *Culex* species with *Ae. albopictus* in Baltimore, Maryland: spatial partitioning and temporal condition-specific competition.

Weaker competitors can escape exclusion if there is resource partitioning in space. Spatial partitioning is likely to occur when there are high numbers of usable habitats and competitors are attracted to different environmental conditions or resource types (Amarasekare and Possingham 2001, Calcagno et al. 2006). Previous studies have observed spatial partitioning of mosquitoes across various land use types, such as between urban, suburban, and rural (Braks et al. 2003), urban vs. rural (Rey et al. 2006), industrial vs. commercial (Leisnham and Juliano 2009), and along urbanization gradients (Carbajo et al. 2006, Rubio et al. 2011). As spatial partitioning is associated with land use characteristics, there is potential for spatial partitioning across neighborhoods that vary in condition due to infrastructure management and community capacity (socio-economic status, SES). Land-use and land cover can vary at relatively fine spatial scales in urban landscapes to create a heterogeneous matrix of conditions (Makse et al. 1995, Cadenasso et al. 2007, Grimm et al. 2008), affecting both the quantity and quality of mosquito habitat. In cities, a majority of mosquito

breeding sites are artificial rather than natural containers (e.g. tree holes) (Leisnham et al. 2005).

Previous studies in Baltimore, MD have shown that there is often a higher abundance of disused container habitats in lower SES neighborhoods, and a higher proportion of larger functional and structural containers in high SES neighborhoods (Dowling et al. 2013, LaDeau et al. 2013, Becker et al. 2014, Little et al. 2017). Lower SES neighborhoods have higher numbers of vacant lots and abandoned buildings that can foster large amounts of trash and vegetative overgrowth, both of which can provide mosquitoes with adult and larval habitats (Chaves et al. 2011, Dowling et al. 2013, LaDeau et al. 2013, Becker et al. 2014). *Aedes albopictus* and resident *Culex* species coexist in urban Baltimore, Maryland, where high SES neighborhoods are juxtaposed with lower SES neighborhoods with a history of decay (LaDeau et al. 2013, Becker et al. 2014). These characteristics allow for the potential patchy environment that may facilitate spatial partitioning between *Ae. albopictus* and resident *Culex* species. Although past work has failed to detect definite spatial partitioning between *Ae. albopictus* and other resident mosquitoes among cemeteries in Florida (Leisnham and Juliano 2009, Leisnham et al. 2014), the urban environments of these studies did not contain the substantial socio-economic disparities and resultant urban decay commonly found in cities in the temperate northeastern United States, which may promote this mechanism of coexistence.

Condition-specific competition describes situations when the outcome of competition is altered or even reversed under different abiotic conditions (Tilman 1982, Chesson 2000b). Abiotic conditions commonly vary temporally, with perhaps

the most common example being seasonal changes in climatic conditions at temperate latitudes. For temporal condition-specific competition to facilitate species coexistence, environments must fluctuate between conditions that favor different species. In the northeastern United States, mosquitoes are generally active during the summer, from May through September, during which there is considerable variation in seasonal temperatures that may differentially affect *Ae. albopictus* and co-occurring *Culex* species. *Culex* spp. almost entirely overwinter as adults (Mitchell 1988), whereas *Ae. albopictus* overwinter as eggs (Hawley et al. 1989). Thus, *Culex* spp. may be expected to have a competitive advantage early in the summer because *Culex* spp. adults also appear to be more active than *Ae. albopictus* at lower temperatures typical of early summer conditions in the mid-Atlantic (Vinogradova 2000, Jackson and Paulson 2006, Kunkel et al. 2006), allowing for which might promote greater ovipositional activity among *Culex* spp. at the start of summer and enhance their overall competitive ability.

In this chapter, I test whether spatial and temporal patterns of immature (larvae, pupae) *Ae. albopictus* and resident *Culex* spp. in Baltimore, Maryland are consistent with the spatial partitioning and temporal conditions-specific competition hypotheses of species coexistence. Both hypotheses lead to testable predictions about the abundances and distributions of *Ae. albopictus* and resident *Culex* species.

1. Spatial partitioning predicts different patterns of *Ae. albopictus* and *Culex* spp. abundances among neighborhoods, with *Ae. albopictus* expected in greater numbers in lower SES neighborhoods that have higher numbers of container habitats and unmanaged space, and higher *Culex* spp. mosquito abundances in

high or middle SES neighborhoods that are dominated by larger volume structural and functional containers.

2. Condition-specific competition predicts strong seasonal patterns of coexistence with *Ae. albopictus* being more abundant in the middle and late mosquito season, and *Culex* spp. being more abundant in the early season.

Materials & Methods

Neighborhood selection

Four neighborhoods in Baltimore City, Maryland (Franklin Square, Harlem Park, Union Square, Bolton Hill) categorized as low, medium, and high SES were selected for this study (**Figure 1**). The neighborhoods were chosen *a priori* using both online data and physical surveillance to ensure that they were primarily residential and fell along a SES gradient centering on, above, and below the 2014 median household income of \$41,819 (FactFinder 2014, Little et al. 2017). Three blocks in each neighborhood (12 total) that were representative of the landcover and average SES of the neighborhood were chosen as study blocks. The blocks cover approximately eight acres of land and are composed of attached row homes. All study blocks were separated by a minimum of one city block between each of the sites. The proportion of occupied buildings was assessed each year of the study. Blocks in the high SES neighborhood had the greatest proportion occupied structures (97.4%, 94.4 – 100), the low SES neighborhoods had the lowest proportion occupied structures (39.4%, 23.5 – 55.6), and the middle SES neighborhood fell between the low and high neighborhoods with 80.4% (68.5 – 93.3) occupancy over the two years. There

was a no change in occupancy in the high SES neighborhood from 2014 to 2015; however, there was a slight increase in occupancy in the middle SES neighborhood and a decrease in occupancy in the low SES neighborhoods.

Ovitraping

Intensive standardized mosquito collections were conducted in each neighborhood from May through November (9 sample weeks) in 2014 and 2015. The majority mosquito activity in the mid-Atlantic typically occurs from May to September (McCardle et al. 2004, Armistead et al. 2014, Shone et al. 2014), however collections were continued until approximately a maximum of 25% of ovitraps showed occupation. Oviposition traps (ovitraps) were deployed every three weeks to identify oviposition activity throughout the summer season. Sample dates corresponded to roughly the same Morbidity and Mortality Weekly Report (MMWR) week each year, however due to shifts in the calendar, the collections are represented by the two weeks in which the sampling occurred (i.e. 21/20 for sample week 1). Collection week dates have Sites were chosen based on vegetation presence, accessibility, safety, and suitable mosquito habitat.

Six ovitraps were deployed in each of the 12 study blocks per collection period (72 total). Three ovitraps were placed in each block half to provide sufficient coverage of the block. Ovitrap consisted of 600 mL black plastic cups with two holes drilled above the waterline to prevent the cups from flooding and prematurely hatching *Aedes* spp. eggs or the loss of *Culex* spp. egg rafts and larvae. Ovitrap were lined with seed germination paper (Nasco Science, Fort Atkinson, WI), filled with 450 mL of tap water and baited with an additional 50 mL of Timothy Hay infusion

(100 g Timothy Hay in 30.3 L of distilled water for five days). Ovitrap were secured in secondary holder cups secured to available substrate allowing for easy collection of samples. Ovitrap were deployed on the same day in all neighborhoods over a period of four hours. After seven days, ovitrap were collected and all larvae were enumerated and identified to species. *Culex* egg rafts and larvae were immediately removed from traps and allowed to mature to 4th instar larvae for identification where their numbers were estimated to the nearest 25 following random sampling of the first 50 larvae. Seed germination paper from each ovitrap was stored for five days in summer conditions (27°C, 90% humidity) and then submerged in a nutrient broth solution to hatch any eggs present. Larvae were reared to 4th instar, identified to species, and enumerated.

Buckets

Prior studies have observed that *Culex* spp. often prefer to oviposit in larger containers than *Ae. albopictus* (Carrieri et al. 2003), therefore, in addition to deploying ovitrap in 2015, four 1-gallon buckets were also deployed in each study block (two per block half) during June and August of 2015. Each bucket was baited with 2L of the same infusion as the ovitrap. This provided a total of 48 additional oviposition traps during each of these sessions, in addition to the 72 traditional ovitrap. Buckets were processed using the same protocol as the traditional ovitrap.

Resident container surveillance

Resident containers in all study blocks of the four neighborhoods were sampled over three collection periods during both the 2014 and 2015 mosquito seasons in mid-June (early season), late July/early August (middle season), and mid-

September (late season). Every parcel in each study block was searched for water-holding containers unless access was denied by the resident. Containers were then evaluated using a standardized data sheet that assessed parcel type, container type, container volume, solar exposure, biotic presence, and mosquito presence/absence (Dowling et al. 2013, LaDeau et al. 2013, Little et al. 2017). If mosquito larvae were present, container contents were homogenized and up to 1L of water was sampled per container. Mosquito samples were taken from all accessible mosquito-positive containers in study blocks. Samples were returned to the laboratory where all mosquito larvae were isolated and then stored in ethanol for later processing. Larvae were identified to the genus or species level based on instar (early vs. late) and presence/absence of species was recorded for each sample. All pupae and any newly emerged adults were identified to genus or species depending on condition of organism (Bodner et al. 2016).

Container indices (CIs) are commonly used to quantify levels of immature mosquito infestation. In this study the container index measures the percent of mosquito positive containers per meter squared within a given block by multiplying the average number of containers per square meter by the percent of total positive containers in that block (Little et al. 2017). Genus level indices (*Aedes* and *Culex*), were calculated for each sample period using the resident container data obtained during the two years of the study.

Statistical analyses

Mosquito abundances from ovitraps were analyzed using a doubly repeated measures univariate ANOVA with the nine sample weeks and two study years

serving as two repeated measures. *Aedes albopictus* and *Culex* spp. abundances were each averaged across ovitraps within each block and log-transformed to meet assumptions of normality and homogeneity of variances. Three species of *Culex* were identified during the study with 97.0% being either *Cx. pipiens* or *Cx. restuans*. *Culex pipiens* and *Cx. restuans* are morphologically difficult to distinguish as adults (DeGaetano 2005, Diuk-Wasser et al. 2006), found in similar environments (Deichmeister and Telang 2011, Johnson et al. 2015), and are considered ecologically equivalent as larvae (Reiskind and Wilson 2008). As the focus of this study is to compare spatial and temporal distributions of *Ae. albopictus* with competitively inferior *Culex* spp., these similarities in life history traits led me to combine the *Culex* spp. for analysis. In all models, block was a random variable to control for block-level variation and was nested within neighborhood because I wanted to compare mosquito populations among neighborhoods.

Follow-up linear models were performed to test the *a priori* hypothesis that *Ae. albopictus* abundance affects *Culex* spp. abundance. For each linear model I used log-transformed mosquito abundances and treated block and sample week as random variables. Fixed variables were neighborhood and year. In the final model I removed the interactive terms of *Ae. albopictus* by season and *Ae. albopictus* by neighborhood ($p > 0.05$) to improve model-fit assessed by AICc values.

Analyses of the CIs were similar to that of the ovitraps, but the repeated term was collection (early, middle, late) for the two study years. Data was log-transformed to meet assumptions of normality and homogeneity of variances. I performed follow up linear models on log-transformed *Aedes* spp. and *Culex* spp. container indices, to

test whether the *Aedes* spp. CI affects the *Culex* spp. CI. Block and collection were treated as random variables and neighborhood and year were treated as fixed variables. Interactive terms remained in the final model. Significant differences were investigated using contrasts.

The bucket data was analyzed as a simple univariate ANOVA between the two collection dates (June and August 2015). Abundances were log-transformed to meet assumptions of normality and homogeneity of variances. Following the methodology for ovitraps and resident container indices, I performed follow up linear models on the bucket data to see if *Ae. albopictus* was a predictor of *Culex* spp. abundances. All analyses were performed using SAS PROC MIXED (SAS 2016). Significant differences in all models ($p < 0.05$) were investigated *a posteriori* by Tukey-Kramer adjustments.

Results

Ovitrapping

A total of 145,309 mosquitoes consisting of five species from two genera were collected across the study neighborhoods during the two years of the study. *Aedes albopictus* was the most abundant species representing 78.1% ($n = 113,471$) of the total mosquitoes. *Culex pipiens* and *Cx. restuans* represented 20.0% ($n = 16,239$ and $12,766$, respectively). The remaining 1.9% consisted of *Ae. japonicus* ($n = 1,913$) and *Cx. territans* ($n = 920$). *Aedes albopictus* abundances were significantly different between the two years, as well as across sample periods and neighborhoods (**Table 1**). Higher mean abundances occurred in 2015 during three weeks in the middle and late summer as compared to 2014 (**Figure 2**). As predicted the highest *Ae. albopictus*

abundances occurred in the mid-summer (July/August), however they peaked slightly earlier in 2014 when compared to 2015 (**Figure 2**). There was also a significant neighborhood by sample week interaction (**Table 1**), with lower mean *Ae. albopictus* abundances in Bolton Hill, the highest SES neighborhood compared to the low SES neighborhood of Harlem Park during two sample weeks in early summer (**Figure 3**). Bolton Hill also had significantly lower mean *Ae. albopictus* abundances than the medium SES neighborhood of Union Square and the low SES neighborhood of Franklin Square in the late summer (**Figure 3**).

ANOVAs on total *Culex* spp. abundances showed a significant three-way interaction between year, sample week, and neighborhood (**Table 1**). Mean *Culex* spp. abundances showed two clear peaks in 2014, but only one in 2015. The peaks in 2014 were earlier and later in the summer compared to the mid-summer peak in 2015. When *Culex* spp. peaked in early summer 2014, there were significantly higher numbers of *Culex* spp. in Franklin Square and Union Square compared to 2015. During the peak in 2015, *Culex* spp. were significantly higher in mid-summer compared to the same time in 2014. Finally, in late summer 2014, there were significantly greater abundances of *Culex* spp. in the medium SES neighborhood Union Square than there were in the low SES neighborhood of Franklin Square (**Figure 4**). Follow-up linear models indicate a significant positive effect of *Ae. albopictus* on total *Culex* spp. abundance (**Table 2**, slope = 0.2903), which was consistent between both study years and across all four neighborhoods (no significant interaction effects).

Buckets

During the two collection dates (June and August 2015) a total of 24,649 mosquitoes were collected. Consistent with collections from ovitraps, *Ae.s albopictus* was the most dominant mosquito collected in buckets, representing 70.6% (n=17,397) of specimens. *Culex pipiens* (n=3,277) and *Cx. restuans* (n=3,175) constituted a greater proportion (26.2%) of total mosquito abundances compared to the ovitraps. The remaining 3.2% was comprised of *Cx. territans* (n=550) and *Ae. japonicus* (n=250). ANOVAs on mean *Ae. albopictus* abundances showed a significant seasonal effect (**Table 3**). Significantly greater mean abundances ($p<0.05$) of *Ae. albopictus* were collected in the August than in the June collection (**Figure 5**). ANOVAs on total *Culex* spp. abundances in the bucket ovitraps yielded a neighborhood by collection interaction (**Table 3**). Upon further examination with adjustments using Tukey's test, the interaction proved insignificant. The main effect of collection month was also significant, with significantly greater mean abundances of *Culex* spp. occurring in the August collection (**Figure 6**). Follow-up linear models showed no significant effect of *Ae. albopictus* abundance on *Culex* spp. abundances in bucket ovitraps ($F_{1,10.4}=0.21$, $p=0.6577$).

Resident container indices (CIs)

A total of 2,103 resident containers were sampled in the study blocks over the 6 collection periods in 2014 and 2015. Of these, 34.8% (n=731) contained immature mosquitoes. *Aedes* spp. were found in 65.7% (n=480) of the mosquito positive containers, while *Culex* spp. were found in 44.7% (n=327) of the mosquito positive

containers. *Aedes* spp. and *Culex* spp. were found together in 25.03% (n=183) of the mosquito positive containers.

Container indices (CIs) for *Ae. albopictus* presence in resident containers differed significantly across collection dates and neighborhoods, and there was a significant collection period by neighborhood interaction (**Table 4**). In the early summer, there were no differences in *Aedes* CIs between neighborhoods. During the middle and late summer collection periods the low SES neighborhood Franklin Square had the highest mean *Aedes* CI, being significantly different than all other neighborhoods in the middle period ($p < 0.05$) and significantly different from Bolton Hill, the highest SES neighborhood, in the late period (**Figure 7**). *Aedes* CI showed the clearest seasonal differences in Franklin Square, with all collection periods being different from each other in this neighborhood. Harlem Park, the other low SES neighborhood, also showed strong seasonal differences in *Aedes* CI, with the early summer collection period being significantly lower than the mid-season period (**Figure 7**).

ANOVAs on the *Culex* spp. CI showed a significant three-way year by collection by neighborhood interaction (**Table 4**). There were noticeable seasonal differences in *Culex* CI in both study years, but only those in 2015 were statistically significant. In 2015, both low SES neighborhoods (Franklin Square and Harlem Park) had their *Culex* CIs peaked in the early summer and then dropped during the middle summer before increasing again at the end of the season (**Figure 8B**). There was a reversal of this trend in the high (Bolton Hill) and middle (Union Square) SES neighborhoods, where *Culex* CI peaked in the middle season and was lowest in the

late summer. In 2015 there was a higher mean *Culex* CI in Franklin Square than Bolton Hill (**Figure 8**). Similar to the *Aedes* CI, *Culex* CI also showed the clearest seasonal differences in Franklin Square in 2015 but a different trend, indicating a clear decline from early to late summer (**Figure 8A**). Harlem Park showed a decrease in its CI from early to mid-season in 2015 (**Figure 8**). Due to the differences being found only in year two in the three-way interaction, I decided to investigate the interactive effect of year by collection and the main effect of neighborhood to further tease apart any patterns in the data.

There was a significant ($p < 0.05$) difference in 2015 between the early and middle collection and the early and late collection, with the early collection showing the highest CI (**Figure 9**). Investigation of the effect of neighborhood on the *Culex* spp. CI showed a significant ($p < 0.05$) difference between the high SES neighborhood, Bolton Hill, and the low SES neighborhood, Franklin Square, with Franklin Square having more *Culex* spp. positive containers than Bolton Hill. While the differences between the other neighborhoods were not significant the general trend shows more *Culex* spp. positive mosquitoes per square meter as SES decreases (**Figure 10**).

The follow-up linear model showed a significant effect of the *Aedes* CI on *Culex* CI, including interaction effects of *Aedes* CI with neighborhood and with year (**Table 5**). The effects of *Aedes* CI on *Culex* CI were positive and strongest in the high (Bolton Hill, slope = 0.8563) and medium (Union Square, slope = 0.876) SES neighborhoods, and significantly different from the effects of *Aedes* CI in the lowest SES neighborhoods (Franklin Square and Harlem Park) that only indicated moderate

positive effects (slopes = 0.4983 and 0.5977, respectively). There was also an overall stronger and significantly different effect of *Aedes* CI on *Culex* CI in 2014 (slope = 0.5134) than in 2015 (slope = 0.4328).

Discussion

Despite the competitive superiority of *Ae. albopictus* to both *Cx. pipiens* and *Cx. restuans*, these mosquitoes continue to coexist in the temperate mid-Atlantic region. The results of this study show limited evidence for condition-specific competition and spatial partitioning between *Ae. albopictus* and resident *Culex* spp. mosquitoes in Baltimore, Maryland.

Seasonal condition-specific competition predicts low abundances of *Ae. albopictus* in the early parts of the summer and rise to a peak in the mid-to-late summer. Results from all three datasets support the seasonal condition-specific hypothesis for *Ae. albopictus* in that seasonal trends show an increase and peak in mid-summer abundances of *Ae. albopictus* from the early summer. Despite the general trend, there is evidence from the ovitraps that there may be yearly differences as the peak abundances of *Ae. albopictus* occurred earlier in 2015 than in 2014 (**Figure 2**). Total *Culex* spp. abundances showed less conclusive evidence that seasonal condition-specific competition is occurring in Baltimore. I predicted early season peak abundances of *Culex* spp. due to earlier adult activity and larval tolerance for cooler temperatures and increasing overall mosquito abundances in the mid-summer. Both container ovitraps and container index analyses showed high variability from year to year. Ovitrap results from 2014 supported my early season prediction, but there was a secondary peak in mid-summer and there was only a

single *Culex* spp. peak in mid-summer 2015 (**Figure 4**), indicating that there are likely other processes influencing mosquito abundances. Higher percentages of total *Culex* spp. were observed in the buckets, however the seasonal effect suggested overall higher abundances in the mid-summer as compared to the early summer. The *Culex* CIs also indicated some evidence of seasonal condition-specific competition, with *Culex* spp. behaving in the same general manner in 2014 and 2015, with peak numbers of *Culex* positive containers occurring in the early summer and decreasing numbers throughout the summer. It should be noted though, that there were significantly higher numbers of *Culex* positive containers in 2015 (**Figure 9**). The differences in seasonal *Culex* spp. abundances could be due to differences in activity for *Cx. pipiens* and *Cx. restuans*. Seasonal oviposition studies of *Cx. pipiens* and *Cx. restuans* studies have shown early seasonal peaks in *Cx. restuans* and mid-summer peak numbers of *Cx. pipiens* (Madder et al. 1980, Lampman et al. 1997, Lee and Rowley 2000, Jackson and Paulson 2006). The crossover between dominance of the two species has been partially attributed to the onset of warmer temperatures (Kunkel et al. 2006). This crossover would explain the two seasonal peaks of *Culex* spp. in 2014.

Yearly differences, especially for *Culex* spp., may be due to climatological changes from year to year, with 2015 having earlier warmer temperatures than 2014 (NOAA 2016), with average early summer temperatures being approximately 2.5°C higher than 2014 during the first collection of 2015, and approximately 3.5°C above the 29 year normal temperatures for May. This temperature change could affect the results of condition-specific competition. The earlier warmer temperatures in 2015

may also suggest that seasonal mosquito activity (traditionally late May to November) could have shifted forward, indicating an earlier peak in *Culex* spp. that may not have been captured by the ovitraps. *Culex* spp. larvae have shown a slight reverse in competitive ability in the laboratory under lower temperatures with the competitive advantage of *Ae. albopictus* being minimized when temperatures are lower than 20°C (Carrieri et al. 2003). This would indicate that *Culex* spp. should show increased abundances early in the season, supporting previous studies showing that both *Cx.restuans* and *Culex p. quinquefasciatus* show adults emerging at temperatures as low as 12°C (Shelton 1973), compared to *Ae. albopictus* which emerges at temperatures closer to 15°C (Delatte et al. 2009). Differences in resident containers, represented by the container indices, show that *Culex* spp. abundances were higher in the early season, particularly in 2015 (**Figure 8** and **Figure 9**). This is supported by the increased early summer temperatures observed in 2015 compared to 2014, especially if *Culex* spp. have earlier ovipositional activity based on their ability to diapause as adults instead of in egg form (Clements 1992). The earlier peak of *Ae. albopictus* abundances from the ovitraps in 2015 compare to 2014 (**Figure 2**) could also be attributed to the warmer mid-summer temperatures, which would support models suggesting that mosquito abundances will continue to increase and peak earlier in the northeastern United States with the onset of climate change (Kraemer et al. 2019).

An alternate explanation for differences in *Culex* spp. abundances could relate to the ovipositional requirements for *Culex* spp. *Culex* spp. eggs are deposited in floating rafts on water surfaces (Vinogradova 2000). Therefore, the success of *Culex*

spp. should be closely tied to wet climatic conditions (Means 1979). Previous studies have shown that larval *Culex* spp. abundances increase under moderate rainfall conditions, while heavy precipitation reduces larval presence through flushing of larvae from their habitats (Gardner et al. 2012). Increased precipitation in a sampling week could significantly decrease the numbers of egg rafts or larvae in an ovitrap, which could explain the results of this study. *Aedes albopictus* abundances have been linked to increased precipitation in the two weeks prior to a sampling event in Baltimore, MD (Little et al. 2017). While the *Ae. albopictus* abundances supported my overall predictions, further investigation of fine scale climatological processes affecting mosquito abundances may assist in explaining temporal variation in urban mosquito abundances.

The data also show limited evidence for spatial partitioning of *Ae. albopictus* from ovitrapping and container indices. Consistent with spatial partitioning, there were overall lower abundances of *Ae. albopictus* in the high SES neighborhood of Bolton Hill throughout the sampling seasons (**Figure 2**). There were higher numbers of *Aedes* spp. positive containers per meter squared in Franklin Square than in any other neighborhood in the mid-summer and those numbers were continued on in the late summer with higher CIs in Franklin Square than in the high SES neighborhood of Bolton Hill, therefore indicating that the low SES neighborhood is associated with higher numbers of *Aedes* spp. positive containers per meter squared than the high SES neighborhood (**Figure 7**). In 2014, *Culex* spp. were highest during the early season peak in the medium SES neighborhood of Union Square, but later in the season abundances were higher in the low SES neighborhoods. In 2015, *Culex* spp.

abundances were highest in Bolton Hill (High SES) during the first collection, however after the first week the highest abundances of *Culex* spp. occurred in the low SES neighborhood of Harlem Park. These results suggest that at certain times of the summer spatial partitioning may be occurring but in an inconsistent manner between years and weeks. Additionally, the follow-up linear models show that in general, *Aedes* spp. abundances are associated with *Culex* spp. abundances across blocks with ovitraps, and among neighborhoods and years with container indices. This suggests that there may be processes working beyond spatial segregation between neighborhoods that may be conducive of increasing numbers of both genera of mosquitoes, such as spatial segregation at the individual container level.

General trends of spatial segregation showed that lower SES neighborhoods supported higher abundances of both *Ae. albopictus* and *Culex* spp. overall; however, they showed that higher SES neighborhoods did not provide a refuge for *Culex* spp. when compared to *Aedes* spp., making a limited case for spatial segregation of mosquitoes among varying SES neighborhoods. This finding is consistent with previous studies showing that low SES neighborhoods have higher mosquito infestation rates (Dowling et al. 2013, LaDeau et al. 2013, Little et al. 2017). The high SES neighborhood of Bolton Hill showed lower overall abundances of both *Aedes* spp. and *Culex* spp. mosquitoes across both ovitraps and container indices compared to the other neighborhoods. The low SES neighborhood of Franklin Square showed overall higher abundances for both mosquito genera based on container indices compared to the other neighborhoods. Some spatial partitioning was observed

throughout the season, but no one neighborhood seemed to provide a refuge to *Culex* spp.

The differences between the lowest SES neighborhood and highest SES neighborhood may be due to differences in habitat availability between blocks, especially as the linear models showed a positive relationship occurring between *Culex* spp. and *Aedes* spp. for both the ovitraps and container indices. There was a neighborhood by *Aedes* spp. interaction that occurred with container indices, which indicated that middle and high SES neighborhoods acted differently than low SES neighborhoods with a stronger positive relationship between the two genera. Previous studies have shown overall trends of high numbers of disused, low-volume containers in low SES neighborhoods and generally lower numbers of container habitats in high SES neighborhoods (Little et al. 2017). Ovipositional preference of mosquitoes may be dependent on the presence of containers in an environment, fewer numbers of resident containers may indicate a lack of options for mosquito oviposition within a neighborhood, therefore increasing the relationship between mosquito genera in neighborhoods with lower habitat availability.

The larger bucket traps showed a higher percentage of *Culex* spp. oviposition than the traditional ovitraps. This supports previous studies showing that *Culex* spp. may preferentially oviposit in larger container habitats (Carrieri et al. 2003, Costanzo et al. 2005). The buckets only showed seasonal effects of mosquito abundances with both *Ae. albopictus* and *Culex* spp. increasing in the mid-summer. Spatial partitioning among neighborhoods was not observed with this dataset, due to the lack of evidence for differing mosquito abundances between neighborhoods. While the results do not

specifically support my hypotheses, larger volume ovitraps may provide better surveillance for *Culex* spp. in the presence of *Ae. albopictus* in urban environments than traditional ovitraps due to ovipositional preference for larger volumes of water.

Increased abundances of both *Aedes* spp. and *Culex* spp. in low SES neighborhoods may have substantial public health implications for residents in these neighborhoods. *Aedes albopictus* is a competent vector for numerous arboviruses, including Dengue, Zika, and West Nile viruses (Gratz 2004, Turell et al. 2005), and both resident *Culex* spp. found in our study area have been implicated in the spread of West Nile virus in the eastern United States (Ebel et al. 2005, Hamer et al. 2008, Kilpatrick et al. 2010, Johnson et al. 2015). West Nile virus transmission has also been associated with lower and middle SES neighborhoods (Ruiz et al. 2007, Harrigan et al. 2010, Rochlin et al. 2011), however entomological data in these studies has been limited to the presence of *Culex* spp. The presence of both *Aedes* and *Culex* mosquitoes in a neighborhood may contribute to increased risk of infection for residents, especially with the generalist feeding patterns of *Ae. albopictus* compared to *Culex* spp. mosquitoes (Savage et al. 1993, Sardelis et al. 2002, Goodman et al. 2018). The increased abundances of mosquitoes in low SES neighborhoods may put the residents disproportionately at risk for mosquito-borne disease when compared to middle and high SES neighborhoods.

In conclusion, I found limited evidence for either condition-specific competition or spatial partitioning as mechanisms of mosquito coexistence in this study. I did observe clear, predictable seasonal patterns of *Ae. albopictus* abundances across all our data sources that is consistent with the seasonal prediction of an

increase from early to mid-late summer. *Culex* spp. abundances varied by year in ovitraps and showed differing patterns from the resident container sampling, thereby indicating that other mechanisms of coexistence are likely occurring. The variability of *Culex* spp. abundances from year to year and across neighborhoods indicates that climate and habitat may play a larger role in their ecology compared to *Ae.*

albopictus, possibly due to overwintering survival or attraction to different container habitats. Consistent with spatial partitioning, I observed lower overall mosquito abundances in the high SES neighborhood, and highest overall mosquito abundances in the low SES neighborhoods; however, patterns of abundance were generally similar across neighborhoods rather than showing potential environmental refuges for *Culex* spp. among neighborhoods. Future studies should examine differences in *Culex* habitat availability in urban environments as well as fine scale climate predictors of mosquito abundance. The disproportionately higher abundances of all mosquito species in low SES neighborhoods highlights the need for targeted vector control in these neighborhoods.

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Error bars removed for clarity.

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Tables

Table 1: Mixed model repeated measures ANOVA results for abundances of *Ae. albopictus* and total *Culex* spp. abundances in standardized ovitraps from Baltimore, MD. Significant results are indicated in bold type.

	<i>Ae. albopictus</i>			<i>Culex</i> spp.		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Year (Y)	1, 23.4	66.94	<0.0001	1, 19.9	2.34	0.1421
Sample Week (SW)	8, 105	322.28	<0.0001	8, 105	27.28	<0.0001
Neighborhood (N)	3, 21.6	23.35	<0.0001	3, 19.2	1.17	0.3485
Y * SW	8, 95.1	8.82	<0.0001	8, 99.4	41.3	<0.0001
Y * N	3, 23.5	1.15	0.3481	3, 20	0.94	0.4409
SW * N	24, 103	2.16	0.0041	24, 103	1.77	0.0263
Y * SW * N	23, 95.1	1.46	0.1035	23, 99.4	3.0	<0.0001

Table 2: Mixed model results of the effect of *Ae. albopictus* on total *Culex* spp. abundances in standardized ovitraps. Significant results are indicated in bold type.

	df	<i>F</i>	<i>P</i>
<i>Ae. albopictus</i>	1, 34.2	4.47	0.0419
Year	1, 212	0.23	0.4096
Neighborhood	3, 208	1.19	0.3132

Table 3: Mixed model ANOVA results for abundances of *Ae. albopictus* and *Culex* spp. in buckets in Baltimore, MD during June and August of 2015. Significant results are indicated in bold type.

	<i>Ae. albopictus</i>			<i>Culex</i> spp.		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Collection	1, 8	164.99	<0.0001	1, 8	8.37	0.0201
Neighborhood	3, 8	2.99	0.0958	3, 8	0.23	0.8746
Collection * Neighborhood	3, 8	2.92	0.1003	3, 8	4.32	0.0435

Table 4: Mixed model repeated measures ANOVA results for *Aedes* spp. and *Culex* spp. standardized container indices. Significant results are indicated in bold type.

	<i>Aedes</i> spp. CI			<i>Culex</i> spp. CI		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Year (Y)	1, 60	0.19	0.6620	1, 60	0.21	0.6514
Collection (C)	2, 60	35.16	<0.0001	2, 60	12.56	<0.0001
Neighborhood (N)	3, 12	13.51	0.0004	3, 12	3.91	0.0368
Y * C	2, 60	1.45	0.2604	2, 60	4.08	0.0218
Y * N	3, 60	1.71	0.2105	3, 60	0.72	0.5418
C * N	6, 60	3.35	0.0092	6, 60	2.15	0.0600
Y * C * N	6, 60	1.82	0.1054	6, 60	2.38	0.0393

Table 5: Mixed model results of the effect of the presence of *Aedes* spp. on *Culex* spp. abundances using standardized container indices (CI). Significant results are indicated in bold type.

	<i>Culex</i> spp. Container Index		
	df	<i>F</i>	<i>P</i>
<i>Aedes</i> spp. Container Index (ACI)	1, 70.9	4.47	<0.0001
Year	1, 58.6	1.11	0.2967
Neighborhood	3, 28.7	5.19	0.0054
ACI * Year	1, 58.6	5.43	0.0233
ACI * Neighborhood	3, 67.6	8.79	<0.0001

Figures

Figure 1:

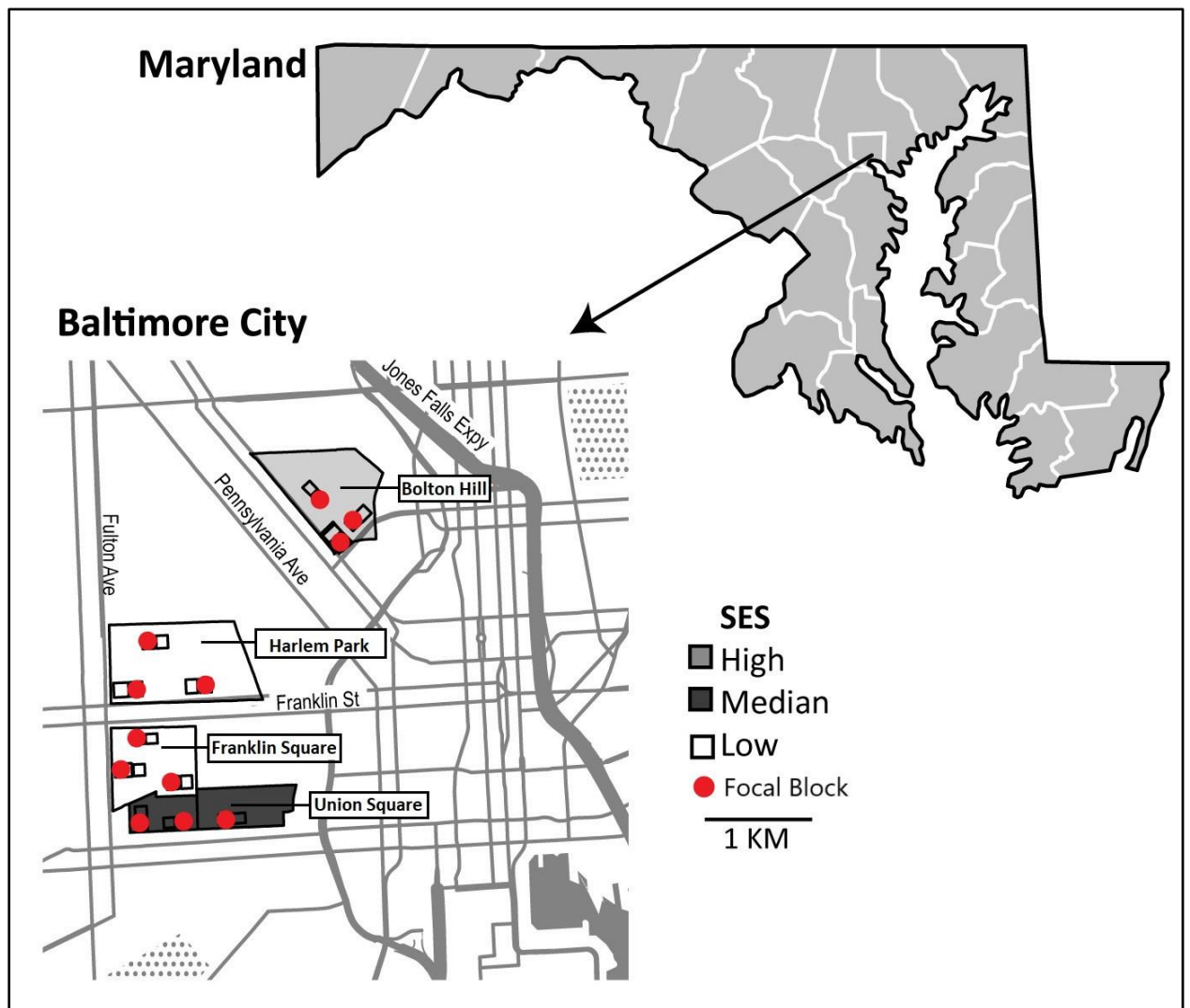


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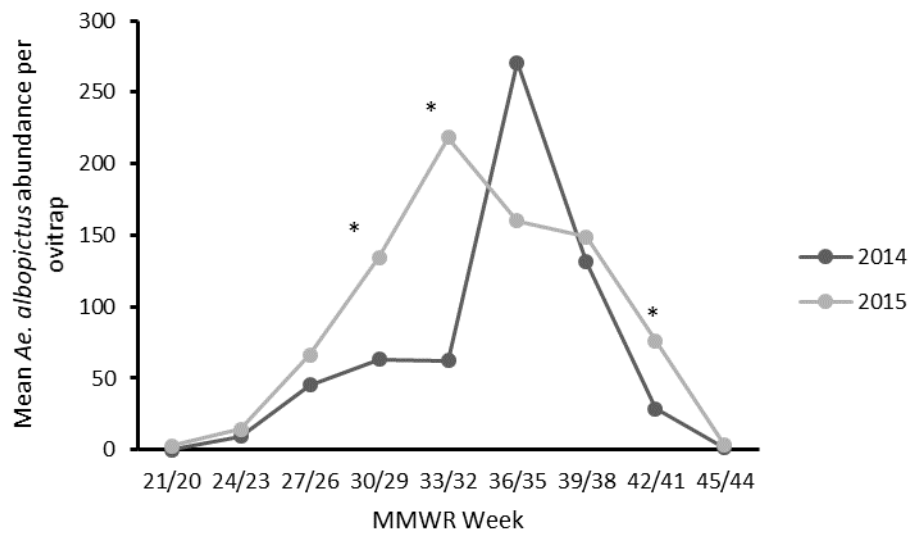


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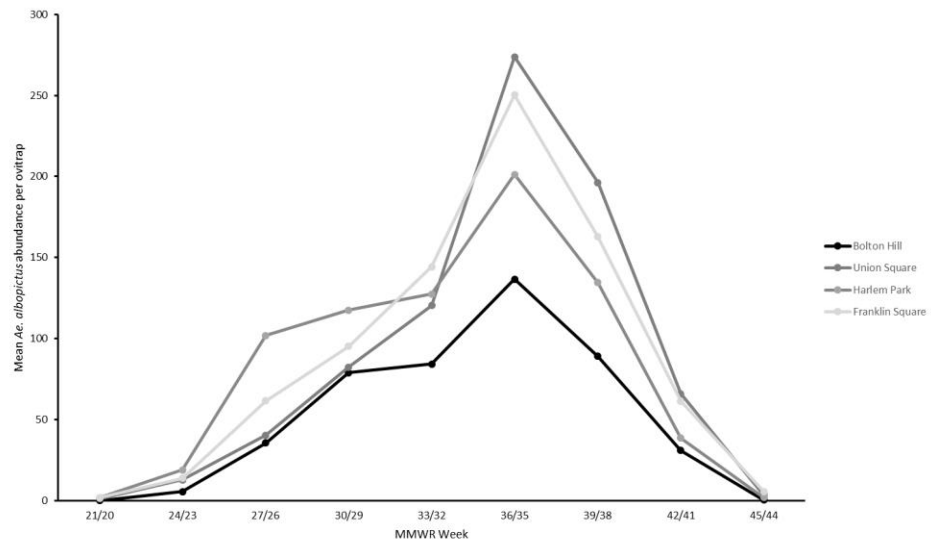
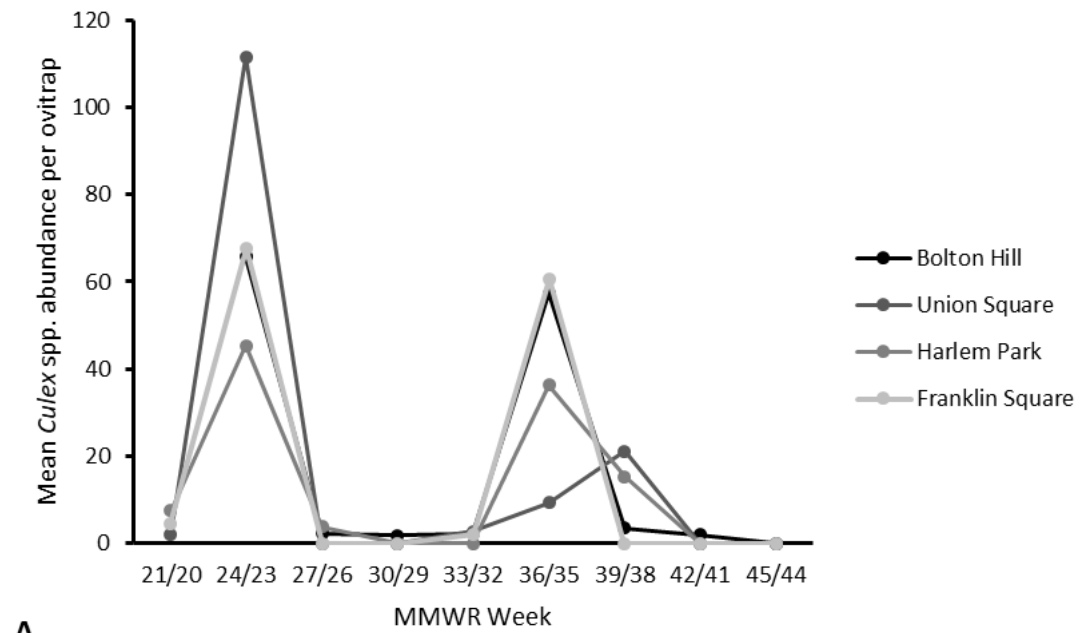
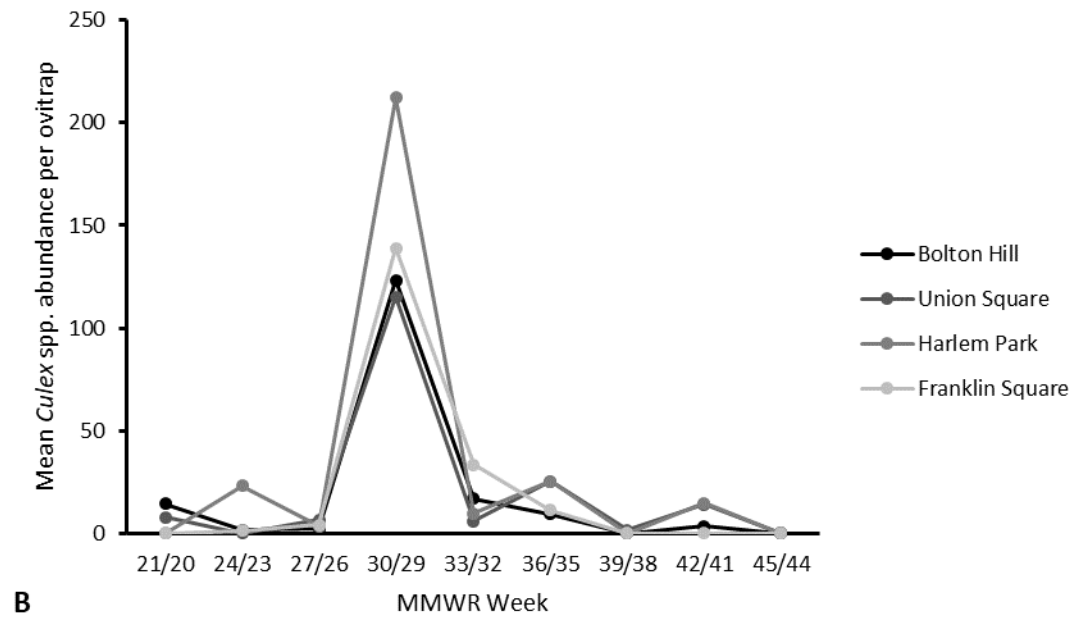


Figure 4:



A



B

Figure 5:

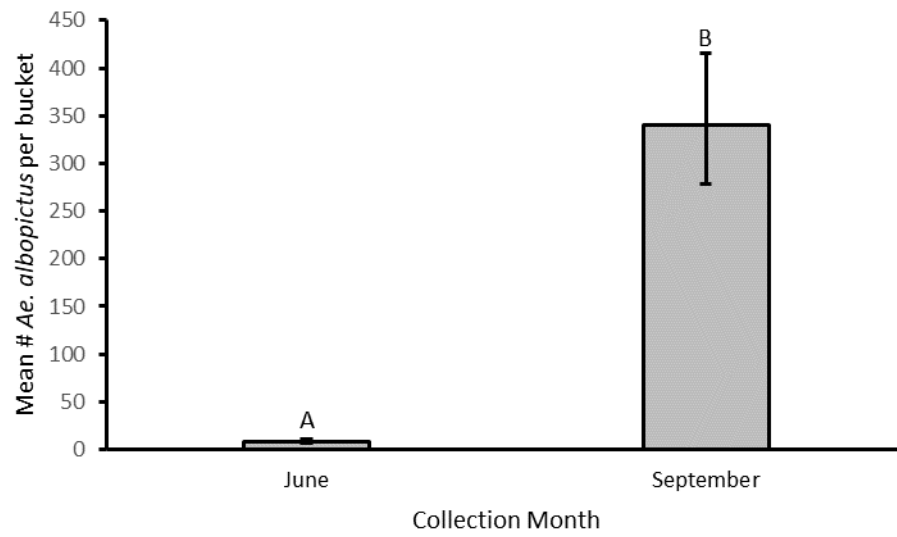


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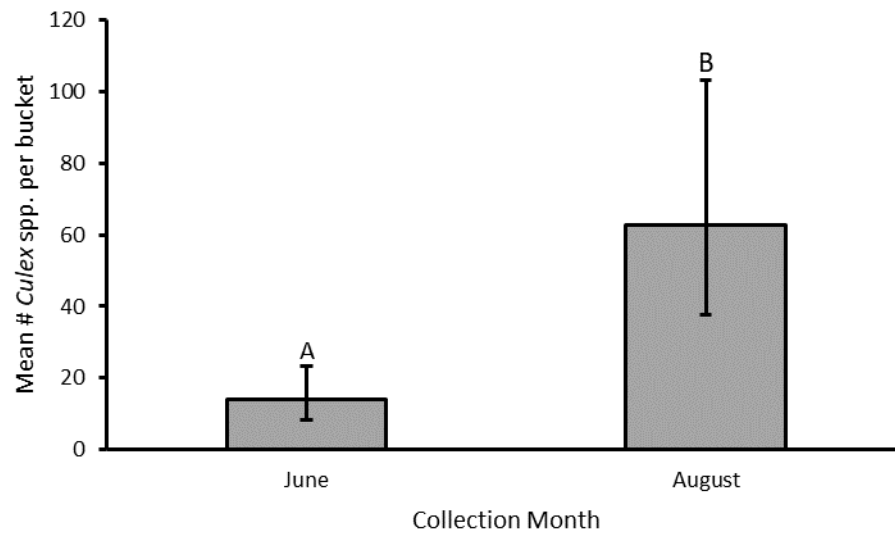


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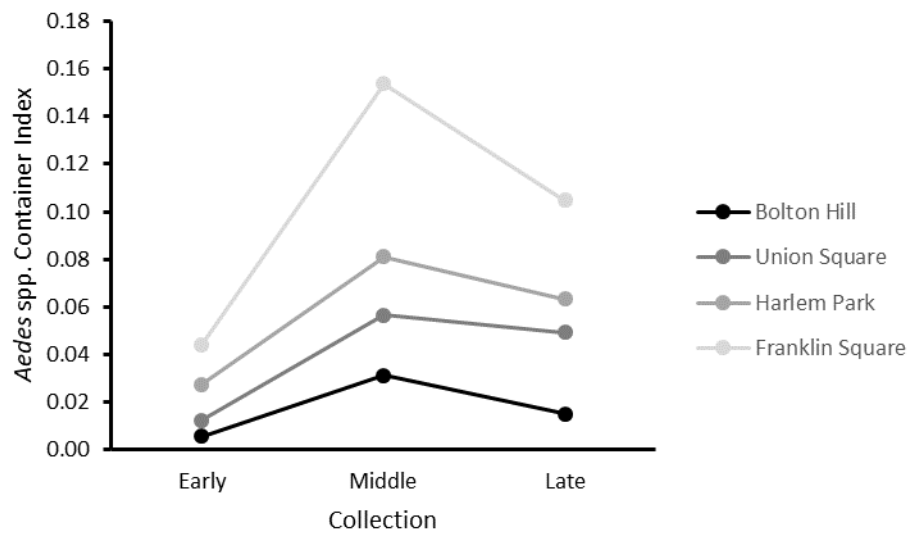
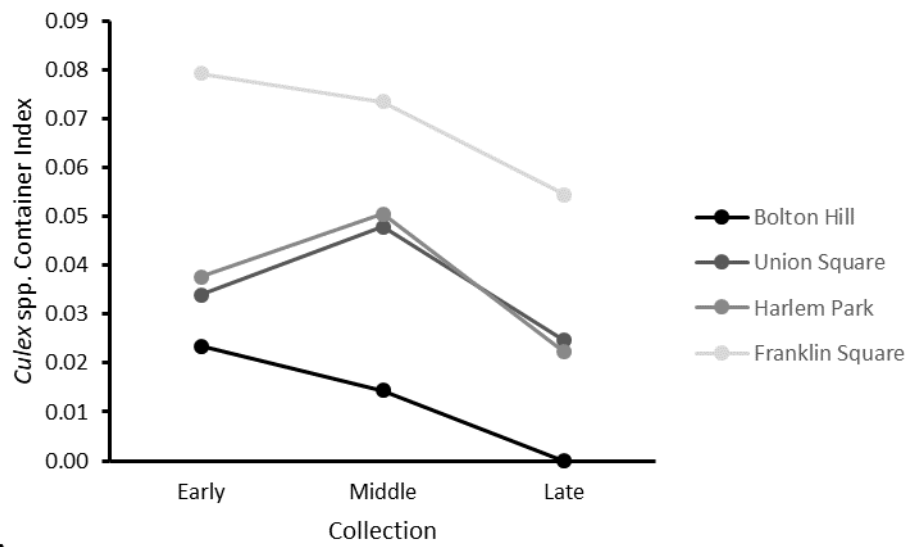
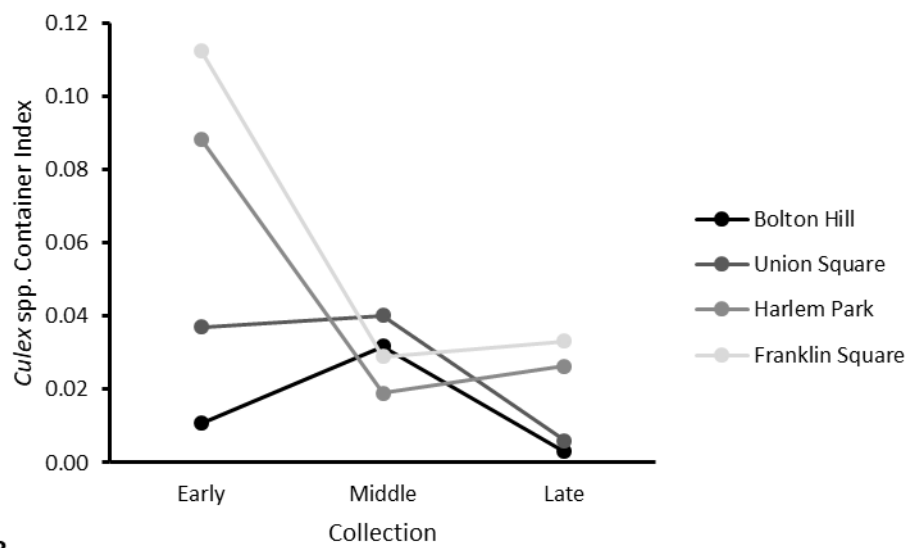


Figure 8:



A



B

Figure 9:

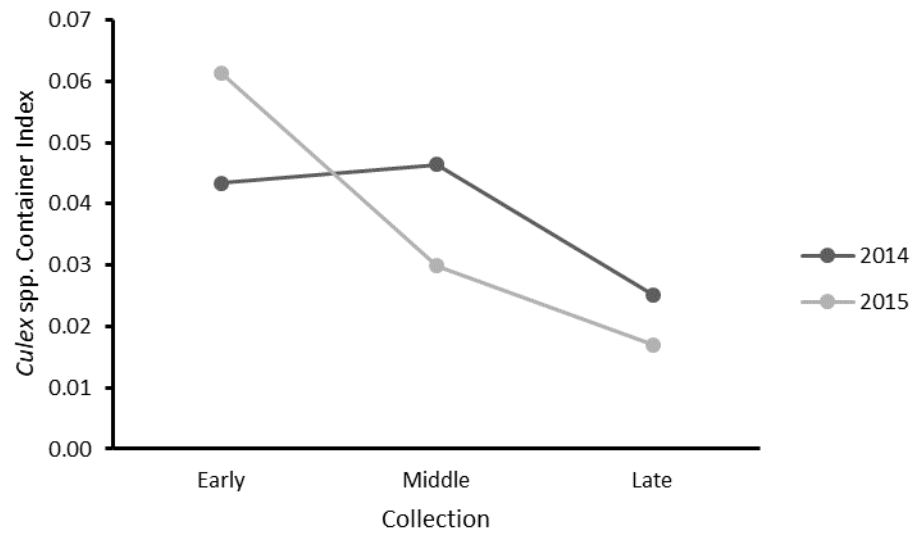
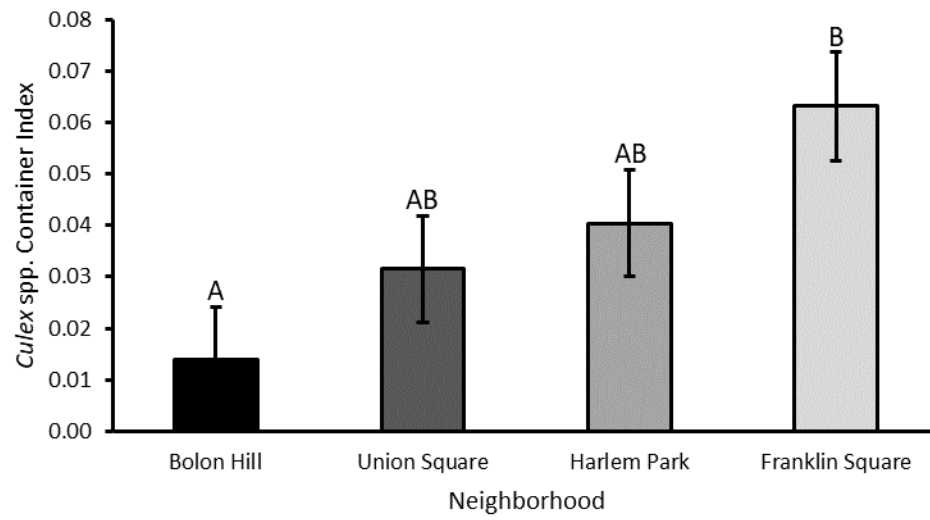


Figure 10:



Chapter 3: A test of the aggregation model of coexistence between *Aedes albopictus* and resident *Culex* spp. in Baltimore, MD

Introduction

Interactions between competing species are often strongly asymmetrical, thereby leading to the exclusion of the weaker competitor (Hardin 1960, Tilman 1982, Chase and Leibold 2003). However, species may escape competitive exclusion via several ecological mechanisms including differential resource use (Tilman 1982), differential susceptibility to predation (Chase and Leibold 2003), condition-specific competition (Tilman 1982, Chesson 2000) and spatial partitioning (Shorrocks et al. 1979). When competing species occupy discrete habitats, spatial partitioning can influence interspecific competition and the likelihood of species coexistence. If a superior competitor's distribution is aggregated among a proportion of available habitats, rather than uniformly or randomly distributed, unoccupied patches can serve as refuges for a weaker competitor. The aggregation model of coexistence predicts that a weaker competitor may regionally persist if intraspecific aggregation increases relative to interspecific aggregation as a result of spatial partitioning (Ives 1988a, b, 1991, Sevenster 1996). If the overlap of the competitors is reduced, the effects of interspecific competition are lessened, therefore allowing for coexistence (Ives 1988a, b, Sevenster 1996). Multiple sources have suggested that if intraspecific aggregation increases relative to interspecific aggregation, competitive interactions will be relaxed and may lead to coexistence (Atkinson and Shorrocks 1981, Ives 1988a, Hartley and

Shorrocks 2002). Aggregation is observed in a number of insect groups that inhabit patchy resources, including drosophilids (Jaenike and James 1991) and carrion flies (Ives 1991), and has been shown to be important to the coexistence of species in these systems.

Following its introduction to the continental United States in the 1980s, the Asian tiger mosquito, *Aedes albopictus* (Skuse), quickly spread throughout the eastern part of the country becoming a common biting pest and vector species in urban temperate environments (Lounibos 2002, Rochlin et al. 2013, Faraji et al. 2014). *Aedes albopictus* utilizes water-filled natural (e.g., tree holes) and artificial (e.g., buckets, vases, birdbaths) container habitats for their developmental stages (eggs, larvae, pupae) where they often compete for limited food resources (detritus and associated microbiota) with resident mosquitoes (Merritt et al. 1992, Barrera 1996, Yee and Juliano 2006). Commensurate with the expansion of *Ae. albopictus* has been the decline of many resident mosquito species consistent with competitive exclusion, and subsequent field and laboratory tests have confirmed that *Ae. albopictus* is competitively superior to almost all other species that it encounters on the North American continent (Barrera 1996, Daugherty et al. 2000, Teng and Apperson 2000, Juliano et al. 2004, Costanzo et al. 2005, Armistead et al. 2008). Nevertheless, in some regions, competitively inferior resident species coexist with *Ae. albopictus*, and various ecological mechanisms have been hypothesized and tested to explain this coexistence.

Aggregation of *Ae. albopictus* among individual container habitats has been hypothesized as a mechanism facilitating the persistence of resident mosquitoes

following the invasion of *Ae. albopictus* but has been rarely studied. The most well-documented impacts of *Ae. albopictus* have been on the yellow fever mosquito, *Aedes aegypti* (L.) in the southeastern United States (Black IV et al. 1989, Braks et al. 2003, Juliano et al. 2004, Rey et al. 2006, Reiskind and Lounibos 2009). *Aedes aegypti* was rapidly displaced by *Ae. albopictus* throughout much of its geographic range in the 1980s and 1990s, but has persisted in some urban areas (O'meara et al. 1995, Comiskey et al. 1999). Aggregation of *Ae. albopictus* and *Ae. aegypti* has been recorded among cemetery vases in Florida (Leisnham and Juliano 2009, Fader and Juliano 2012), and a laboratory experiment revealed that increased intraspecific aggregation of *Ae. albopictus* relaxed the effects of competition on *Ae. aegypti* sufficiently enough to allow species coexistence (Fader and Juliano 2012). Aggregation between *Ae. albopictus* and another established invasive mosquito, *Aedes japonicus* (Theobald) has been documented among used tire and tree hole habitats in temperate urban and peri-urban environments in Maryland (Freed and Leisnham 2014). These few studies suggest that aggregation may be a viable mechanism of coexistence among mosquito species but additional studies are needed to investigate the role of aggregation in other systems, particularly with *Ae. albopictus* and other co-occurring species in different genera.

The geographic range of *Ae. albopictus* in the United States overlaps with that of several resident *Culex* spp., including *Culex pipiens* L. and *Culex restuans* (Theobald) (Reiskind and Wilson 2004, Yee et al. 2004, Costanzo et al. 2005). While competitively inferior to *Ae. albopictus* at the larval stage (Carrieri et al. 2003, Costanzo et al. 2005, Costanzo et al. 2011), these *Culex* spp. frequently coexist in

temperate urban environments and are known to utilize similar larval habitats, such as abandoned tires and buckets (Carrieri et al. 2003, Costanzo et al. 2005). In addition to its ecological importance, regional coexistence of *Cx. pipiens* and *Cx. restuans* with *Ae. albopictus* has important implications for disease transmission. *Culex pipiens* and *Cx. restuans* are the principal vectors of West Nile virus (WNV) in the eastern United States, amplifying WNV among local avian populations, but rarely biting humans (Apperson et al. 2004, Kilpatrick et al. 2005, Kilpatrick et al. 2010). Their coexistence with *Ae. albopictus* is likely to increase human WNV transmission as the invasive mosquito is an efficient bridge vector of the virus from bird to human populations (Turell et al. 2001, Sardelis et al. 2002, Turell et al. 2005, Farajollahi and Nelder 2009, Faraji et al. 2014).

In this chapter, I investigate the role of aggregation in the coexistence between the invasive mosquito *Ae. albopictus* with resident *Cx. pipiens* and *Cx. restuans* in four neighborhoods in Baltimore, Maryland (USA). I sampled *Ae. albopictus*, *Cx. pipiens*, and *Cx. restuans*, in both existing container habitats and experimentally positioned oviposition containers in each neighborhood to determine if they were associated and aggregated, and if their aggregation may be sufficient to support their coexistence. Sampling was conducted over two active mosquito seasons (May-November) of 2014 and 2015 to determine if aggregation varied with changing seasonal conditions. I also conducted a controlled laboratory competition experiment that manipulated the aggregation of the competitively superior *Ae. albopictus* to test if intraspecific aggregation of *Ae. albopictus* could relax the effects of interspecific

competition on the competitively inferior *Cx. pipiens* and increase the likelihood of persistence in these discrete container habitats.

Materials & Methods

Field surveys

Field sampling was conducted in 2014 and 2015, between May and November of each year, when adult mosquitoes are active in the region. Four neighborhoods (Franklin Square, Harlem Park, Union Square, and Bolton Hill) in West Baltimore, Maryland were chosen because they were relatively equidistant from the Baltimore Harbor and from large forested parks and included a broad range of socioeconomic status (SES) conditions as described in Little et al., 2017. Following Little et al. (2017), I categorized study neighborhoods along a SES gradient relative to median household income. For the purposes of this study, Franklin Square and Harlem park were classified as low SES neighborhoods; Union Square was a medium SES neighborhood; and Bolton Hill a high SES neighborhood (**Figure 1**), based on the median household income level reported by Baltimore City (<https://bniajfi.org/>). Three study blocks were randomly chosen to give 12 total blocks for this study. All study blocks are comprised of attached row homes, with an average block area of eight acres. Neighborhoods were identified using online data from Baltimore City and the US Census Bureau (<http://bniajfi.org/> and <https://www.census.gov>). The occupancy of each block and neighborhood was also assessed, as a previous study showed occupancy to be a strong predictor of mosquito infestation (Little et al. 2017). The highest SES neighborhood, Bolton Hill, had an average of 97.4% (94.4 – 100.0) occupancy over the two-year study period, the middle SES neighborhood, Union

Square, showed an average of 80.4% (68.5 – 93.3) occupancy, and the low SES neighborhoods, Harlem Park and Franklin Square, averaged 39.4% (23.5 – 55.6) occupancy over the two years. The low SES neighborhoods showed the most variation in occupancy with Franklin Square having an average of 46.4% occupancy and Harlem Park averaging 32.4% occupancy during the study period, suggesting potential differences of suitability in habitat for mosquitoes even in neighborhoods with similar SES indicators.

Existing container habitats were surveyed during three survey periods in each year corresponding to early (June), middle (July/August), and late (September) summer. Each survey period took approximately five days utilizing teams of trained personnel, employing similar methodology to past studies (e.g. Dowling et al. 2013, Bodner et al. 2016, Little et al. 2017). All privately-owned and public parcels in each study block were thoroughly searched for all water-holding containers that could be sampled unless access was restricted by the occupant. Information on parcel type, container type, container volume, and larval presence/absence was also collected. If larvae were present and the container was accessible, the water was homogenized and a sample was taken. Larvae were returned to the University of Maryland, College Park for identification. Larvae, pupae, and adults in the samples were enumerated and identified to the genus or species level dependent on the life stage of the organism (Bodner et al. 2016).

In addition to surveying existing containers, which reflects not only female oviposition but the outcome of biotic and abiotic interactions of larvae and eggs within habitats that likely vary in carrying capacity, I also sampled populations using

standardized oviposition traps, which measure pre-interaction habitat choice. Black 600-mL oviposition cups were deployed in each study block every three weeks from May through November for a total of nine collection weeks each year. During each collection, three cups were placed in each of the two halves of each block, giving a total of six cups per block (see **Chapter 2** for details).

Oviposition cups were lined with seed germination paper (Nasco Science, Fort Atkinson, WI) and baited with 500 mL of 10:1 Timothy Hay infusion water. For each collection event, oviposition cups were collected after seven days of deployment and brought back to the laboratory. Any larvae in the cups were identified to species and enumerated. If *Culex* species egg rafts were present they were hatched, identified, and numbers estimated to the nearest 25 individuals. Seed germination paper was stored in 90% humidity for 5 days prior to being flooded with a nutrient broth to hatch *Aedes* eggs. Larvae were reared to 4th instar and then identified and enumerated in the laboratory (Leisnham and Juliano 2009).

Aggregation of *Ae. albopictus*, *Cx. pipiens*, and *Cx. restuans* identified in the ovitraps was calculated among ovitraps on each study block for each of the 18 total sample weeks. Aggregation was assessed by calculating the index of intraspecific aggregation (J), which is the proportionate increase in the average number of conspecifics found in a patch relative to the number of competitors per container expected of a random distribution; an index of interspecific aggregation (C), which is proportionate increase in the average number of heterospecifics found in a patch relative to the number expected of a random distribution; and an index of relative aggregation (T), which is the relative strength of intraspecific vs. interspecific

aggregation (Ives 1991, Sevenster 1996). The T index indicates whether competitor x could persist given the aggregation of the stronger competitor (J_y) and the association of the two species (C_{xy}), with a $T_{xy} < 1.0$ being necessary and sufficient for coexistence (Sevenster 1996) (see details in **Appendix A**). For both *Cx. pipiens* and *Cx. restuans* on each block, I calculated the percentage of sampling weeks for which there were coexistence conditions ($T_{xy} < 1.0$). An index of relative aggregation (T) could not be calculated for *Cx. pipiens* or *Cx. restuans* for some collections on each block. This was almost always because there were no *Culex* spp. sampled for those collections. There were 15 instances when *Ae. albopictus* was absent from a collection and T could not be calculated, which occurred at the extremes of the sampling season.

Laboratory experiment

Experimental units consisted of 80 first instar larvae per species distributed among eight experimental microcosms that consisted of 400 mL tri-pour beakers with 350 mL distilled water provisioned with 0.7 ± 0.002 g senescent *Quercus alba* (white oak) leaf litter. Because I am interested in the effect of varying *Ae. albopictus* aggregation on the population performance of *Cx. pipiens*, *Ae. albopictus* density was manipulated across the eight microcosms within in each unit. *Cx. pipiens* density remained constant across the 8 microcosms with 10 larvae each. Each replicate consisted of a control treatment with zero *Ae. albopictus* larvae, and four aggregation treatments as follows: uniform (10, 10, 10, 10, 10, 10, 10, and 10 larvae/microcosm), low (20, 20, 20, 20, 0, 0, 0, and 0 larvae/microcosm), medium (40, 40, 0, 0, 0, 0, 0,

and 0 larvae/microcosm), and high (80, 0, 0, 0, 0, 0, 0, and 0 larvae/microcosm) aggregation of *Ae. albopictus* (Fader and Juliano 2012). These aggregation treatments represented a range of *Ae. albopictus* aggregation (J_{al} = -0.1 to 6.9) which were broadly representative of *Ae. albopictus* intraspecific aggregation observed in the field (see **Appendix B**). This experiment was conducted with five replicates for a total of 200 individual microcosms. Microcosms were conditioned for three days prior to the introduction of newly hatched (<24-hr old) larvae.

Larvae were sourced from *Ae. albopictus* and *Cx. pipiens* colonies (F1-3 generations) housed at the University of Maryland, College Park. Eggs were synchronously hatched in a solution of 0.15 g of bovine liver powder per liter of distilled water. Within 24 hours of hatching, larvae were rinsed and transferred into the microcosms. Treatments were randomly assigned to microcosms, and microcosms were randomly assigned to six shelves within two environmental chambers that were both set at 26°C with a 14:10 light:dark photoperiod to mimic mean summer conditions in the region. Microcosms were systematically shuffled among shelves and chambers daily to control for any variations between the shelves and chambers. Pupae were removed from microcosms daily and placed into individual vials until adult emergence. Emergence date, sex, and species were recorded for each adult mosquito. Following emergence mosquitoes were dried (>24h, 50°C) and wing lengths were measured as a proxy for fecundity. Proportion survivorship, median female development time, and median female wing length were calculated for both species in each replicate aggregation treatment of 80 larvae. These fitness parameters were used to calculate the finite rate of population increase (λ') (Livdahl and Sugihara 1984,

Juliano 1998). Leaf litter (0.7 g) was added to each microcosm on days 7, 14, and 21 to avoid total depletion of nutrients and to mimic regular additions in the field.

Statistical analyses

Associations in the occupancy (presence/absence of larvae) of *Aedes* spp. and *Culex* spp. among resident container samples was assessed using Mantel-Haenszel tests on multiple 2x2 tables (Sokal and Rohlf 1995). Tests were conducted across blocks within each neighborhood as well as after combining neighborhoods for each of the three survey periods in both 2014 and 2015 to yield 24 and 6 total tests, respectively. Mantel-Haenszel tests control for differences in block effects even when the individual blocks contain few observations - provided the overall sample size is adequate.

Four 1-way ANOVAS for each study year were used to test for differences among neighborhoods in the percent of total ovitrap collections where coexistence conditions were met ($T_{xy} < 1.0$) for *Cx. pipiens* and *Cx. restuans* in each study year.

The effect of aggregation of *Ae. albopictus* on *Cx. pipiens* in the laboratory experiment was analyzed using one-way ANOVAs. Proportion female survivorship, median female development time, and median female wing length were analyzed for differences between aggregation treatments for both *Ae. albopictus* and *Cx. pipiens*. The finite rate of population increase (λ') was calculated using proportion female survivorship, median female development time, and median wing length and was then $\log_{10}(y+1)$ transformed to meet assumptions of normality and homogeneity of variances and analyzed for differences between aggregation treatments. *Culex*

pipiens, λ' did not meet assumptions of normality and homogeneity of variances even after various transformations, thus a non-parametric randomization wrapper was used to test for significance between treatments (Cassell 2002).

In addition to ANOVAs, linear models were used to test for a linear relationship between *Ae. albopictus* and *Cx. pipiens* λ' . Intraspecific levels of *Ae. albopictus* aggregation (J_{al}) were calculated for each aggregation treatment (uniform, low, medium, high) and treated as a continuous variable. I then tested to see if the slope was significantly different from zero to test for the effect of aggregation of *Ae. albopictus* on *Cx. pipiens* λ' . All analyses were done using SAS 9.4 PROC GLM or PROC MIXED (SAS 2016) with an experiment-wise $\alpha = 0.05$.

Results

Field surveys

A total of 2,103 existing containers were surveyed in the study. A total of 59.1% (n=1,242) of the water-holding containers came from 2014, while 2015 rendered 42.0% (n=861). The two low SES neighborhoods, Franklin Square and Harlem Park, provided 65.9% (n=818) of all water holding containers in 2014, and they provided 72.5% (n=624) of water holding containers in 2015. Mosquito larvae were found in 34.8% (n=731) of the containers. *Aedes* spp. larvae were found in 65.7% (n=480) of the positive containers, while *Culex* spp. were found in 44.7% (n=327) of the positive containers. Both genera were present in 25.0% (n=183) of the positive containers.

For all survey events, the combined percentage of total containers with either only *Aedes* or *Culex* mosquitoes was higher than the percentage of containers with both *Aedes* and *Culex*, indicating a negative association between the two genera. A total of 14 of the 24 tests within the four neighborhoods and all six tests combining neighborhoods confirmed significant negative associations ($p < 0.05$) (**Table 1**). Negative associations between the genera were observed more frequently in 2014 than in 2015. *Aedes* and *Culex* spp. were negatively associated during all six survey periods in the low SES neighborhood of Harlem Park, whereas they were only negatively associated in one survey period (July 2014) in the highest SES neighborhood Bolton Hill. The proportions of containers with *Culex* spp. were higher early in the season, whereas the proportion of containers with *Aedes* were higher in the middle and later parts of the season. Nevertheless, during all survey periods, a substantial percentage of the containers a genus occupied were in isolation to the other genus (**Table 1**).

When J , C , and T indices were calculated, there was no clear pattern of independent aggregation for *Ae. albopictus* with either *Cx. pipiens* or *Cx. restuans* among blocks (**Table 2**). However, an expectation of *Culex* spp. coexistence with *Ae. albopictus* was observed (i.e. $T_{xy} < 1.0$) for some weeks in every block throughout the study (see **Appendix B**), except for block 3 in Franklin Square, which showed no probability of coexistence between *Cx. restuans* and *Ae. albopictus* in 2014 and no probability of coexistences between *Cx. pipiens* and *Ae. albopictus* in 2015 (**Table 2**). ANOVAs showed no significant effects of neighborhood on the likelihood of *Culex*

spp. coexistence with *Ae. albopictus* when averaging across blocks, indicating that all neighborhoods were acting in a similar manner.

Laboratory experiment

ANOVAs did not show any significant effects of *Ae. albopictus* aggregation on female *Cx. pipiens* survival ($F_{4,20} = 2.0$, $p = 0.1333$), female development time ($F_{4,19} = 1.01$, $p = 0.4290$), female wing length ($F_{4,19} = 0.86$, $p = 0.5061$), or λ' ($F_{4,20} = 0.91$, $p = 0.4796$) (**Figure 2**). *Cx. pipiens* survival was highest in the medium and high aggregation treatments, despite no significant effects of treatment. The uniform and low aggregation treatments resulted in $\lambda' < 1.0$ for *Cx. pipiens*, indicating that these populations would be expected to decline, while the control, medium, and high treatments yielded mean $\lambda' > 1.0$. Trends, while not significant, did show slight increases in performance of *Cx. pipiens* with increasing *Ae. albopictus* aggregation. The randomization test for main effects of treatment showed a significance level of 0.1810, indicating no differences between treatments.

Results from the linear model treating *Ae. albopictus* aggregation as a continuous variable was not significant with $R^2 = 0.14$. The slope was not significantly greater than zero ($F_3 = 0.83$, $p = 0.4965$), indicating a lack of a positive linear relationship between $J_{Ae. albopictus}$ and *Cx. pipiens* λ' .

Ae. albopictus performance was negatively affected by its own aggregation (**Figure 3a**). There were significant effects of treatment for female survival ($F_{3,16} = 46.17$, $p < 0.0001$). The high aggregation treatment was significantly lower than all other treatments. Development time for *Ae. albopictus* females also showed a

significant treatment effect ($F_{3,16} = 96.67$, $p < 0.0001$). The uniform treatment showed the most rapid development time while the high aggregation treatment showed the slowest development time (**Figure 3b**). Median female wing length showed significant differences between treatments ($F_{3,16} = 5.77$, $p = .0072$). Females from the high aggregation treatment showed significantly greater wing lengths than those in the low and medium aggregation treatments, although there was no difference between the high and uniform treatments (**Figure 3c**). Population performance (λ') for *Ae. albopictus* showed significant differences between aggregation treatments ($F_{3,16} = 107.85$, $p < 0.0001$). There were significant differences between all treatments, with λ' decreasing as aggregation increased (**Figure 3d**). Despite the decreasing population performance as aggregation increased, *Ae. albopictus* λ' remained above 1.0, indicating that the population would increase.

Discussion

Despite the establishment of the competitively superior *Ae. albopictus*, *Cx. pipiens* and *Cx. restuans* continue to coexist with the invasive species in the mid-Atlantic region of the United States. My study provided some evidence that aggregation of *Ae. albopictus* in larval habitats is a mechanism of coexistence for these species. Among standardized ovitraps in the field, there was little evidence for consistent egg aggregation, indicating that other mechanisms of coexistence may be occurring. Among resident containers, there was evidence of independent aggregation, especially in the first year of my study (2014). My laboratory study, which manipulated aggregation treatments, showed that there was little effect of aggregation of *Ae. albopictus* on the survival, development time, or population

performance of *Cx. pipiens*, while consistent with other studies, *Ae. albopictus* was negatively impacted by increased intraspecific competition (Costanzo et al. 2005, Fader and Juliano 2012).

Standardized ovitrapping showed that there were no consistencies over time or spatially among neighborhoods that supported the aggregation hypothesis as a mechanism for coexistence. While conditions supporting coexistence were present at times during the two years (**Table 2**) there was insufficient evidence to support aggregation as a driver of coexistence between *Ae. albopictus* and *Culex* spp. *Aedes albopictus* is known to demonstrate skip oviposition, where eggs from a single gonotrophic cycle are distributed among multiple oviposition sites, and in the presence of multiple high-quality sites a female is more likely to evenly distribute her eggs among the sites (Davis et al. 2015, Fonseca et al. 2015). The use of ovitraps for the surveillance of *Ae. albopictus* is well documented (Silver 2007); however, this method of surveillance may not truly replicate the conditions that would lead to egg aggregation in nature, especially in the presence of the inferiorly competitive *Cx. pipiens*. Another possible explanation for the lack of aggregation could be the presence of superior habitats for *Cx. pipiens* oviposition in the focal blocks. Studies have shown that *Cx. pipiens* larvae are frequently found in larger container habitats, such as tanks and storm water catch basins (Vinogradova 2000, Carrieri et al. 2003, Gardner et al. 2012), which may be more attractive than standardized ovitraps to ovipositing females.

Resident container sampling showed that in several neighborhoods *Aedes* spp. and *Cx.* spp. were negatively associated, suggesting that while there may not be

independent aggregation in the standardized ovitraps, the conditions present in resident containers may affect larval interactions over time causing a negative association. Studies in Florida cemeteries have shown that resident containers can vary greatly in their biotic and abiotic conditions (O'meara et al. 1995) which in turn may favor one species over another. It is likely that oviposition choice depends on species specific environmental cues and that these cues may be independent, contributing to the observed aggregation (Chesson 2000). Studies have shown that *Cx. pipiens* is more likely to be found in larger containers (Carrieri et al. 2003, Costanzo et al. 2005), and tends to perform better in the presence of quickly decomposing nutrient resources (Costanzo et al. 2011), while *Ae. albopictus* is frequently found in smaller containers (Carrieri et al. 2003) and performs well in the presence of most nutrient sources, including those with slow decay rates and low nutrient content (Murrell and Juliano 2008, Costanzo et al. 2011).

While previous studies have shown no association between SES and mosquito habitat abundance per yard, disused or trash containers are more frequently found in low SES neighborhoods and are likely to be unmanaged, thereby leading to high mosquito production (Dowling et al. 2013, Little et al. 2017). In my study, independent aggregation in resident containers was most frequently observed in the low SES neighborhoods that produced the most container habitats, suggesting that the increased availability of oviposition sites may contribute to aggregation. The high SES neighborhood showed the least amount of independent aggregation and had the least number of container habitats. Aggregation may be more likely to occur when there are more oviposition sites available to mosquitoes. More water holding

containers were identified in neighborhoods in 2014 than 2015, indicating that there may be climatological differences between the two years.

The results of my laboratory study demonstrated that aggregation of *Ae. albopictus* did not significantly affect the survival, fitness, or rate of population increase of *Cx. pipiens*. Survival of *Cx. pipiens* in the study never rose above 20%, indicating that the mosquitoes were generally poor competitors in this system. This is consistent with the *Cx. pipiens* survival under a single source elm leaf treatment used by Costanzo et al. (2011) in competition experiments between *Ae. albopictus* and *Cx. pipiens*. In this study's experimental microcosms, I used white oak leaf litter as the food source as it is a common detrital element in the region (Trexler et al. 1998, Freed and Leisnham 2014); however, previous studies have shown that slowly decomposing detritus negatively affects survivorship of *Cx. pipiens* (Costanzo et al. 2011). Future studies with different forms of detritus may show shifts in the population of *Cx. pipiens*. *Aedes albopictus* survivorship was not impacted by the use food source, but was affected by the increased aggregation treatments, which is in agreement with other studies investigating intraspecific competition among *Ae. albopictus* (Costanzo et al. 2005, Fader and Juliano 2012).

The finite rate of population increase (λ') for *Cx. pipiens* hovered around or below 1.0 for all treatments, with slightly lower numbers for the uniform and low aggregation treatments, indicating that though survival was poor the population would be expected to persist. The lack of significant differences between treatments does not support aggregation as a mechanism of coexistence between *Cx. pipiens* and *Ae. albopictus*. Increasing aggregation did affect λ' for *Ae. albopictus*, with

increasing aggregation negatively affecting the performance. This result is consistent with previous studies; however, the relative effect of aggregation was not as extreme in this system as it was when *Ae. albopictus* was in competition with *Ae. aegypti* (Fader and Juliano 2012), with λ' remaining well above 1.0 for all treatments. The differences between the two species responses to the treatments supports previous evidence that *Cx. pipiens* is an inferior competitor to *Ae. albopictus* (Carrieri et al. 2003, Costanzo et al. 2005).

This study shows that there is some evidence for aggregation being a driving force of coexistence between *Ae. albopictus* and *Cx. pipiens* in the eastern United States. While aggregation has been suggested as a mechanism to support coexistence of other mosquito species in direct competition (Fader and Juliano 2012), this study has shown that the aggregation of *Ae. albopictus* in this system, along with its indiscriminate usage of container habitats, does not influence coexistence at the block level in Baltimore, MD. Aggregation was observed in resident containers and may contribute to mosquito coexistence at the landscape or regional level although other forms of spatial segregation may also be contributing to local persistence of species (Rey et al. 2006). While *Ae. albopictus* aggregation did not affect the measures of performance of *Cx. pipiens* in this study, it did show that *Cx. pipiens* is a relatively poor competitor both alone and in the presence of *Ae. albopictus*, with little variation in λ' under all experimental conditions. In concurrence with previous studies, *Ae. albopictus* was negatively affected by increased intraspecific competition, but not to the same level as when in direct competition with species such as *Ae. aegypti* (Costanzo et al. 2005, Fader and Juliano 2012). While there was limited evidence of

competition being relaxed for *Cx. pipiens* by the aggregation of *Ae. albopictus*, this study has shown that it may be one of many non-mutually exclusive spatial and temporal mechanisms contributing to the regional persistence of these vector species.

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Figure 1:

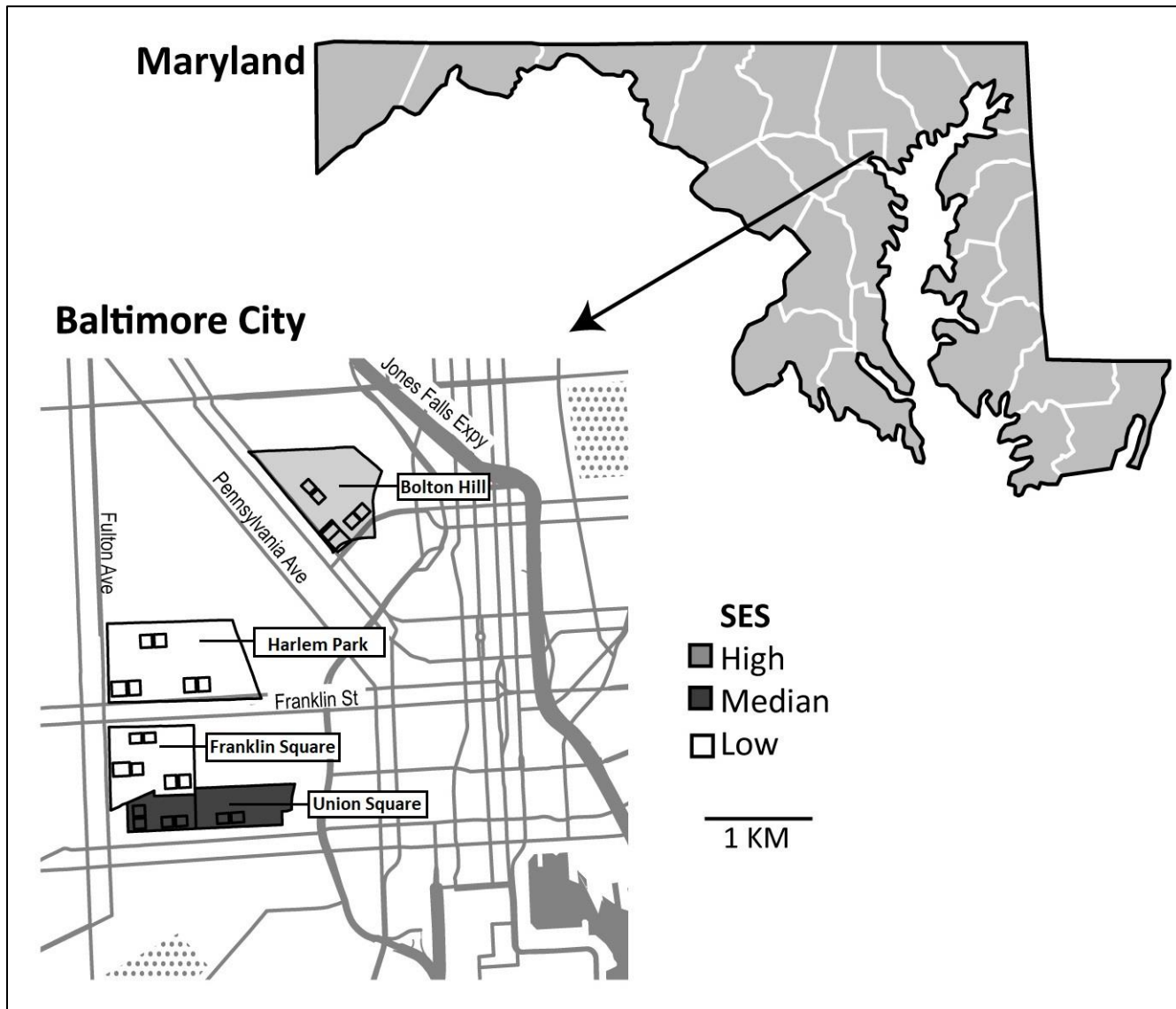


Figure 2:

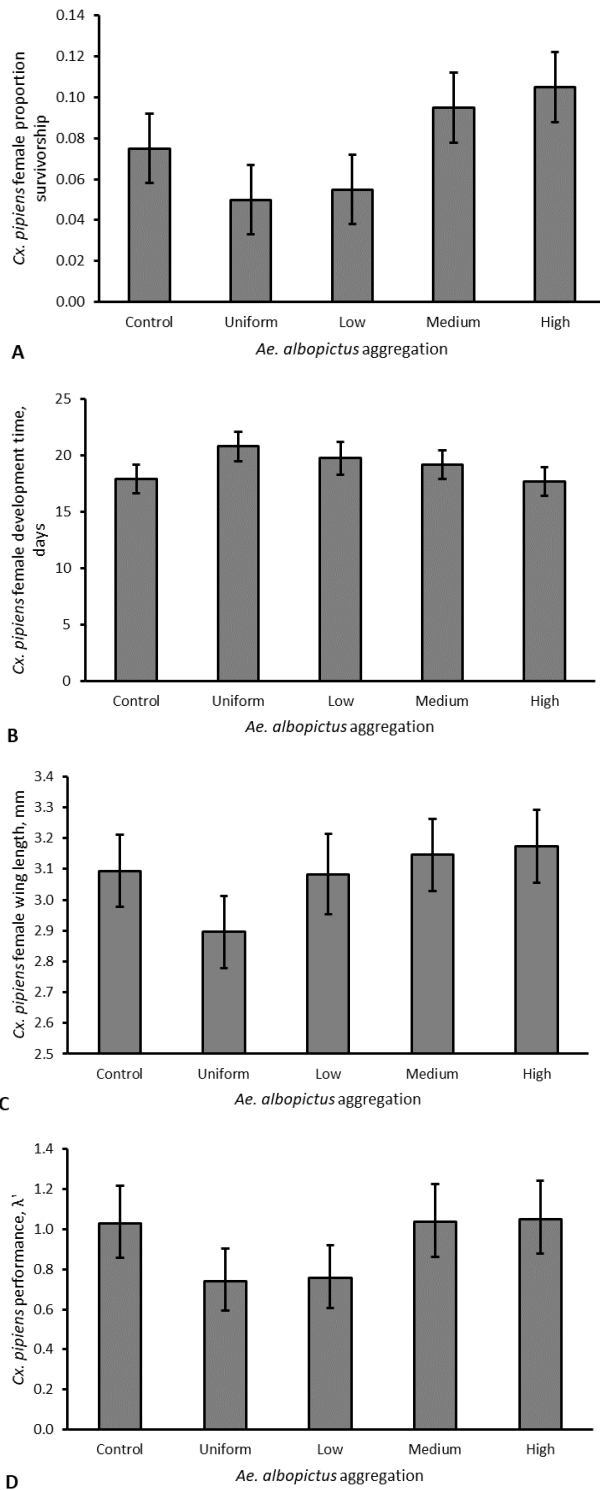
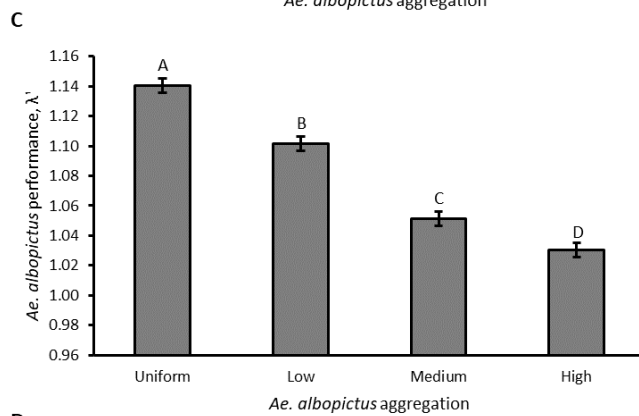
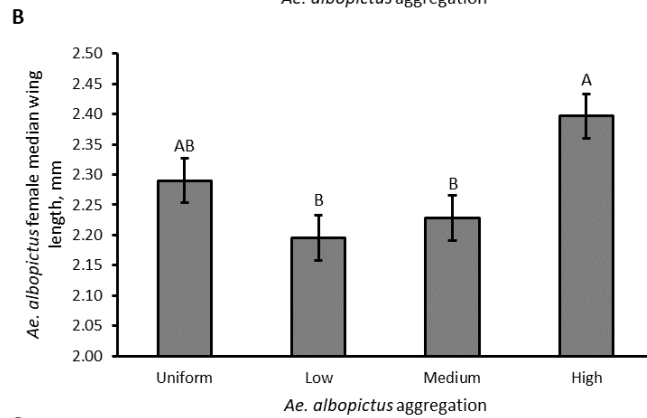
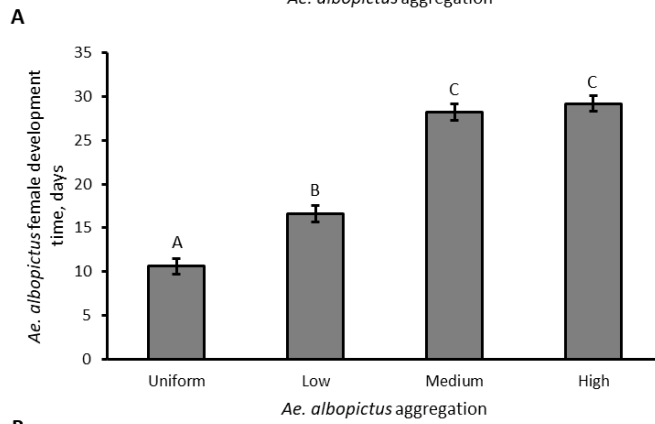
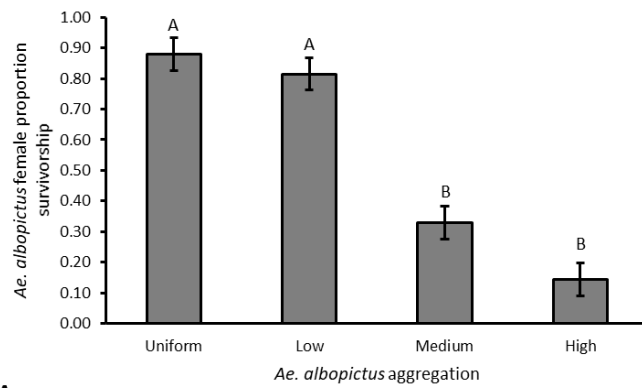


Figure 3:



D

Ae. albopictus aggregation

Tables

Table 1: Association of *Aedes* spp. and *Culex* spp. using Mantel-Haenszel tests for resident containers. For Mantel-Haenszel tests, $df=1$. There was insufficient data for a test of containers in Bolton Hill in late 2014.

Year	Season	Neighborhood	<i>Aedes</i> only (%)	<i>Culex</i> only (%)	Both (%)	None (%)	X^2	p
2014	Early	Franklin Square	6 (3.1)	22 (11.2)	6 (3.1)	162 (82.7)	11.08	0.0009*
		Harlem Park	3 (2.6)	10 (8.7)	5 (4.3)	97 (84.3)	10.25	0.0014*
		Union Square	2 (2.6)	9 (11.8)	2 (2.6)	63 (82.9)	0.74	0.3910
		Bolton Hill	0 (0.0)	6 (11.8)	2 (3.9)	43 (84.3)	1.22	0.2700
		Total	11 (2.5)	47 (10.7)	15 (3.4)	365 (83.3)	29.17	<0.0001*
	Middle	Franklin Square	43 (26.5)	5 (3.1)	21 (13.0)	93 (57.4)	16.04	0.0001*
		Harlem Park	19 (26.8)	2 (2.8)	19 (26.8)	31 (43.7)	14.45	0.0001*
		Union Square	7 (7.7)	5 (5.5)	11 (12.1)	68 (74.7)	13.77	0.0002*
		Bolton Hill	4 (9.1)	1 (2.3)	4 (9.1)	35 (79.5)	6.16	0.0130*
		Total	73 (19.8)	13 (3.5)	56 (15.2)	227 (61.7)	55.47	<0.0001*
	Late	Franklin Square	30 (18.2)	5 (3.0)	16 (9.7)	114 (69.1)	18.35	<0.0001*
		Harlem Park	11 (19.3)	2 (3.5)	7 (12.3)	37 (64.9)	5.41	0.0200*
		Union Square	10 (10.1)	2 (2.0)	6 (6.1)	81 (81.8)	12.38	0.0004*
		Bolton Hill	1 (3.2)	0 (0.0)	0 (0.0)	30 (96.8)	-	-
		Total	52 (14.8)	9 (2.6)	29 (8.2)	262 (74.4)	39.66	<0.0001* †
2015	Early	Franklin Square	8 (7)	24 (21.1)	16 (14.0)	66 (57.9)	10.46	0.0012*
		Harlem Park	1 (0.9)	23 (21.5)	13 (12.1)	70 (65.4)	25.17	<0.0001*
		Union Square	1 (2.5)	9 (22.5)	3 (7.5)	27 (67.5)	2.52	0.1130
		Bolton Hill	0 (0.0)	2 (11.1)	2 (11.1)	14 (77.8)	2.61	0.1060
		Total	11 (4.4)	44 (17.7)	23 (9.3)	170 (68.5)	20.98	<0.0001*
	Middle	Franklin Square	43 (32.8)	4 (3.1)	6 (4.6)	78 (59.5)	1.49	0.2220
		Harlem Park	22 (26.5)	1 (1.2)	7 (8.4)	53 (63.9)	7.46	0.0063*
		Union Square	7 (10.8)	1 (1.5)	12 (18.5)	45 (69.2)	27.18	<0.0001*
		Bolton Hill	10 (27.8)	5 (13.9)	7 (19.4)	14 (38.9)	0.04	0.8450
		Total	82 (26)	11 (3.5)	32 (10.2)	190 (60.3)	24.61	<0.0001*
	Late	Franklin Square	26 (35.6)	5 (6.8)	6 (8.2)	36 (49.3)	0.43	0.5120
		Harlem Park	26 (25.5)	3 (2.9)	8 (7.8)	65 (63.7)	6.42	0.0110*
		Union Square	15 (30.6)	0 (0.0)	2 (4.1)	32 (65.3)	0.00	1.0000
		Bolton Hill	9 (34.6)	0 (0.0)	1 (3.8)	16 (61.5)	0.01	0.9390
		Total	76 (30.4)	8 (3.2)	17 (6.8)	149 (59.6)	8.01	0.0047*

* indicates a significant effect at experiment-wise $\alpha=0.05$

† calculations done with block 1 of Bolton Hill removed, only 1 container with larvae was found and there can be no association calculated (divisor = 0)

Table 2: Percentage of weeks demonstrating $T < 1.0$, the persistence criterion of *Cx. pipiens* and *Cx. restuans* in the presence of *Ae. albopictus*, at the block level for 2014 and 2015.

Block	2014		2015	
	T_{pi*al}	T_{re*al}	T_{pi*al}	T_{re*al}
FS1	11.1	22.2	22.2	11.1
FS2	22.2	11.1	22.2	22.2
FS3	22.2	0.0	0.0	11.1
HP1	22.2	11.1	22.2	11.1
HP2	11.1	33.3	11.1	33.3
HP3	22.2	11.1	44.4	33.3
US1	11.1	22.2	22.2	44.4
US2	22.2	11.1	11.1	22.2
US3	33.3	33.3	11.1	33.3
BH1	12.5	25.0	25.0	12.5
BH2	50.0	37.5	22.2	22.2
BH3	12.5	12.5	11.1	33.3

* calculated using abundance data from standardized ovitraps

Chapter 4: Priority effects competition between *Ae. albopictus* and resident *Culex* spp. in Baltimore, MD

Introduction

Theoretical and empirical ecology has shown that in the presence of a superior competitor, a weaker competitor should be excluded under limited resources. Despite this prediction, inferior competitors often manage to avoid exclusion via a number of mechanisms including condition-specific competition (Chesson 2000), differential resource use (Tilman 1982), and spatial resource partitioning (Chesson 2000). The order and timing that species colonize habitats can also influence the structure and compositions of communities (Fukami 2015). Priority effects refer to the impacts that a species may have on its own ecology as well as that of later colonizers due to prior arrival into a habitat. While priority effects can refer to a wide range of species interactions, the most important may be those that alter or reverse the outcome of interspecific competition and facilitate species coexistence (Fukami 2015, Weidlich et al. 2017). Prior studies show that priority effects due to interspecific competition are often inhibitory for the later cohort, wherein secondary colonizers are negatively affected by a previous colonizer (Connell and Slatyer 1977, Alford and Wilbur 1985, Fukami 2015). Interspecific competition within habitats that are patchy and resource-limited is often highly asymmetrical and I expect that the outcomes of interspecific competition in these habitats to be highly modified by the order of colonization (Wellborn et al. 1996, Juliano 2009). Insects commonly colonize patchy, resource-limited habitats and engage in strongly asymmetric competition, yet few studies have

directly tested the role of priority effects on species coexistence (Shorrocks and Bingley 1994, Sunahara and Mogi 2002, Padeffke and Suhling 2003, Rasmussen et al. 2014).

Priority effects and inter-stage competition have been shown to be important in a number of systems including fungal inhabiting drosophilids (Shorrocks and Bingley 1994, Hodge et al. 1996), salamanders (Eitam et al. 2005), anurans and dipterans (Blaustein and Margalit 1996), odonates (Padeffke and Suhling 2003, Rasmussen et al. 2014), and single-species mosquito populations (Livdahl 1982, Maciá and Bradshaw 2000), with the secondary colonizer experiencing strong inhibitory effects from the first species colonizing the habitat. Less is known about the interspecific consequences of priority effects in container-utilizing mosquito communities, however in a study between two bamboo-stump breeding mosquitoes, inhibitory priority effects were noticed across the second cohort to colonize a container (Sunahara and Mogi 2002). Further investigation into container-utilizing mosquito communities is needed to understand priority effects and inter-stage competition in these systems.

The Asian tiger mosquito, *Aedes albopictus* (Skuse), invaded the United States in the 1980's and in the following three decades has firmly established itself throughout the country (Moore and Mitchell 1997, Hahn et al. 2016, Hahn et al. 2017). The spread of this mosquito is due in part to its ability to successfully utilize container habitats for its aquatic stages (egg, larvae, pupae). *Aedes albopictus* has been shown to be competitively superior to many resident mosquito species in the United States, with the majority of studies focusing on its interactions with other

container-utilizing *Aedes* species (Juliano 2009). This species is also of public health importance as it vectors a number of arboviral pathogens including dengue, Chikungunya virus, and West Nile virus (Ibáñez-Bernal et al. 1997, Turell et al. 2001, Sardelis et al. 2002, Paupy et al. 2009). In addition to being a competent arboviral vector, *Ae. albopictus* is also one of the most common human-biting mosquitoes in the eastern United States, increasing its interest to mosquito control professionals (Moore and Mitchell 1997, Braks et al. 2003, Benedict et al. 2007).

In the northeastern United States *Ae. albopictus* frequently encounters two other common container-utilizing mosquitoes, *Culex pipiens* (L.), and *Cx. restuans* (Theobald), in urban environments (LaDeau et al. 2013, Bodner 2014, Little et al. 2017). *Culex pipiens* was introduced to the United States in the 1700's and is a now common mosquito in urban areas of the northeastern United States. (Turell et al. 2001, Lounibos 2002, Darsie Jr and Ward 2005). *Culex restuans*, a North American resident mosquito, occupies a similar ecological niche to *Cx. pipiens*, although seasonal patterns of abundance differ between the two species with *Cx. restuans* dominating in the early summer and *Cx. pipiens* abundances increasing in the mid to late summer seasons (Harrington and Poulson 2008, Reiskind and Wilson 2008, Kilpatrick et al. 2010, Johnson et al. 2015). Both *Culex pipiens* and *Culex restuans* have been implicated as important vectors of West Nile virus in the Northeast, making them both of ecological and public health interest (Andreadis et al. 2004, Kilpatrick et al. 2010, Johnson et al. 2015). *Aedes albopictus* has been shown to be a superior competitor to *Cx. pipiens* and *Cx. restuans* under most conditions (Carrieri et al. 2003, Costanzo et al. 2005, Costanzo et al. 2011, Muturi et al. 2011). However,

despite this superiority, both *Cx. pipiens* and *Cx. restuans* have managed to escape exclusion in urban centers in the northeastern United States, indicating that ecological mechanisms other than competitive exclusion are at play.

The overall goal of my chapter is to test the hypothesis that priority effects competition within individual container habitats can facilitate the regional coexistence of *Culex* spp. mosquitoes with *Ae. albopictus*. The first objective of this chapter is to test the primary predictions of priority effects competition: that asynchronous colonization of habitats by two competitors will relax the competitive impact of the superior competitor on the inferior competitor. I expect to see the greatest effects of when the primary colonizer is the competitively inferior species, providing this species with a developmental head-start before colonization by the superior competitor. To test this prediction, I conducted a controlled laboratory competition experiment in which I manipulated the order of colonization of *Ae. albopictus* and *Cx. pipiens* in microcosms under high and low competition conditions and tested the population performance of the priority colonizer and its effects on the population performance of the second cohort. The second objective of this chapter was to determine the effects of priority cohorts of *Ae. albopictus* and *Cx. pipiens* on the subsequent colonization of each species in natural populations. To test this, I measured the field oviposition of both species in experimentally positioned containers with high and low resources and seeded with either single or mixed species cohorts of larvae, or no larvae. Even if the primary predictions of priority effects competition are met in a laboratory environment, prior cohorts may inhibit interspecific oviposition and species co-occurrence within the same habitats in the field; thus, promoting

habitat segregation and limiting the role of priority effects competition as a mechanisms of species coexistence in this system.

Materials & Methods

Laboratory experiment

The experiment was a completely randomized factorial design that consisted of experimental microcosms of 500 mL tri-pour beakers filled with 450 mL distilled water and provisioned with low (10g) or high (50g) amounts dried senescent white oak (*Quercus alba*) leaf litter. *Aedes albopictus* (F₁₋₃ generation; University of Maryland, College Park, MD) and *Culex pipiens* (F₁₋₃ generation; University of Maryland, College Park, MD) were synchronously hatched in a nutrient broth to make up the initial (priority) cohorts. Within 24 hours, larvae were rinsed and transferred into experimental microcosms. One of 4 initial cohort treatment densities (20:0, 10:10, 0:20, 0:0 *Ae. albopictus* : *Cx. pipiens*) was added on day 0. Ten additional first instar larvae of either species were added to each microcosm on 10 days after the start of the experiment as a late cohort. Additional dried white oak leaf litter was added to each microcosm every at the same days to avoid complete resource depletion and to mimic the natural condition of continuous resource inputs to containers. Microcosms were established 4 days prior to the initiation of the experiment. Each treatment combination was replicated 5 times yielding 40 microcosms with a late cohort of *Ae. albopictus*, and 40 containing a late cohort of *Cx. pipiens* for a total of 80 microcosms. In this chapter treatment densities refer to

the initial cohort, while second cohort analyses are referred to as late cohort species for the laboratory experiment.

The experiment was housed in two environmental chambers set at 22°C with a light:dark 13:11 photoperiod to approximate early summer conditions. Treatments were randomly assigned to microcosms, and microcosm position was randomly assigned within each incubator. Microcosms were shuffled daily, both within and between incubators to ensure randomization and control for any incubator effects. Each microcosm was examined daily for pupation. All pupae were removed from microcosms and placed in individual vials until adult emergence. Adults were identified, sexed, and dried, then wing lengths were measured. Proportion survivorship, median female development time, and mean female wing length were calculated for both initial and second cohort *Ae. albopictus* and *Cx. pipiens*. These fitness parameters were used to calculate the finite rate of population increase, λ' , for each species and cohort.

Field study

Three blocks in each of the neighborhoods of Franklin Square and Harlem Park, Baltimore were chosen for a total of six replicate field sites for this experiment. Study blocks were chosen based on the presence of *Ae. albopictus*, *Cx. pipiens*, and *Cx. restuans* as determined by seasonal ovitrapping and resident container sampling (**Chapter 2**). Study blocks are characterized by having rowhouses with easily distinguishable parcels. Within each block, eight experimental mesocosms were randomly placed in abandoned lots of approximately 49 meters square, for a total of

48 experimental mesocosms. Mesocosms were composed of 8 L buckets willed with 3 L of water and lined with seed germination paper (Nasco Science, Fort Atkinson, WI). In 2016, one block was removed from the experiment due to demolition and construction within the site, for a total of 40 experimental mesocosms.

The experimental design was a 2x4 factorial completely randomized design, with all treatment combinations applied to each set of eight mesocosms in each of the study blocks. Each mesocosm included either low (48 hr rested tap water only) or high resources (48 hour rested tap water with 10% hay infusion) and one of four treatment densities of *Ae. albopictus* : *Cx. pipiens* (60:0, 30:30, 0:60, 0:0), thus testing the effects of prior cohorts of single and mixed species larvae on the subsequent oviposition of wild mosquitoes at low and high resource levels. F₁₋₃ generation third instar *Ae. albopictus* and *Cx. pipiens* larvae raised at the University of Maryland were randomly applied to each treatment combination. After experimental set up and placement, mesocosms were examined every other day for pupae from the initial cohort which were collected into individual vials and held in the laboratory until adult emergence. After seven days, all mesocosms were returned to the laboratory where they were examined for the presence of *Aedes* and *Culex* spp. eggs and larvae. As initial mosquito cohorts were seeded into mesocosms as third instars, it was easy to differentiate any remaining individuals of these initial cohorts from subsequently oviposited individuals that had newly hatched. All larvae that hatched in the field were enumerated and identified to species. *Culex* spp. egg rafts were collected and hatched in a nutrient broth, and larvae were identified to species and enumerated. Seed germination papers were removed from mesocosms and *Aedes* spp. eggs were

hatched in a nutrient broth after being stored for four days to allow embryonation. All larvae were enumerated and identified to species

Statistical Analyses

To test the relationships between initial cohort treatments, secondary cohorts, and resource levels in the laboratory study, two-way ANOVAs were performed on survival, median development time, median wing length and λ' for *Ae. albopictus* and *Cx. pipiens* initial and second cohorts using SAS 9.4 PROC GLM (SAS 2016). Differences among treatments were investigated using Tukey-Kramer adjustments for all pairwise comparisons. λ' was $\log_{10}(y + 1)$ transformed to account for assumptions of normality and homogeneity of variances.

I used two-way ANOVAs to test the effects of initial cohort and resource level on field abundances of *Ae. albopictus* and *Culex* spp. Due to seasonal differences in the presence of *Culex* spp. across seasons **Chapter 2**), I analyzed field data separately by season. *Aedes albopictus* and *Cx. restuans* abundances were the dependent variable in the early summer survey. *Ae. albopictus* and *Cx. pipiens* were the dependent variable in the late summer survey. All abundance data was $\log_{10}(y + 1)$ transformed to account for assumptions of normality and homogeneity of variances. Pairwise comparisons were investigated with *a posteriori* Tukey-Kramer adjustments. Analysis for the laboratory experiment was performed using SAS 9.4 PROC GLM and analysis for the field survey was performed using SAS 9.4 PROC MIXED (SAS 2016). Significance was set with $\alpha = 0.05$ for all analyses.

Results

Laboratory experiment

Initial cohort female *Ae. albopictus* survival was not significantly affected by resource level, treatment density, or second cohort (**Table 1**). Initial cohort *Ae. albopictus* survival was lowest with high conspecific densities (20:0) under low resources but was not statistically different from other treatments (**Figure 1A**). There was an initial treatment density by resource level effect on median female development time of initial cohort *Ae. albopictus* (**Table 1**). Under high resource conditions development time was significantly shorter than under low resource conditions, with the longest development times occurring in the low nutrient conspecific treatment (**Figure 1B**). There was a significant resource level effect on median female wing length of initial cohort *Ae. albopictus* (**Table 1**) with larger wing lengths occurring in the high resource level treatments (**Figure 1C**). Initial cohort *Ae. albopictus* λ' was not significantly affected by any treatment or the second cohort (**Table 1, Figure 1D**). λ' for all combinations was greater than 1, indicating that populations would increase.

Female survival of the initial cohort of *Cx. pipiens* showed a significant resource level effect (**Table 1**). There was significantly higher survival of the initial cohort of *Cx. pipiens* under high resource conditions compared to low resource conditions (**Figure 2A**). There was also a significant resource level effect on median female wing length, but no significant effects were noted for development time (**Table 1**). Initial cohort *Cx. pipiens* λ' was significantly affected by resource level

(**Table 1, Figure 2B**). Under high resource conditions first cohort *Cx. pipiens* λ' was significantly higher than under low resource conditions irrespective of initial cohort treatment densities. Initial cohort *Cx. pipiens* λ' was below 1 at both resource levels indicating decreasing populations.

Survival for the second cohort of *Ae. albopictus* was not affected by initial cohort treatment densities or resource levels (**Table 2, Figure 4A**). There was a significant resource level by treatment density interaction for female development time (**Figure 4B**). Low resource levels with the 20:0 conspecific treatment and the 10:10 conspecific treatment densities had significantly longer development times than all other treatments. No significant differences were observed in female median wing lengths (**Table 2, Figure 4C**). There was a significant resource level by treatment density interaction for *Ae. albopictus* λ' (**Table 2, Figure 4D**). At high resource levels, λ' of the second cohort of *Ae. albopictus* was affected by treatment density with *Ae. albopictus* λ' being significantly lower in the 0:20 heterospecific treatment than in the control. At low nutrient levels *Ae. albopictus* was affected by the presence of conspecific larvae more than heterospecific larvae (**Figure 4D**). Second cohort *Ae. albopictus* λ' was greater than 1.0 for all treatment combinations indicating that populations would increase under all situations, however they were on average lower than λ' for the first cohort of *Ae. albopictus*.

Survival for the second cohort of *Cx. pipiens* showed a strong resource level by treatment density interaction (**Table 2**). Under high resource conditions with an initial cohort of any species, *Cx. pipiens* survival was significantly different than the control (**Figure 5A**). Under high resource conditions there were no significant

differences between treatments. Survival in the low resource treatments was significantly different from the high resource treatments, except for the control (**Figure 4A**). Significant differences were observed between treatments and resource levels for both female median development of 2nd cohort *Cx. pipiens*, but were not graphed due to inestimable LS mean values (**Table 2**). No significant differences were observed for median female wing lengths of 2nd cohort *Cx. pipiens* (**Table 2**). *Culex pipiens* λ' in the second cohort was significantly higher under high resource levels than low resource levels (**Table 2, Figure 4B**). On average, λ' was lower in the 2nd cohort of *Cx. pipiens* than it was in the first cohort.

Field study

A total of 6,005 mosquito larvae were identified in the experimental mesocosms in the early summer (June) session. *Culex restuans* represented 64.6% (n=3,880) of the total mosquito abundances. *Aedes albopictus* comprised 32.1% (n=1,925) of the total. Other mosquito species identified were *Ae. japonicus* (n=1,149), *Cx. pipiens* (n=100), and *Cx. territans* (n=50), representing 19.1%, 1.7%, and 0.8% of total mosquitoes collected, respectively.

During the late summer (September) collection, a total of 14,577 mosquitoes were identified. The most abundant species was *Ae. albopictus*, representing 88.2% (n=12,863) of the total. The second most common species was *Cx. pipiens*, comprising 9.3% (n=1,350) of the total abundances. Other species represented were *Cx. restuans* (n=200) and *Ae. japonicus* (n=164), representing 2.5% of the total mosquito abundance.

There were no significant effects on oviposition by *Ae. albopictus* in either the early or the late season field study, however there were higher mean seasonal abundances of *Ae. albopictus* in the September collection which is consistent with previous findings for both neighborhoods in Baltimore City (see **Chapter 2**). In the June collection there were significant resource level and treatment density effects on *Cx. restuans* oviposition (**Table 3**). The high resource mesocosms showed significantly ($p < 0.05$) higher abundances of *Cx. restuans* than the low resource mesocosms (**Figure 5A**) during the early season. The effect of treatment density on *Cx. restuans* oviposition also showed significant differences among treatments with the mixed treatment (30:30 *Ae. albopictus* : *Cx. pipiens*) being significantly more attractive to ovipositing *Cx. restuans* than either the negative control or the *Ae. albopictus* only treatment (**Figure 5B**). There were significant resource level effects on *Cx. pipiens* oviposition in the September collection (**Table 3**). Further examination showed a significant difference between the high and low resource levels, with the high resource level having significantly higher abundances of *Cx. pipiens* (**Figure 6**).

Discussion

Though *Ae. albopictus* is competitively superior to both *Cx. pipiens* and *Cx. restuans* under most situations, they continue to coexist in the urban mid-Atlantic. One possible explanation of this phenomenon could be priority colonization by an inferior competitor, otherwise known as priority effects. In this study I examined priority colonization under different resource levels and with different treatment densities of both *Ae. albopictus* and *Cx. pipiens* in both the laboratory and the field.

Consistent with my hypotheses, the results of our laboratory study suggest that under high resource conditions, there are clear inhibitory priority effects of the initial cohort on the second cohort. Priority colonization by either *Ae. albopictus* or *Cx. pipiens* increased their population performance (λ') as compared to being the secondary colonizer in a high resource habitat. This result supports prior studies of natural container utilizing mosquitoes (Livdahl 1982, Sunahara and Mogi 2002). In habitats where resources are plentiful, priority colonization should provide the colonizer ample opportunity to exploit and deplete available nutrient resources in the environment prior to the arrival of subsequent species. *Culex pipiens* survivorship and population performance for both cohorts were significantly decreased in the laboratory under low resource conditions (**Figures 2, 4**), bringing λ' below 1.0 indicating declining populations. While *Ae. albopictus* showed decreased survivorship and λ' under low resource conditions compared to high resource availability, λ' remained above 1.0 indicating that resource availability is likely more important for the competitively inferior *Cx. pipiens*. These results are consistent with previous studies of resource competition between *Ae. albopictus* and *Cx. pipiens* with low resource availability providing greater negative effects on *Cx. pipiens* fitness (Carrieri et al. 2003, Costanzo et al. 2005).

Ae. albopictus population performance was more negatively influenced by the presence of heterospecific larvae under low resource conditions than it was by conspecific larvae – either in the initial cohort or in the second cohort, suggesting that *Ae. albopictus* is more strongly influenced by intraspecific competition than it is by priority effects. This finding supports prior studies which have shown that

intraspecific competition affects *Ae. albopictus* survival and population performance more strongly than competition with *Cx. pipiens* (Costanzo et al. 2005, Yoshioka et al. 2012). Under high resource conditions there was some evidence that heterospecific competition affected the performance of *Ae. albopictus* compared to *Cx. pipiens*, this may be due to *Cx. pipiens*' ability to perform well under higher nutrient conditions (Vinogradova 2000). This also supports results showing that *Ae. albopictus* population performance decreases with aggregation or increased densities (see **Chapter 3**) (Costanzo et al. 2005, Fader and Juliano 2012). Under low resource conditions there was not a significant effect of the either species second cohort on *Ae. albopictus*. Despite seeing negative effects of intraspecific competition, λ' for all cohorts of *Ae. albopictus* under all resource levels and treatment densities was greater than 1.0, indicating that populations would continue to increase, demonstrating the competitive superiority of this invasive mosquito.

In contrast, first cohort *Cx. pipiens*' population performance varied greatly between low and high resource levels, with both survival and λ' increasing in the high resource treatments (**Figure 2**). *Culex pipiens* is known to perform well in high nutrient environments (Vinogradova 2000), which supports my findings. This study found that first cohort *Cx. pipiens* showed better survivorship in the presence of *Ae. albopictus* than in treatments with only conspecifics. This contradicts findings from previous studies which have shown decreased survivorship of *Cx. pipiens* in the presence of *Ae. albopictus* and in the presence of conspecific competitors (Costanzo et al. 2005). While survivorship was affected by treatment density, there were only significant influences of resource level on the overall population performance, with λ'

being just below 1.0 under high resource conditions for both initial and 2nd cohorts of *Cx. pipiens*. This suggests that priority colonization in nutrient rich habitats could significantly benefit *Cx. pipiens* persistence in an environment, even though our laboratory experiment showed a λ' of just under 1.0 due to differences in nutrient type (Costanzo et al. 2011).

The second cohort of *Cx. pipiens* showed the best survivorship under high resource conditions (**Figure 4**) supporting findings from previous studies (Carrieri et al. 2003, Costanzo et al. 2005). While the differences were not significant among treatments within a resource level, the trends were the same for both low and high resource treatments, with high resource mesocosms having significantly higher levels of survival. Second cohort *Cx. pipiens* survival was also just below 1.0 in high resource treatments, but significantly below 1.0 in low resource treatments, once again suggesting that resource level is important in *Cx. pipiens* development. These results indicate that there may be other factors influencing the outcomes of competition between *Ae. albopictus* and *Cx. pipiens*, possibly differential resource use or larval feeding behavior. *Ae. albopictus* has been shown to exhibit grazing behavior in larval habitat while *Cx. pipiens* gathers food in the water column (Merritt et al. 1992), which could influence the quantity and quality of resources obtained by each species. Additionally, studies have shown that different mosquito species may show niche partitioning in the water column, which may support our finding that heterospecific competition at lower densities may support the coexistence of *Cx. pipiens* with *Ae. albopictus* (Yee et al. 2004). Differential feeding patterns or niche partitioning may explain why intraspecific competition of *Ae. albopictus* second

cohorts negatively affected initial cohorts of *Ae. albopictus*. If *Ae. albopictus* preferentially feeds on the surfaces of a container and/or submerged materials while *Cx. pipiens* feeds on suspended material, the mosquitoes may be released from the effects of direct competition.

Field studies did not support my hypothesis that mosquitoes would be more likely to oviposit in containers previously inhabited by conspecific mosquito larvae, however they did confirm the results of the laboratory study that high resource levels attracted more *Culex* spp. in both the early and late mosquito seasons (**Figures 5, 6**), this is in accordance to previous oviposition studies of *Culex* spp. (Lampman and Novak 1996). *Aedes albopictus* displayed no significant preference in oviposition choice, possibly due to their behavior of skip oviposition, although studies have shown that *Ae. albopictus* will oviposit more evenly among high quality habitats than low quality (Davis et al. 2015). There was some influence of the priority cohort on oviposition for early season *Cx. restuans* (**Figure 5**). *Culex restuans* were more likely to oviposit in containers containing a mixed population of mosquito larvae, rather than a population of either only *Ae. albopictus* or no prior colonizers. Prior studies of *Cx. restuans* oviposition choice have shown that ovipositing females are attracted to nutrient rich habitats and that the presence of conspecifics reduces the number of egg rafts in a container (Reiskind and Wilson 2004), however there is little known about the presence of heterospecific effects on oviposition. Prior colonization by *Culex* spp. mosquitoes may serve as an oviposition attractant to conspecific species. Interestingly, the treatments without prior colonization by mosquito larvae were not attractive to *Cx. restuans* or *Cx. pipiens* – indicating that priority colonization of a

habitat may not be an important factor in mosquito coexistence. If priority colonization was to be a mediator of coexistence, I would expect significantly higher numbers of *Culex* spp. mosquitoes to be ovipositing in uncolonized habitats – especially in the early season when *Culex restuans* is most abundant.

In conclusion, priority colonization of a container does provide inhibitory effects on the secondary colonizer; however, the competitive superiority of *Ae. albopictus* does not provide sufficient evidence that this is a strong mechanism of coexistence for *Ae. albopictus* and *Cx. pipiens* in container habitats. Resource availability seems to be the driving force in *Culex* spp. population performance, with resource level being a strong influence on survivorship and population performance. Interestingly I found that in the field, *Culex restuans* preferred to oviposit in the presence of *Ae. albopictus*, suggesting that niche partitioning or differential resource use within a container may be important to mosquito coexistence, especially in species that utilize different feeding behaviors even resource level seems to be the driving force for *Cx. pipiens* performance. *Aedes albopictus* was negatively impacted by intraspecific competition at all instar levels, indicating that *Ae. albopictus* should show avoidance behaviors to previously colonized habitats, however our oviposition study showed no difference among treatments at ovipositional attraction. Further studies should examine the effects of feeding behavior on interstage competition on the population performance of both *Ae. albopictus* and container-utilizing *Culex* spp. to better understand competition dynamics.

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on 2nd cohort *Ae. albopictus* female median development time. **C.** Effects of treatment density and resource level on 2nd cohort *Ae. albopictus* female median wing length. **D.** Effects of treatment density and resource level on 2nd cohort *Ae. albopictus* λ' . Significant pairwise differences are indicated by different letters above bars. Least square means values are \pm SE.

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5. Figure 5: **A.** Field collected *Cx. restuans* abundances in experimental mesocosms by resource level. **B.** Field collected *Cx. restuans* abundances in experimental mesocosms by treatment density. Least square means values are \pm SE. Significant pairwise differences are indicated by different letters above bars.

6. Figure 6: Field collected *Cx. pipiens* abundances in experimental mesocosms by resource level. Significant pairwise differences are indicated by different letters above bars. Least square means values are \pm SE.

Tables

Table 1: ANOVA table for female survival, median development time, median wing length, and λ' of the initial cohorts of *Ae. albopictus* and *Cx. pipiens* from the priority laboratory study. TD indicates initial cohort treatment density.

		<i>Ae. albopictus</i>			<i>Cx. pipiens</i>		
		df	F	P	df	F	P
Survival	Resource level (RL)	1	0.56	0.4595	1	41.52	<0.0001*
	Treatment density (TD)	1	3.19	0.0837	1	1.29	0.2639
	Late cohort species (LCS)	1	0.75	0.3941	1	0.05	0.8215
	RL x TD	1	0.96	0.3349	1	2.07	0.1595
	RL x LCS	1	0.08	0.7752	1	0.05	0.8215
	TD x LCS	1	1.20	0.2820	1	0.05	0.8215
	RL x TD x LCS	1	0.00	0.9544	1	0.14	0.7072
Median development time	RL	1	106.04	<0.0001*	1	54.38	<0.0001*
	TD	1	35.70	<0.0001*	1	0.66	0.4276
	LCS	1	0.22	0.6443	1	0.00	0.9490
	RL x TD	1	4.86	0.0349*	1	0.66	0.1416
	RL x LCS	1	1.41	0.2441	1	2.38	0.1416
	TD x LCS	1	0.03	0.8665	1	1.30	0.2693
	RL x TD x LCS	1	0.02	0.8996	0	-	-†
Median wing length							
	RL	1	35.54	<0.0001*	1	0.03	0.8640
	TD	1	2.01	0.1657	1	0.30	0.5924
	LCS	1	1.54	0.2235	1	0.04	0.8457
	RL x TD	1	0.16	0.6901	1	0.09	0.7726
	RL x LCS	1	0.21	0.6534	1	0.02	0.8871
	TD x LCS	1	0.17	0.6860	1	1.62	0.2204
	RL x TD x LCS	1	0.09	0.7708	0	-	-†
λ'							
	RL	1	2.61	0.1161	1	25.43	<0.0001*
	TD	1	0.79	0.3803	1	0.55	0.4639
	LCS	1	1.01	0.3230	1	0.66	0.4238
	RL x TD	1	0.68	0.4159	1	2.69	0.1108
	RL x LCS	1	0.94	0.3408	1	2.54	0.1210
	TD x LCS	1	0.94	0.3383	1	0.75	0.3929
	RL x TD x LCS	1	0.92	0.3440	1	2.35	0.1352

* Significant effect

† Non-estimable result

Table 2: ANOVA table for the survival, median development time, median wing length, and λ' of second cohort females of *Ae. albopictus* and *Cx. pipiens* from the priority laboratory study.

		<i>Ae. albopictus</i>			<i>Cx. pipiens</i>		
		df	F	P	df	F	P
Survival	Resource level (RL)	1, 32	0.67	0.4191	1, 32	10.39	0.0029*
	Treatment density (TD)	3, 32	1.97	0.1385	3, 32	1.21	0.3212
	RL x TD	3, 32	1.09	0.3678	3, 32	1.25	0.3091
Development time (median)	RL	1, 32	49.95	<0.0001*	1, 17	7.50	0.0140*
	TD	3, 32	21.76	<0.0001*	3, 17	4.68	0.0147*
	RL x TD	3, 32	14.48	<0.0001*	3, 17	0.80	0.4635
Wing length (median)	RL	1, 23	3.39	0.0751	1, 17	0.59	0.4545
	TD	3, 32	0.58	0.6304	3, 17	1.51	0.2491
	RL x TD	3, 32	2.19	0.1082	3, 17	1.54	0.2430
λ'	RL	1, 32	17.31	0.0002*	1, 32	17.61	0.0002*
	TD	3, 32	8.98	0.0002*	3, 32	0.89	0.4553
	RL x TD	3, 32	3.57	0.0247*	3, 23	1.64	0.1990

*Significant effect

Table 3: ANOVA table of effects on early mean field collection abundances of *Cx. restuans* and late field collection abundances of *Cx. pipiens*.

		<i>Cx. restuans</i>			<i>Cx. pipiens</i>		
		df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Mean abundances							
	Resource level (RL)	1, 32	3.6	0.0240*	1, 78	1.56	0.0224*
	Treatment density (TD)	3, 32	11.46	0.0019*	3, 78	5.42	0.2058
	RL x TD	3, 32	1.82	0.1634	3, 78	1.12	0.3455

*Significant effect

Figures

Figure 1:

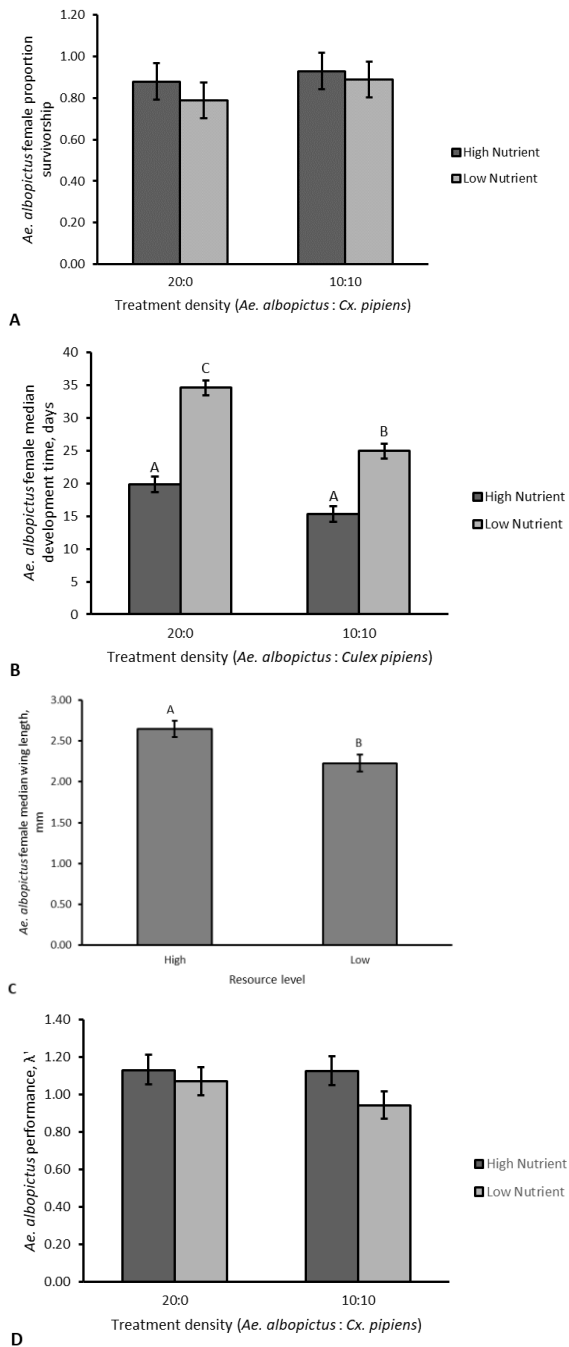
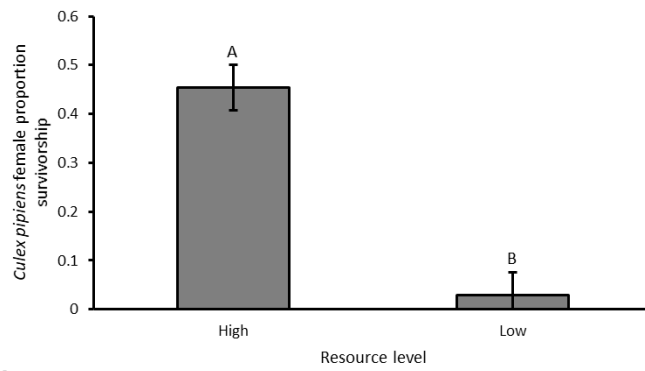
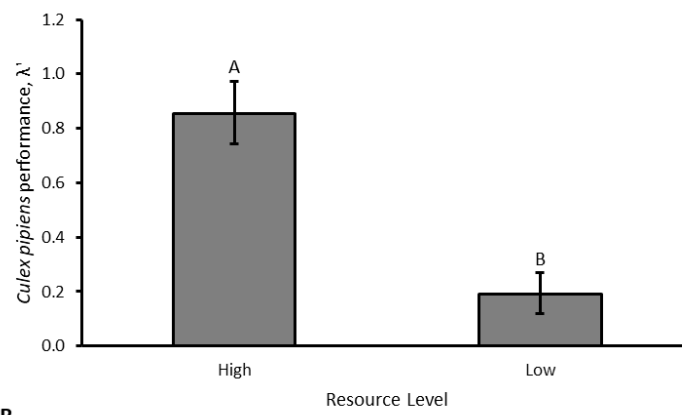


Figure 2:



A



B

Figure 3:

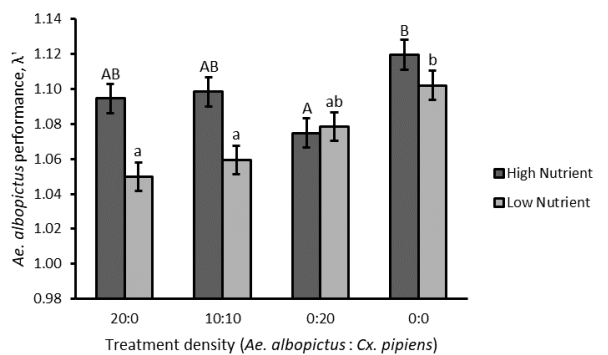
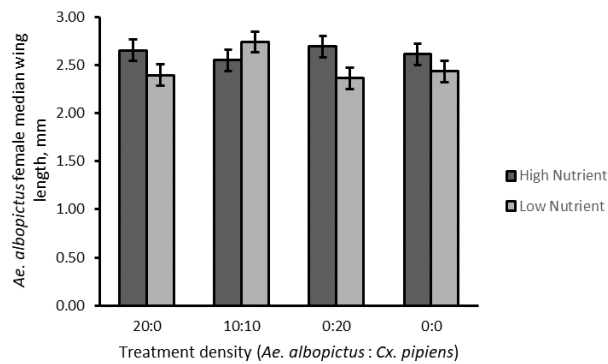
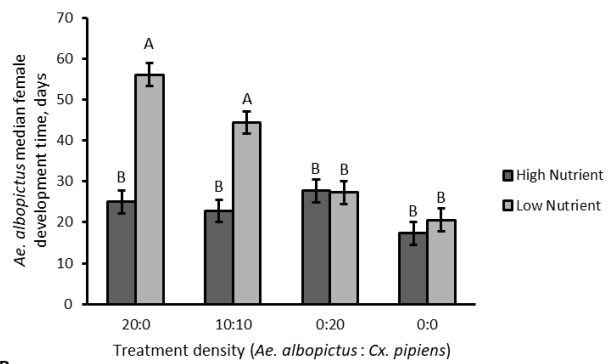
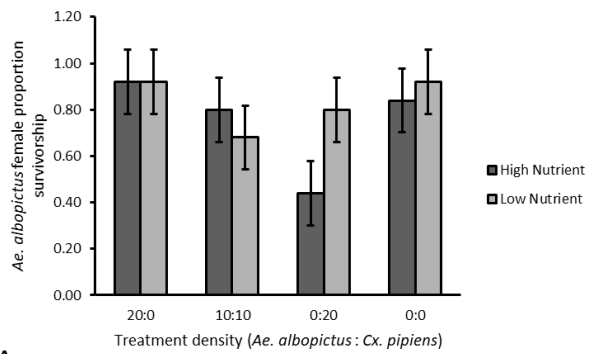
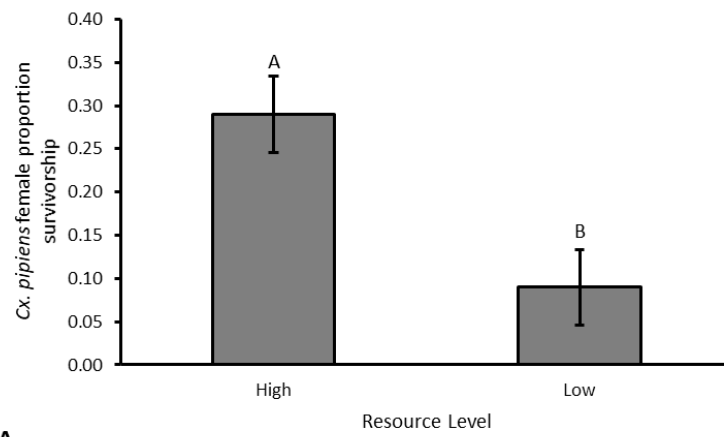
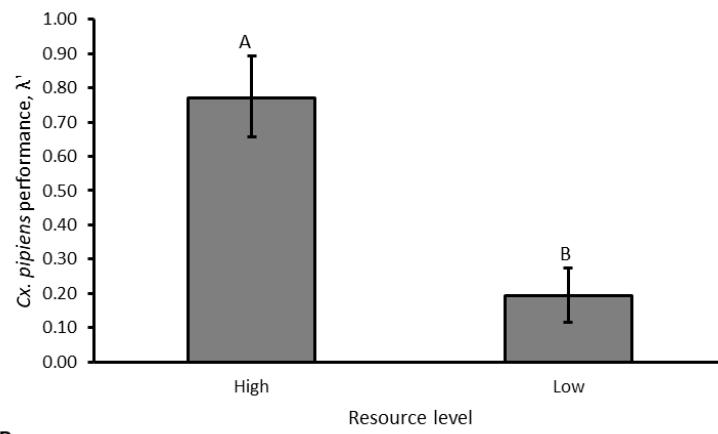


Figure 4:

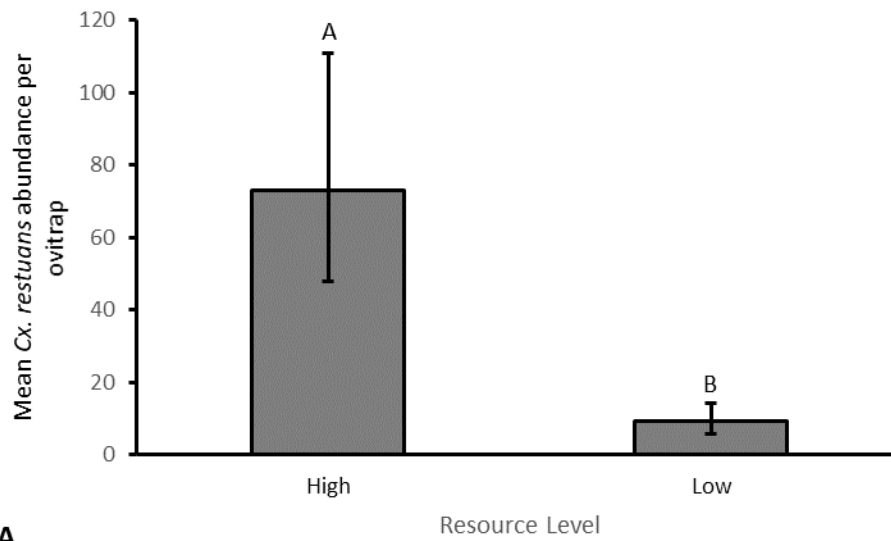


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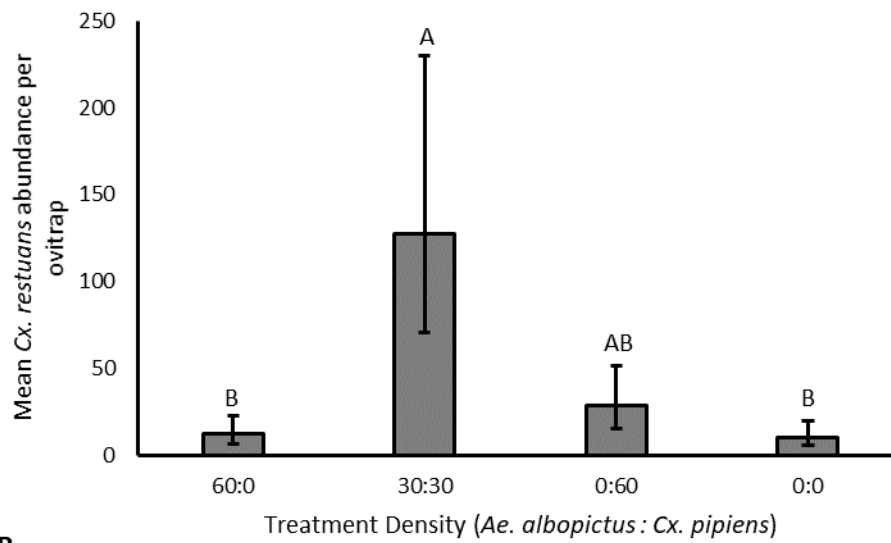


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Figure 5:

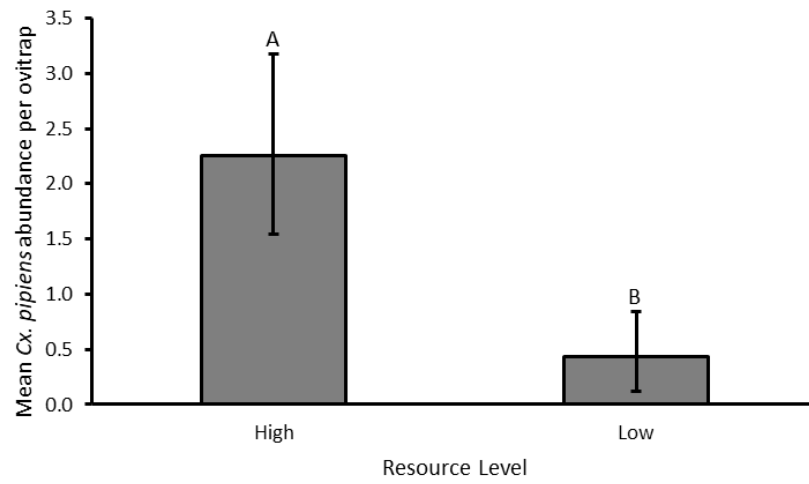


A



B

Figure 6:



Appendices

Appendix A

Calculations for *J*, *C*, and *T* indices

Aggregation indices were calculated for *Ae. albopictus*, *Cx. pipiens*, and *Cx. restuans* at the block level for each of the 18 collection dates. First, the index of intraspecific aggregation (*J*) (Ives 1991) was calculated in each block for each date:

$$J_x = \frac{V_x}{m_x^2} - \frac{1}{m_x}$$

where m_x is the mean, and V_x is the variance of species X per patch. When $J = 0$, species X is randomly distributed among containers. When $J > 0$, species X is aggregated, and if $J < 0$ there is a uniform distribution. The *J* index assumes that all patches are identical, a criteria that was met in this study through standardized ovitrapping.

I used the index of interspecific aggregation (*C*) (Ives 1991) to quantify interspecific association between *Ae. albopictus* and the two *Culex* species:

$$C_{xy} = \frac{\text{Cov}_{xy}}{(m_x * m_y)}$$

where *C* is the index of interspecific aggregation (the proportionate increase in the mean number of heterospecific competitors found in a container relative to the number of heterospecifics predicted in a random distribution. In this scenario Cov_{xy} is the covariance between species X and species Y. When $C = 0$, the competing species are independently distributed, while at $C > 0$, the two species are positively associated and at $C < 0$, the competing species are negatively associated.

Finally, I calculated the index of relative aggregation (T) to test the relative strength of intraspecific versus interspecific aggregation:

$$T_{xy} = \frac{(J_x + 1)(J_y + 1)}{(C_{xy} + 1)^2}$$

where interspecific aggregation is present at $T < 1.0$, and intraspecific aggregation dominates at $T > 1.0$ (Ives 1991, Sevenster 1996, Fader and Juliano 2012, Freed and Leisnham 2014). T_{xy} determines the persistence of a species of interest (X) could persist given the aggregation of a competitor (Y). For our T calculations, *Cx. pipiens* and *Cx. restuans* served as our species of interest while *Ae. albopictus* served as our competitor. Similar to prior studies on aggregation, in this study I attempted only to test whether competing life stages were aggregated, but did not attempt to distinguish aggregation of ovipositing females from aggregation due to multi-egg clutches (Hartley and Shorrocks 2002, Leisnham and Juliano 2009, Freed and Leisnham 2014).

Appendix B

***J*, *C*, and *T* indices for *Ae. albopictus*, *Cx. pipiens*, and *Cx. restuans* for each collection date at the block level.**

*NA indicates the index is not calculable based on the lack of a species in the block

*BS = Bolton Hill, FS = Franklin Square, HP = Harlem Park, US = Union Square

*€ indicates sampling was unable to occur on the block for that date

Date	Block	<i>J</i> _{<i>Ae.</i>} <i>albopictus</i>	<i>J</i> _{<i>Cx.</i>} <i>pipiens</i>	<i>J</i> _{<i>Cx.</i>} <i>restuans</i>	<i>C</i> _{<i>ae</i>} * <i>cspi</i>	<i>C</i> _{<i>ae</i>} * <i>cxre</i>	<i>T</i> _{<i>ae</i>} * <i>cspi</i>	<i>T</i> _{<i>ae</i>} * <i>cxre</i>
5/21/2014	BS1	€	€	€	€	€	€	€
	BS2	€	€	€	€	€	€	€
	BS3	€	€	€	€	€	€	€
	FS1	4.4	NA	4.88	NA	-1.2	NA	-0.037
	FS2	NA	NA	4.60241	NA	NA	NA	NA
	FS3	4.33333	NA	NA	NA	NA	NA	NA
	HP1	NA	NA	1.97	NA	NA	NA	NA
	HP2	NA	NA	4.94	NA	NA	NA	NA
	HP3	NA	NA	NA	NA	NA	NA	NA
	US1	4.4	NA	-1	NA	6	NA	1.2963
	US2	NA	NA	NA	NA	NA	NA	NA
	US3	NA	NA	2.29333	NA	NA	NA	NA
6/11/2014	BS1	0.36983	NA	0.28963	NA	0.04545	NA	0.7632
	BS2	0.33333	NA	1.12148	NA	1.46667	NA	1.85
	BS3	1.29333	NA	0.64667	NA	0.08	NA	0.47093
	FS1	1.38171	4.94	1.55429	2.64545	-0.5688	1.5306	0.18103
	FS2	2.15272	NA	2.17882	NA	-0.0985	NA	0.28594

	FS3	0.30196	1.97	1.22	0.26939	0.39796	0.97498	1.07374
	HP1	0.32699	NA	0.25063	NA	0.18103	NA	0.89001
	HP2	0.13449	NA	1.97	NA	-0.5242	NA	0.41941
	HP3	2.5607	4.88	1.97	-0.2328	-0.7164	0.21545	0.07964
	US1	1.153	4.94	0.02036	1.48916	-0.0923	1.15614	0.42159
	US2	0.7955	4.94	1.235	-0.5388	-0.8143	0.25688	0.10343
	US3	2.1137	4.94	0.82816	-0.6122	0.69971	0.12453	0.54588
7/2/2014	BS1	2.45239	NA	NA	NA	NA	NA	NA
	BS2	0.11059	4.88	2.29333	-0.7819	-0.3329	0.19635	0.60067
	BS3	0.37912	NA	NA	NA	NA	NA	NA
	FS1	0.27413	NA	NA	NA	NA	NA	NA
	FS2	0.3063	NA	NA	NA	NA	NA	NA
	FS3	0.09365	NA	NA	NA	NA	NA	NA
	HP1	0.63506	NA	4.76	NA	1.25647	NA	1.38005
	HP2	0.64796	NA	1.112	NA	0.61542	NA	0.98025
	HP3	0.77506	NA	NA	NA	NA	NA	NA
	US1	0.89662	NA	NA	NA	NA	NA	NA
	US2	0.69613	NA	NA	NA	NA	NA	NA
	US3	0.21363	NA	NA	NA	NA	NA	NA
7/23/2014	BS1	0.19824	NA	NA	NA	NA	NA	NA
	BS2	0.17309	1.112	NA	-0.0716	NA	0.79145	NA
	BS3	1.67013	NA	NA	NA	NA	NA	NA
	FS1	0.1611	NA	NA	NA	NA	NA	NA
	FS2	0.26103	NA	NA	NA	NA	NA	NA
	FS3	0.45087	NA	NA	NA	NA	NA	NA
	HP1	0.46361	NA	NA	NA	NA	NA	NA
	HP2	0.52143	NA	NA	NA	NA	NA	NA

	HP3	0.25101	NA	NA	NA	NA	NA	NA
	US1	1.17239	NA	NA	NA	NA	NA	NA
	US2	1.83749	NA	NA	NA	NA	NA	NA
	US3	0.58322	NA	NA	NA	NA	NA	NA
8/13/2014	BS1	0.39964	NA	NA	NA	NA	NA	NA
	BS2	1.03266	NA	1.97	NA	-1.2	NA	-0.0984
	BS3	1.16015	NA	NA	NA	NA	NA	NA
	FS1	0.35969	NA	NA	NA	NA	NA	NA
	FS2	0.21952	3.8	NA	-0.1661	NA	0.68383	NA
	FS3	0.81514	4.92	NA	1.76862	NA	1.52529	NA
	HP1	1.98659	NA	NA	NA	NA	NA	NA
	HP2	0.19693	NA	NA	NA	NA	NA	NA
	HP3	0.76142	NA	NA	NA	NA	NA	NA
	US1	0.39767	NA	NA	NA	NA	NA	NA
	US2	1.91402	NA	NA	NA	NA	NA	NA
	US3	0.34023	4.96	4.96	-0.3571	0.18571	0.47966	0.88471
9/5/2014	BS1	0.06663	1.31333	NA	0.00673	NA	0.94384	NA
	BS2	0.34711	0.57333	NA	-0.2914	NA	0.52599	NA
	BS3	0.22798	0.97333	NA	-0.1365	NA	0.70315	NA
	FS1	0.22052	0.158	NA	-0.1021	NA	0.73566	NA
	FS2	0.01111	0.12775	NA	-0.0341	NA	0.95527	NA
	FS3	0.32054	2.30667	NA	-0.1958	NA	0.60899	NA
	HP1	0.14391	0.776	NA	-0.2712	NA	0.63715	NA
	HP2	0.04933	2.29333	NA	-0.01	NA	0.94347	NA
	HP3	0.22175	0.51537	NA	-0.0356	NA	0.78937	NA
	US1	0.16169	3.032	NA	-0.4589	NA	0.4658	NA
	US2	0.05141	NA	NA	NA	NA	NA	NA

	US3	0.19128	1.23	NA	0.69416	NA	1.42213	NA
9/26/2014	BS1	0.62064	NA	4.88	NA	-0.2442	NA	0.46633
	BS2	0.20854	4.88	NA	-0.3271	NA	0.55682	NA
	BS3	0.60635	NA	NA	NA	NA	NA	NA
	FS1	0.1486	NA	NA	NA	NA	NA	NA
	FS2	0.16942	NA	NA	NA	NA	NA	NA
	FS3	0.07925	NA	NA	NA	NA	NA	NA
	HP1	0.4508	4.94	NA	-0.3184	NA	0.46983	NA
	HP2	0.21209	NA	2.29333	NA	-0.2314	NA	0.63413
	HP3	0.34304	4.88	NA	1.25015	NA	1.67541	NA
	US1	0.22994	NA	4.94	NA	-0.3657	NA	0.51573
	US2	0.32393	4.94	NA	-0.5444	NA	0.3441	NA
	US3	0.30673	4.94	4.94	-0.2557	-0.7436	0.56956	0.19621
10/17/2014	BS1	4.82857	NA	NA	NA	NA	NA	NA
	BS2	2.08493	NA	1.96	NA	0.09496	NA	0.35494
	BS3	0.51769	NA	NA	NA	NA	NA	NA
	FS1	1.25625	NA	NA	NA	NA	NA	NA
	FS2	1.84417	NA	NA	NA	NA	NA	NA
	FS3	0.16257	NA	NA	NA	NA	NA	NA
	HP1	0.05554	NA	NA	NA	NA	NA	NA
	HP2	0.79302	NA	NA	NA	NA	NA	NA
	HP3	2.70523	NA	NA	NA	NA	NA	NA
	US1	0.19963	NA	NA	NA	NA	NA	NA
	US2	0.25309	NA	NA	NA	NA	NA	NA
	US3	1.03571	NA	NA	NA	NA	NA	NA
11/6/2014	BS1	NA	NA	NA	NA	NA	NA	NA
	BS2	NA	NA	NA	NA	NA	NA	NA

	BS3	NA	NA	NA	NA	NA	NA	NA
	FS1	2.49941	NA	NA	NA	NA	NA	NA
	FS2	2.0375	NA	NA	NA	NA	NA	NA
	FS3	NA	NA	NA	NA	NA	NA	NA
	HP1	1.66667	NA	NA	NA	NA	NA	NA
	HP2	3.32	NA	NA	NA	NA	NA	NA
	HP3	3.5	NA	NA	NA	NA	NA	NA
	US1	2.04592	NA	NA	NA	NA	NA	NA
	US2	NA	NA	NA	NA	NA	NA	NA
	US3	4.33333	NA	NA	NA	NA	NA	NA
5/18/2015	BS1	€	€	€	€	€	€	€
	BS2	NA	NA	NA	NA	NA	NA	NA
	BS3	4.625	NA	1.98513	NA	-1	NA	2E-17
	FS1	NA	NA	NA	NA	NA	NA	NA
	FS2	1.03137	NA	NA	NA	NA	NA	NA
	FS3	2.13333	NA	NA	NA	NA	NA	NA
	HP1	4.94393	NA	NA	NA	NA	NA	NA
	HP2	1.25	NA	NA	NA	NA	NA	NA
	HP3	0.93419	NA	NA	NA	NA	NA	NA
	US1	0.83673	NA	3.96667	NA	-1.25	NA	-0.1361
	US2	1.78571	NA	NA	NA	NA	NA	NA
	US3	4.53846	NA	4.96	NA	-1.2	NA	-0.0361
6/8/2015	BS1	0.94087	NA	NA	NA	NA	NA	NA
	BS2	4.80577	NA	NA	NA	NA	NA	NA
	BS3	4.77778	NA	NA	NA	NA	NA	NA
	FS1	1.009	NA	NA	NA	NA	NA	NA
	FS2	0.91558	NA	4.92	NA	1.53103	NA	1.32129

	FS3	0.51378	NA	NA	NA	NA	NA	NA
	HP1	1.28831	NA	1.96	NA	-0.48	NA	0.22724
	HP2	0.78172	NA	4.94	NA	-1.2	NA	-0.1123
	HP3	0.91672	NA	2.02694	NA	-0.12	NA	0.45912
	US1	2.17949	NA	NA	NA	NA	NA	NA
	US2	0.24211	NA	NA	NA	NA	NA	NA
	US3	1.16992	NA	NA	NA	NA	NA	NA
6/29/2015	BS1	0.35534	NA	NA	NA	NA	NA	NA
	BS2	0.17011	NA	4	NA	-0.8064	NA	0.16547
	BS3	0.23225	NA	NA	NA	NA	NA	NA
	FS1	0.44788	2.29333	NA	-0.9719	NA	0.01938	NA
	FS2	0.15688	4.76	NA	-0.2494	NA	0.64882	NA
	FS3	0.29561	NA	NA	NA	NA	NA	NA
	HP1	0.12722	4.5	NA	0.7884	NA	1.58655	NA
	HP2	0.18969	NA	2.02694	NA	-0.4623	NA	0.45193
	HP3	0.89263	NA	NA	NA	NA	NA	NA
	US1	0.43399	NA	4.92	NA	-0.3918	NA	0.42411
	US2	0.38692	4.76676	4.76	-0.4226	-0.3446	0.41633	0.47259
	US3	0.26578	NA	NA	NA	NA	NA	NA
7/20/2015	BS1	0.11192	1.97	NA	-0.1606	NA	0.75495	NA
	BS2	0.04056	0.788	4.94	-0.0589	-0.077	0.90445	0.88704
	BS3	1.06693	0.62545	2.04408	-0.4532	0.88075	0.26456	0.90992
	FS1	0.01418	4.97	0.59956	0.27977	-0.0826	1.26188	0.90462
	FS2	0.06009	1.98	0.94343	0.37808	-0.0818	1.29996	0.86615
	FS3	0.11723	1.58249	3.056	0.49846	-0.3965	1.34123	0.54016
	HP1	0.30745	0.25901	NA	0.09915	NA	0.84068	NA
	HP2	0.04629	0.77208	4.97	0.02406	-0.2555	0.97876	0.7116

	HP3	0.13124	0.35818	4.985	-0.1418	-0.4352	0.75867	0.49926
	US1	0.29934	4.97	4.97	-0.669	0.74447	0.25476	1.34258
	US2	0.09415	2.31333	2.0237	0.31377	-0.1174	1.20073	0.80669
	US3	0.20204	1.01184	1.235	0.34653	-0.4457	1.1202	0.46112
8/10/2015	BS1	0.17794	4.88	NA	0.64447	NA	1.39606	NA
	BS2	0.15404	4.94	NA	-0.184	NA	0.7071	NA
	BS3	0.53554	4.94	4.94	1.06167	-0.7746	1.34263	0.14676
	FS1	0.07175	1.55429	NA	0.32943	NA	1.24042	NA
	FS2	0.19217	4.88	4.76	0.20234	-0.599	1.00853	0.33636
	FS3	0.0967	1.31333	NA	0.20329	NA	1.0972	NA
	HP1	0.13746	4.7	NA	-0.1026	NA	0.78898	NA
	HP2	0.03412	2.02694	NA	0.05199	NA	1.01728	NA
	HP3	0.34117	4.88	NA	-0.2394	NA	0.56715	NA
	US1	0.05211	NA	4.88	NA	-0.2365	NA	0.72566
	US2	0.04286	NA	NA	NA	NA	NA	NA
	US3	0.1737	NA	4.97	NA	0.66374	NA	1.41751
8/31/2015	BS1	0.23144	4.97	NA	-0.7382	NA	0.21262	NA
	BS2	0.08478	NA	NA	NA	NA	NA	NA
	BS3	0.43155	4.97	NA	1.47429	NA	1.7284	NA
	FS1	0.05957	4.976	NA	-0.1243	NA	0.8265	NA
	FS2	0.03681	4.976	NA	-0.0681	NA	0.89884	NA
	FS3	0.20983	NA	NA	NA	NA	NA	NA
	HP1	0.28274	2.02694	NA	0.89304	NA	1.47577	NA
	HP2	0.09784	4.88	NA	0.29446	NA	1.1791	NA
	HP3	0.40204	0.78935	NA	-0.1389	NA	0.61417	NA
	US1	0.40224	4.94	NA	1.33869	NA	1.66782	NA
	US2	0.32284	4.94	NA	0.37664	NA	1.04068	NA

	US3	0.10929	NA	2.04408	NA	-0.0092	NA	0.89318
9/21/2015	BS1	0.11246	NA	NA	NA	NA	NA	NA
	BS2	0.65448	NA	NA	NA	NA	NA	NA
	BS3	1.1755	NA	NA	NA	NA	NA	NA
	FS1	0.39869	NA	NA	NA	NA	NA	NA
	FS2	0.59337	NA	NA	NA	NA	NA	NA
	FS3	0.26263	NA	NA	NA	NA	NA	NA
	HP1	0.19904	NA	NA	NA	NA	NA	NA
	HP2	0.1644	NA	NA	NA	NA	NA	NA
	HP3	0.22495	NA	NA	NA	NA	NA	NA
	US1	0.33243	NA	4.94	NA	-0.18	NA	0.61542
	US2	0.34916	NA	NA	NA	NA	NA	NA
	US3	0.03647	NA	NA	NA	NA	NA	NA
10/12/2015	BS1	0.1707	2.31333	1.97	0.59772	-0.2008	1.36476	0.6827
	BS2	0.83797	NA	NA	NA	NA	NA	NA
	BS3	0.59378	NA	NA	NA	NA	NA	NA
	FS1	0.85138	NA	NA	NA	NA	NA	NA
	FS2	0.14869	NA	NA	NA	NA	NA	NA
	FS3	0.8572	NA	NA	NA	NA	NA	NA
	HP1	0.57992	NA	NA	NA	NA	NA	NA
	HP2	0.1097	2.04408	4.94	0.21577	0.37968	1.09559	1.24329
	HP3	0.23839	4.96	4.96	0.1374	-0.2656	0.91845	0.59299
	US1	0.42533	NA	NA	NA	NA	NA	NA
	US2	0.44179	4.98	NA	1.10796	NA	1.46205	NA
	US3	0.05456	2.735	NA	-0.3062	NA	0.65794	NA
11/2/2015	BS1	NA	NA	NA	NA	NA	NA	NA
	BS2	4.78571	NA	NA	NA	NA	NA	NA

	BS3	NA	NA	NA	NA	NA	NA	NA
	FS1	2.27784	NA	NA	NA	NA	NA	NA
	FS2	1.13053	NA	NA	NA	NA	NA	NA
	FS3	2.67128	NA	NA	NA	NA	NA	NA
	HP1	4	NA	NA	NA	NA	NA	NA
	HP2	4.14286	NA	NA	NA	NA	NA	NA
	HP3	4.53846	NA	NA	NA	NA	NA	NA
	US1	1.40333	NA	NA	NA	NA	NA	NA
	US2	0.50465	NA	NA	NA	NA	NA	NA
	US3	4.4	NA	NA	NA	NA	NA	NA

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