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The University of Southern Mississippi

INFLUENCE OF DETRITUS LEVELS AND ORGANIC POLLUTION ON INTERSPECIFIC RESOURCE COMPETITION, OVIPOSITION BEHAVIOR, AND LARVAL SURVIVAL OF TWO TIRE-INHABITING MOSQUITO SPECIES (DIPTERA: CULICIDAE)

by

David Wayne Allgood

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

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December 2011

ABSTRACT

INFLUENCE OF DETRITUS LEVELS AND ORGANIC POLLUTION ON INTERSPECIFIC RESOURCE COMPETITION, OVIPOSITION BEHAVIOR, AND LARVAL SURVIVAL OF TWO TIRE-INHABITING MOSQUITO SPECIES (DIPTERA: CULICIDAE)

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December 2011

Mosquitoes (Diptera: Culicidae) are vectors of disease in the adult stage, but understanding the factors affecting distributions of the immature stages is important to the understanding and control of adult populations. Discarded automobile tires comprise important larval mosquito habitats. The Asian tiger mosquito (*Aedes albopictus*) and the southern house mosquito (*Culex quinquefasciatus*) are two medically important species commonly found in tires, but factors affecting their larval distributions in tires have not been studied, nor have their interspecific interactions. I investigated the effects of chemicals associated with organic pollution on oviposition preferences and larval survival of both species, and the effects of resource limitation, interspecific density, and chemical pollution on interspecific competition between both species. I conducted field oviposition bioassays in tires containing different pollution concentrations, and laboratory larval survivorship bioassays in the same concentrations. Both species laid significantly more eggs in higher pollution concentrations, but there was no relationship between oviposition preference and larval survival in polluted water. In the laboratory, I measured larval survivorship, development time, adult mass, and population growth of both species under different resource levels, interspecific larval densities, and pollution

concentrations. *Culex quinquefasciatus* survivorship and population growth were more detrimentally affected at low resource levels and at high interspecific densities, indicating that *Ae. albopictus* is a superior resource competitor. The presence of pollution did not affect the competitive outcome. My results indicate that organic pollution increases the susceptibility of tires to colonization by these species, and that larval competition between these species may affect adult populations.

ACKNOWLEDGMENTS

I would like to thank my thesis director, Dr. Donald Yee, and my committee members Dr. Kevin Kuehn and Dr. Carl Qualls for providing valuable advice on my experimental designs and statistical analyses, and for their helpful comments and suggestions on earlier versions of this document. I also thank Dr. Donald Yee, Dr. Sandra Allan, Dr. Jocelyn Millar, Dr. Katie Costanzo, Sean McCann, Dr. Margaret C. Wirth, Ebony Murrell, and Lynn Landrum for advice and instruction on mosquito rearing and chemical preparation.

I am grateful to Dr. Sandra Leal for use of her equipment and for providing fruit flies for my experiments; Dr. Douglas Masterson for providing chemicals and for use of his lab; and the USDA/ARS Center for Medical, Agricultural and Veterinary Entomology in Gainesville, FL for providing mosquitoes. I thank Elizabeth Himel, Anna Reiber, Francis Ezeakacha, Brad Hopkins, Thomas Bocek, Kris Pitcher, William Glasgow, Jeff Skiff, Jim Beard, Tim Stockstill, Ashton Reeves, and Tiffany Pleasant for assistance in the lab and in the field. This research was funded by the University of Southern Mississippi's Department of Biological Sciences and the Mississippi Entomological Association.

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INTRODUCTION

Problem Statement

Mosquitoes are of major research interest due to their role as hosts for various pathogens that are capable of causing disease (e.g., malaria, dengue fever, yellow fever, and West Nile virus) in vertebrates, including humans and livestock (Clements 2000). Although mosquitoes are biting nuisances and disease vectors in the adult stage, understanding the biology and ecology of immature stages, which inhabit lentic aquatic habitats, is integral to the understanding and control of adult populations.

Although the presence of larvae in aquatic habitats is thought to be due to female oviposition preferences rather than habitat suitability (Macan 1961, Mian and Mulla 1986, Roberts 1996, Clements 1999), the absence of a species in a habitat may also be due to unattractive or toxic conditions, or antagonistic biotic interactions, such as predation or competition (Macan 1961). Understanding intra- and interspecific interactions, and biotic and abiotic factors that influence oviposition and survival of offspring are crucial to understanding the population dynamics of these organisms.

Mosquito species respond differently to various oviposition cues (Clements 2000). Factors that affect oviposition behavior of mosquitoes include salinity (Roberts 1996), water color (Ikeshoji 1975, Beehler et al. 1993b), presence of conspecific or congeneric eggs (Bruno and Laurence 1979, Laurence and Pickett 1982, Allan and Kline 1998), presence of conspecific or congeneric larvae (Allan and Kline 1995, 1998, Clements 1999, Allan et al. 2005), habitat structure (Subra 1981), container type (Chambers et al. 1986), and container opening size (Chambers et al. 1986). Much of the research on mosquito oviposition behavior focuses on the effects of detritus and its

associated chemicals (e.g., Ikeshoji 1975, Du and Millar 1999, Allan et al. 2005). Mosquito oviposition behavior may not be influenced by detritus *per se*, but by the intermediate metabolites generated by bacteria as they decompose the detritus (Ikeshoji 1975, Kramer and Mulla 1979). The potential of detrital infusions to affect oviposition behavior changes over time (Isoe et al. 1995, Mboera et al. 2000), and is likely related to temporal changes in microbial biomass and activity (Ikeshoji 1975, Kramer and Mulla 1979). Detrital infusions that elicit positive oviposition responses may act as attractants, arrestants, oviposition stimulants, or some combination of the three. Briefly, attractants orient movements toward the source, repellants orient movements away from away from the source, and arrestants inhibit movement (i.e., cause mosquitoes to land on the oviposition substrate) (Dethier et al. 1960). Oviposition stimulants and deterrents initiate and inhibit oviposition, respectively.

It is generally accepted that the distributions of larval mosquitoes are due to adult female oviposition behavior, but certain factors associated with the larval environment are known to influence larval development and survival, and may lead to selection for increased oviposition responses in optimal larval habitats (Ellis 2008). Availability of resources, habitat size, and larval density are known to affect larval survival and adult body size (Mori 1979, Smith et al. 1995, Agnew et al. 2000, Wynn and Paradise 2001, Dieng et al. 2002). The types of detritus present in an aquatic environment are also important to mosquito larvae. Certain types of detritus may constitute more suitable resources than others (Daugherty et al. 2000, Yee and Juliano 2006, Reiskind et al. 2009). Additionally, detritus, or the bacteria that decompose it, may release chemicals that are detrimental to mosquitoes, and some species may be more susceptible to certain chemicals than others (David et al. 2000, Murrell and Juliano 2008). While it is clear that

certain conditions are important to both adult oviposition behavior and larval survival, few studies have simultaneously made direct quantitative comparisons between mosquito oviposition response and larval survival under a given set of conditions.

This work was designed to investigate the relationship between mosquito oviposition responses and larval survival in polluted water for two species of tire-breeding mosquitoes (*Aedes albopictus* Skuse and *Culex quinquefasciatus* Say), and how interspecific larval interactions between these two species are affected by resource abundance and the presence of chemicals associated with organic pollution (i.e., excessive detritus, animal excreta, and waste).

Study Organisms

Taxonomy

Mosquitoes (Family Culicidae) are true flies (Order Diptera) in the suborder Nematocera. Like other dipterans, mosquitoes possess only one pair of wings; the hindwings are modified into knoblike halteres, which aid in flight coordination. Mosquitoes are distinguished from other dipterans by their elongated proboscis, coupled with the presence of scales on the body and wing veins (Triplehorn and Johnson 2005).

Life History and General Biology

Mosquitoes undergo complete metamorphosis, meaning they have four distinct life stages: an egg, larva, pupa, and adult (Clements 2000). The larval and pupal stages are aquatic, and the adult stage is terrestrial. Mosquito eggs are deposited on or near lentic bodies of water, including shallow pools, marshes, natural containers (e.g., rock pools, phytotelmata), and artificial containers (e.g., discarded tires, barrels, buckets) (Clements 2000). Hatching may occur when development is completed, or for some species, when the water level rises sufficiently to immerse the egg (Clements 2000).

Larval mosquitoes molt four times during their development; the first three molts give rise to the second, third, and fourth larval instars, and the final molt produces the pupa (Clements 2000). When adult development is completed, usually within two days, the adult will emerge from the pupal case onto the water surface, and females of most species will mate and then take a blood meal from a vertebrate host (Clements 2000). After digesting the blood meal, a single female may lay between 50 to 500 eggs in one batch (Clements 2000). In some species, the next gonotrophic cycle will begin after oviposition, and the female will seek another blood meal in order to provision its next batch of eggs (Clements 2000).

Mosquito larvae feed by using their brush-like mouthparts to either create water currents that bring particulate matter to the mouth (i.e., filtering), or by using them to remove particles from surfaces (i.e., browsing) (Clements 2000). Larvae mainly feed on aquatic microorganisms (e.g., bacteria, protists) that colonize organic detritus (e.g., senescent leaves, bark, fruits, invertebrate carcasses), as well as consuming tiny fragments of detritus itself (Merritt et al. 1992). Adult mosquitoes possess an elongated proboscis that is used to obtain sugar from nectar and other plant juices to meet their nutritional needs, and in the case of females, to obtain blood meals from vertebrate hosts (Clements 2000).

Mosquito eggs may be laid singly or in clusters on or above the water surface. Some mosquito species are known to distribute a single ‘batch’ of eggs in ‘clutches’ over multiple sites (Clements 1999), a behavior known as skip oviposition. A batch of mosquito eggs is the entirety of matured eggs laid by a female in a given gonotrophic cycle, and a clutch is a group of eggs deposited by one female at a single location (Clements 1999).

Study Species

This research focuses on the Asian tiger mosquito (*Aedes albopictus* Skuse) and the southern house mosquito (*Culex quinquefasciatus* Say), both of which are competent vectors of important diseases. I chose these species because they are common in my study system (vehicle tires, discussed below), and although both species commonly occur in tires and other container habitats, their interspecific interactions have not been studied.

Aedes albopictus is a member of the subfamily Culicinae, and belongs to the tribe Aedini (Clements 2000) and the subgenus *Stegomyia* (Hawley 1988). *Aedes albopictus* is endemic to east Asia and numerous islands in the Indian Ocean (Hawley 1988). It was first described from present-day West Bengal, India in 1894, and the species is thought to have originated in southeast Asia (Hawley 1988). In recent decades, *Ae. albopictus* has spread throughout the world due to the international shipping of artificial containers (e.g., tires), and now occurs on all continents except mainland Australia and Antarctica (Paupy et al. 2009). The species is found predominantly in rural and suburban locations, and in vegetated urban areas (Hawley 1988, Braks et al. 2003, Lopes et al. 2004). *Ae. albopictus* is a seasonal species, with a peak abundance in late summer and early fall (Joy 2004, Costanzo et al. 2005a).

Ae. albopictus is an invasive species in North America. Breeding populations in the U.S. were first documented in 1985 in Harris County, TX (Sprenger and Wuithiranyagool 1986). By 1988, *Ae. albopictus* had spread throughout much of the eastern U.S. (Hawley 1988). Since its discovery in the U.S., *Ae. albopictus* has become the most abundant species in tires in the southeastern U.S. (Sprenger and Wuithiranyagool 1986, Yee 2008).

Ae. albopictus deposit desiccation-resistant eggs singly on container walls above the water line, and the eggs hatch when flooded (Hawley 1988). Development time from egg to adult varies by habitat under field conditions. Gomes et al. (1995) found that development time ranged from approximately 20 to 38 days, depending on container type. In the lab, development from egg to adult takes approximately a week with adequate food at 27 °C (Gerberg et al. 1994).

Adult female *Ae. albopictus* bite predominantly mammals (Hawley 1988, Mitchell et al. 1992). Blood feeding and oviposition occur primarily during the day (Hawley 1988, Trexler et al. 1997). A single *Ae. albopictus* female may lay 40 to 90 eggs in a single batch (Hawley 1988), with larger females laying more eggs (Armbruster and Hutchinson 2002). *Aedes albopictus* is capable of distributing a single batch of eggs over multiple sites (i.e., skip oviposition) (Clements 1999).

Temperature, detritus, water chemistry, and larval density appear to be largely responsible for determining the larval performance of *Ae. albopictus*. Higher temperatures lead to faster larval development and more rapid adult population growth (Alto and Juliano 2001, Neto and Navarro-Silva 2004). More labile detritus positively affects development rate and adult body size (Dieng et al. 2002), but high concentrations of labile detritus (e.g., invertebrate carcasses) may negatively affect larval survival and performance (Murrell and Juliano 2008). Finally, high densities of conspecific larvae negatively affect larval performance via intraspecific competition (Dieng et al. 2002, Costanzo et al. 2005a, Murrell and Juliano 2008) and release of chemicals that suppress conspecific larval development (Mori 1979).

In its native range, *Ae. albopictus* is a vector of dengue virus (Hawley 1988), and could potentially serve as a dengue vector in the U.S. if the virus were to become

established (Moore and Mitchell 1997). Although there are no confirmed cases of disease transmission to humans by *Ae. albopictus* in the U.S., La Crosse virus (Gerhardt et al. 2001, Lambert et al. 2010), eastern equine encephalitis (Mitchell et al. 1992), and Cache Valley virus (Moore and Mitchell 1997) have been isolated from naturally infected field specimens in the U.S., and *Ae. albopictus* is a competent laboratory vector of these diseases (Tesh and Gubler 1975, Grimstad et al. 1989, Scott et al. 1990, Moore and Mitchell 1997). *Aedes albopictus* is a competent laboratory vector of a number of other diseases (reviewed in Moore and Mitchell 1997, Paupy et al. 2009), but these diseases are not prevalent in North America or are primarily carried by avian hosts (Mullen and Durden 2002).

Culex quinquefasciatus is a member of the subfamily Culicinae, and belongs to the tribe Culicini (Clements 2000). It was originally described from the western U.S. in 1823 and is thought to have originated from Africa, dispersing to other continents by ship sometime prior to its discovery (Vinogradova 2000). *Culex quinquefasciatus* is predominantly an urban species (Subra 1981, Lopes et al. 2004) and occurs in the southern U.S., southern Japan, throughout Africa, and throughout other tropical and subtropical regions (Subra 1981, Vinogradova 2000). In the U.S., *Cx. quinquefasciatus* is mainly active during warmer months (Tesh et al. 2004, Harbison et al. 2009).

Culex quinquefasciatus females lay their eggs in rafts that float on the water surface (Subra 1981). Development times for *Cx. quinquefasciatus* range from 6 to 62 days in the lab and 11 to 47 days in the field, with more rapid development occurring at higher temperatures and in the presence of suspended solids (de Meillon et al. 1967, Hayes and Hsi 1975, Harbison et al. 2009). Larval development time increases when

food is withheld from first instars (de Meillon et al. 1967) and when larval density is high (Smith et al. 1995, Agnew et al. 2000).

Culex quinquefasciatus larvae are found mainly in anthropogenic habitats including latrines, septic tanks (Subra 1981), polluted ponds, storm drains (Barr 1965, Strickman and Lang 1986, Harbison et al. 2009), tires and other (Barr 1965, Subra 1981, O'Meara and Evans 1983) artificial containers (Barr 1965, Subra 1981, Chambers et al. 1986), wells, ditches, and gutters (Subra 1981). Several surveys of tires and other artificial containers in urban areas in the range of *Cx. quinquefasciatus* have found it to be the most abundant species collected (Lopes et al. 2004), or the second most abundant species collected after the predominant *Aedes* species (Chambers et al. 1986, Sprenger and Wuithiranyagool 1986). *Culex quinquefasciatus* is one of the few pollution-tolerant mosquito species (Subra 1981, Clements 2000); larvae are usually found in water containing high amounts of organic pollution, especially human and animal excreta (Barr 1965, Subra 1981). Chemical factors positively associated with larval abundance include free ammonia, organic carbon, nitrates, higher salt concentrations, and slightly alkaline waters (pH ranges of 7.2-7.7) (Sinha 1976).

Adult *Cx. quinquefasciatus* disperse usually only a short distance from the site of emergence (Subra 1981). Females usually blood feed at night, and may bite humans either indoors or outdoors (Subra 1981). Different populations are known to be anthropophilic (human biting) or ornithophilic (bird biting), depending on location (Subra 1981). Females oviposit nocturnally with a peak around dusk, but findings vary on whether a second peak in oviposition occurs around dawn (Beehler et al. 1993a, Mboera et al. 2000). An average *Cx. quinquefasciatus* egg raft consists of about 155 eggs (Subra

1981); larger females have higher fecundity (McCann et al. 2009). A female usually takes another blood meal within half a day of ovipositing (Subra 1981).

Culex quinquefasciatus is an important pest of humans and livestock (Barr 1965). In the U.S., it is a competent vector of West Nile virus (Sardelis et al. 2001, Goddard et al. 2002), and has been implicated as a primary West Nile virus vector for human infection in the southern U.S., due to its propensity to feed on both birds and mammals (Molaei et al. 2007). *Culex quinquefasciatus* is also a vector of St. Louis encephalitis (Hardy et al. 1984), and the virus was isolated from field collected *Cx. quinquefasciatus* specimens during an epidemic that occurred in Pine Bluff, AR in 1991 (Savage et al. 1993).

Study System

Water-filled vehicle tires are an important habitat for larval mosquitoes (Yee 2008). Scirtid beetles, various crustaceans, and dipteran families including Chironomidae, Psychodidae, Ceratopogonidae, Syrphidae, Corethrellidae, Chaoboridae, and Stratiomyidae may also be found in tires (Yee 2008). Although tire surveys for mosquito larvae are common in the literature, the biotic and abiotic factors that influence mosquito oviposition and community structure in tires are seldom investigated (Yee 2008). Many environmental parameters that affect mosquito communities in other aquatic systems differ among tires, including detritus type (Kling et al. 2007), chemical properties, pH, turbidity, alkalinity, conductivity, water color, and temperature (Beier et al. 1983b). Environmental parameters that affect mosquito presence, abundance, and community composition in tires include canopy/shading, water color, turbidity, ammonia (Beier et al. 1983a, Beier et al. 1983b), solute concentration (Costanzo et al. 2005a), amount and type of detritus (Kling et al. 2007), and the site where the tire is stored or

discarded (Lopes et al. 2004, Costanzo et al. 2005a, Kling et al. 2007). Factors associated with the tire itself, such as rim diameter, bead gap (i.e., the gap between the inner edges), and a tire's location in a pile also affect susceptibility to colonization and community structure (Morris and Robinson 1994).

Tires are important to the study of vector dynamics for two reasons. First, tires may constitute long lasting habitats for vector mosquitoes in close proximity to humans and livestock, due to their ubiquity and durability (Yee 2008). Tire dealerships are common in the U.S. with respect to human population density; large dealers, which are concentrated in urban areas, ship tires to smaller dealers in towns and rural areas (Reiter and Sprenger 1987). Tires stored outdoors for long periods of time, such as those awaiting shipment or discarding at dealerships, become subject to mosquito infestation (Reiter and Sprenger 1987). In addition to tires stored at dealerships, discarded tires are one of the most common types of artificial container habitats utilized by larval mosquitoes, and they are especially abundant in low income areas (Chambers et al. 1986). Bunker tires used in agriculture are also important larval mosquito habitats (Kaufman et al. 2005).

Second, the shipping of tires, both within and between areas that are geographically isolated, facilitates range expansion and introduction of invasive container breeding species. Mosquito infested tires have been arriving at seaports for decades, with recorded observations dating back to the 1940s (Reiter and Sprenger 1987). In recent decades, increasing ease of shipping and handling and lax standards for the inspection and treatment of insect-infested tires have led to more frequent importations of insect-infested cargos at U.S. ports (Reiter and Sprenger 1987). Many mosquito invasions and range expansions in the U.S. have been by container breeding mosquitoes (Lounibos

2002), but vector species may be imported into any country involved in the trade of containers (Reiter and Sprenger 1987). As previously discussed, *Ae. albopictus* is an excellent example of an invasive species that has expanded its range to other continents via the shipping of tires.

Research Questions

The main objective of this research was to understand how resource abundance, larval interspecific interactions, and presence of chemicals associated with organic pollution affect adult oviposition, larval development, and survival to adulthood of the co-occurring tire-breeding mosquito species *Ae. albopictus* and *Cx. quinquefasciatus*. To accomplish this objective, I conducted experiments to answer the following questions: 1) Does the concentration of chemicals associated with organic pollution and detrital decomposition affect oviposition response by either species, and do oviposition responses regarding organic pollution correspond to suitability of the larval habitat?; 2) Does interspecific resource competition occur between larval *Ae. albopictus* and *Cx. quinquefasciatus*?; 3) Does the concentration of certain chemicals associated with organic pollution and detrital decomposition affect larval development, survivorship, and interspecific competition when *Ae. albopictus* and *Cx. quinquefasciatus* co-occur?

Significance of Study

This is the first study to examine the nature of interspecific interactions between larval *Ae. albopictus* and *Cx. quinquefasciatus*. The results obtained from this study also contribute to a better understanding of the factors that affect susceptibility of tires to colonization by *Ae. albopictus* and *Cx. quinquefasciatus*, and how detritus types and chemical composition within the larval rearing environment influence interspecific interactions and survival to adulthood of these species. This, in turn, contributes to a

better understanding of how the size and structure of adult vector mosquito populations are affected by the larval rearing environment, especially in locations where tires represent the majority of available mosquito breeding sites.

CHAPTER II

OVIPOSITION RESPONSES AND THEIR RELATIONSHIP WITH LARVAL SURVIVAL IN POLLUTED WATER

Introduction

Oviposition decisions made by female insects are an important determining factor in the distributions of immature stages, especially in situations where the immature stages are limited to the habitat in which they hatch. Such is the case for container-breeding mosquitoes, which are confined to water-filled containers in the larval and pupal stages. Understanding cues that influence oviposition decisions of mosquitoes is integral to mosquito surveillance and control, as such knowledge allows for predictions for where mosquitoes are mostly likely to breed. Additionally, attractive oviposition cues (e.g., organic infusions and synthetic chemicals that mimic them) may be used to bait gravid traps and oviposition traps (ovitraps). Gravid traps are designed to selectively capture gravid (i.e., blood fed and potentially infected) females (e.g., Reiter 1983) and are especially important when surveying for disease-infected mosquitoes in field populations (e.g., Savage et al. 1993). Ovitraps are designed to detect the presence of mosquitoes by collecting their eggs, and may be used to detect transovarial transmission of arboviruses from female mosquitoes to offspring in naturally infected populations (e.g., Gerhardt et al. 2001). Lethal ovitraps are designed to kill gravid mosquitoes by exposing them to a lethal insecticide dose during oviposition, and have been used to reduce *Aedes* mosquitoes in areas with high dengue and Chikungunya virus activity (reviewed in Zeichner 2011).

The oviposition behaviors of the medically important mosquitoes *Aedes albopictus* and *Culex quinquefasciatus* have been studied extensively. Surveillance for

Cx. quinquefasciatus using gravid traps is effective due to the high selectivity of this species for certain organic infusions, but captures of *Ae. albopictus* using this method have been less effective, as *Ae. albopictus* seems to be less selective in its oviposition decisions than *Cx. quinquefasciatus* (Burkett-Cadena and Mullen 2007, 2008, McPhatter and Debboun 2009) and less influenced by olfactory cues (Trexler et al. 1998).

Aedes albopictus show increased oviposition responses to organic infusions such as hay, oak leaves, and pine (Holck et al. 1988, Allan and Kline 1995, Obenauer et al. 2009) when compared to water controls. Additionally, *Ae. albopictus* females oviposit more often in containers with conspecific or congeneric larvae compared to no larvae (Allan and Kline 1995, 1998). Oviposition responses of female *Cx. quinquefasciatus* are positively affected by a number of plant-based infusions (e.g., Bermuda grass, cattail; Allan et al. 2005), wastewater effluent (Mian and Mulla 1986, Allan et al. 2005), and human and animal excreta (Kramer and Mulla 1979, Blackwell et al. 1993, Mboera et al. 1999). Dark colored waters (Beehler et al. 1993b), the presence of conspecific larvae (Allan et al. 2005), and conspecific egg rafts (Bruno and Laurence 1979) also illicit increased oviposition responses. Some infusions elicit greater oviposition responses from *Cx. quinquefasciatus* as they age and become increasingly malodorous (Isoe et al. 1995), which is consistent with the observed affinity of this species for polluted water (Subra 1981).

Aquatic environments may become polluted by high concentrations of fermenting plant or animal detritus, animal excreta, or waste from other anthropogenic sources. Organic pollution in discarded automobile tires and other containers, where *Ae. albopictus* and *Cx. quinquefasciatus* occur, would most likely originate from fermenting detritus, which at high concentrations can putrefy water. Additionally, organic

compounds may leach from the rubber of tires themselves (Evans 1997), and in situations where tires are discarded in landfills or other dumping sites, pollution may originate from inputs of garbage or contaminated runoff.

Millar et al. (1992) identified skatole (3-methylindole), *p*-cresol (4-methylphenol), indole, phenol, and 4-ethylphenol as important chemical constituents of fermenting Bermuda grass (*Cynadon dactylon* L.) infusions, which have been found to be attractive to gravid *Cx. quinquefasciatus* in field surveys (e.g., Allan et al. 2005, Burkett-Cadena and Mullen 2007). A blend of these five chemicals elicited a greater oviposition response than clean water from *Cx. quinquefasciatus* in the lab (Millar et al. 1992) and in the field (Beehler et al. 1994). The five-chemical blend also interacts synergistically with dark colored water to increase oviposition response (Beehler et al. 1993b). The five-chemical blend and its individual constituents do not affect oviposition responses of *Ae. albopictus* to the same degree as *Cx. quinquefasciatus* in the field (Allan and Kline 1995).

In a follow-up study to Millar et al. (1992), Du and Millar (1999) isolated 10 chemicals from the headspace odors above fermenting Bermuda grass; the 10 chemicals consisted of the five chemicals previously isolated by Millar et al. (1992), in addition to nonanal, 2-undecanone, 2-tridecanone, naphthalene, and dimethyl trisulfide. When the 10 compounds were tested individually, nonanal and skatole elicited the greatest oviposition response from *Cx. quinquefasciatus*; however, the 10 chemical blend elicited significantly greater oviposition response from *Cx. quinquefasciatus* than any of the individual components (Du and Millar 1999). In addition to their presence in decaying plant material, skatole and indole are found in animal feces (O'Neil et al. 2006), and skatole, indole, naphthalene, phenol, and *p*-cresol have been identified in landfill leachates (Harmsen 1983, Öman and Hynning 1993, Schwarzbauer et al. 2002). Although

the 10-chemical blend has been used to test oviposition responses of *Cx. quinquefasciatus*, its effects on larval mosquito performance have not been investigated, nor has its effect on oviposition responses of *Cx. quinquefasciatus* or *Ae. albopictus* under field conditions.

The majority of studies on mosquito oviposition cues are conducted in the interest of enhancing trapping and surveillance techniques, with few studies having investigated the relationship between oviposition behaviors and larval survival. According to optimal oviposition theory, the oviposition decisions of insects should be associated with optimal larval performance (Jaenike 1978). In phytophagous insects, oviposition decisions of some females reflect suitability of habitat for offspring performance, while the decisions of other females do not (reviewed in Thompson 1988). This topic is less studied for mosquito taxa. Oviposition decisions of the pitcher plant mosquito (*Wyeomyia smithii* Coquillett) and the eastern tree hole mosquito (*Ae. triseriatus* Say) indicate that these species show some, but not total preference for optimal larval habitat (Heard 1994, Edgerly et al. 1998, Ellis 2008). *Aedes albopictus* shows higher oviposition responses to leaf detritus that supports better larval performance (Reiskind et al. 2009). In contrast, *Cx. quinquefasciatus* does not seem to show increased oviposition preference for optimal larval habitat, a disconnect that has been observed in several tire-inhabiting *Culex* species (Yee et al. 2010). Oviposition responses of an individual female *Cx. quinquefasciatus* appear to be influenced by the chemical properties of that female's larval rearing environment (McCall and Eaton 2001). Mian and Mulla (1986) reported that although *Cx. quinquefasciatus* always oviposited in secondary sewage effluent as opposed to distilled water, larval survival to adulthood was not significantly higher in secondary sewage effluent. Additionally, Roberts (1996) found that *Cx. quinquefasciatus* virtually

always oviposited in freshwater as opposed to saline water, even though larval survival was higher in slightly saline water.

In this chapter, my objectives were to determine if different concentrations of certain chemicals associated with fermenting detritus and organic pollution (hereafter, pollution) affect oviposition responses of *Ae. albopictus* and *Cx. quinquefasciatus* under field conditions, and if there is an association between the oviposition preferences of these species and the survival of their larvae in these chemicals. I hypothesized that, 1) oviposition responses of both species will be affected by different concentrations of pollution, and 2) oviposition responses of females will have different associations with the suitability of larval habitat for different species. Based on current knowledge, I predicted that, 1) *Cx. quinquefasciatus* would oviposit more often in polluted water as opposed to reverse osmosis (RO) filtered water, and *Ae. albopictus* would oviposit more often in RO water as opposed to polluted water, and 2) *Cx. quinquefasciatus* oviposition responses would not correspond to suitability of larval habitat, but *Ae. albopictus* would oviposit more often in habitats more suitable to larval survival.

Methods

Mosquito Rearing

Colonies used to generate mosquitoes for experiments were established from *Ae. albopictus* and *Cx. quinquefasciatus* eggs and larvae collected from aquatic habitats in and around Hattiesburg, MS. A laboratory acclimated strain of *Cx. quinquefasciatus* (hereafter, lab *Cx. quinquefasciatus*) from Gainesville, FL that has been in colony since 1995 was provided by the USDA/ARS Center for Medical, Agricultural and Veterinary Entomology in Gainesville, FL. A colony of lab *Cx. quinquefasciatus* was established at USM in July 2010 and maintained using the methods described below; previous

Table 1

Daily feeding schedule (mg puppy chow per larva) for mosquito rearing.

	Cx. quinquefasciatus	*Ae. albopictus*
Day 0	0.1500	0.2000
Day 1	0.2500	0.2000
Day 2	0.2500	0.3000
Day 3	0.3000	0.4000
+Yeast	+0.1100	$+4.5x10^{-5}$
Day 4	0.3000	0.6000
Day 5	0.4000	0.6000
Day 6	0.5000	0.6000
Day 7	0.7000	0.6000

Note. Amounts of puppy chow per larva are taken from Gerberg et al. (1994).

generations were maintained using the methods described in Allan et al. (2006). Field-collected larvae were identified using keys by Darsie and Ward (2005) and reared to adults in the laboratory. Larvae of the two species were reared to adults on Purina® Puppy Chow® and brewers yeast (Acros Organics, Morris Plains, NJ, USA) on an eight-day feeding schedule (Table 1). Adults were maintained in a colony room kept at approximately 27 °C on a 14:10 hour light:dark cycle with one hour of dawn and one hour of twilight and were provided with a cotton pad soaked with 10 % sugar solution. Anesthetized guinea pigs were used to blood feed *Ae. albopictus* and lab *Cx. quinquefasciatus* (IACUC #A3851-01, 14 Aug 2009), and the arm of the experimenter was used to blood feed Hattiesburg-collected *Cx. quinquefasciatus*. *Ae. albopictus* were provided black cups lined with paper towels and filled to 2.5 cm with RO water for oviposition, and *Cx. quinquefasciatus* were provided black bowls filled to 2.5 cm with larval rearing water. Eggs were used in experiments and to establish new colonies. Mosquito colonies were continually maintained and stocked using these methods.

Although oviposition preferences appear to be learned rather than inherited with each generation based on larval rearing conditions (McCall & Eaton 2001), I attempted to standardize any possible differences in long-term conditioning by using mosquitoes two generations removed from field populations (F_2) in oviposition bioassays. Both F_2 (hereafter, wild) and lab *Cx. quinquefasciatus* were used in oviposition bioassays in order to test for effects of lab acclimation on oviposition response; controlled oviposition bioassays involving this species often use strains that have been selected for laboratory rearing to generate gravid females (e.g., Kramer and Mulla 1979, Isoe et al. 1995, Allan et al. 2005), as wild strains are difficult to blood feed in captivity, and may not feed in sufficient numbers to generate enough gravid females for experiments. Lab *Cx. quinquefasciatus* eggs were collected in larval rearing water for two generations prior to oviposition bioassays. Eggs of this strain were normally collected with fresh (tap or RO) water, but wild strains will not readily oviposit in fresh water, so larval water was used for consistency between strains.

Chemical Blend

The synthetic infusion used to mimic polluted water was the blend of 10 chemical compounds (Du and Millar 1999). The blend was prepared by dissolving chemicals in diethyl ether to make stock solutions that produced the low and high concentration chemical blends (Table 2) when added to water in the appropriate amounts (Du and Millar 1999). Concentrations of compounds in the low concentration treatment reflect concentrations in headspace extracts above infusions containing 4.5 g/L Bermuda grass fermented with 0.27 g/L lactalbumin hydrolyzate and brewers yeast for nine days (Du and Millar 1999). This concentration was most effective for eliciting oviposition responses from *Cx. quinquefasciatus* (Du and Millar 1999); the blend at high

Table 2

Concentration of each chemical present in low and high concentration pollution treatments.

Chemical	Low	High
Dimethyl trisulfide	576 ng/L	57.6 µg/L
Phenol	29 ng/L	2.9 µg/L
p-Cresol	980 ng/L	98.0 µg/L
Nonanal	39 ng/L	3.9 µg/L
4-Ethylphenol	5 ng/L	0.5 µg/L
Naphthalene	25 ng/L	2.5 µg/L
Indole	52 ng/L	5.2 µg/L
2-Undecanone	22 ng/L	2.2 µg/L
3-Methylindole	804 ng/L	80.4 µg/L
2-Tridecanone	15 ng/L	1.5 µg/L

Note. Concentrations are based on those used by Du and Millar (1999).

concentration (100x the low concentration) was repellent (but not deterrent) to gravid *Cx. quinquefasciatus*.

Field Bioassays

Female *Ae. albopictus* and *Cx. quinquefasciatus* were blood fed 2-8 days and 5-12 days, respectively, post-emergence. Mosquitoes were removed from colony cages via aspiration after blood feeding and knocked out with CO_2, and blood-engorged females were sorted from other mosquitoes and transferred to separate colony cages. Blood-fed females of *Ae. albopictus* and *Cx. quinquefasciatus* were held for 3 and 7 days, respectively, after which time they were presumed gravid. At this time, gravid females were knocked out with CO_2 and counted, transferred to 40 mL vials stopped with cotton, and introduced to field bioassay cages within two hours.

Field bioassays were conducted beneath a wood-framed structure with a shade-cloth ceiling and a concrete floor (hereafter, pad) at the USM Science Park in

Hattiesburg, MS. The area immediately surrounding the pad was a grassy lawn, leaving no vegetative canopy above the pad.

Experimental tires (passenger car or light truck tires with radial construction and a wheel diameter of 16 inches) were set up 24 h prior to the introduction of gravid females. Tire interiors were treated with 10% bleach solution, scrubbed with a scour pad, and thoroughly washed with tap water before each run of the experiment. Chemical concentrations and mosquito species were randomly assigned for each tire and tire pair, respectively, for each run of the experiment. Each tire received 3.5 L RO water and 3.5 mL of appropriately concentrated stock solution; control tires received 3.5 mL of diethyl ether. Each experimental unit consisted of a pair of tires containing differing chemical concentrations (control and low concentration, control and high concentration, or low and high concentration) covered with a cage made from plastic PVC piping (1.5 x 0.8 x 0.8 m), mosquito netting, and clear plastic covering on top to prevent inputs of organic detritus, rainwater, intrusion by other animals, and escape of adult female mosquitoes. Forty-eight tires were used, yielding 24 experimental units for each run of the experiment. The inner surfaces of tires receiving *Ae. albopictus* eggs were lined with brown paper towels, as *Aedes* mosquitoes oviposit on container walls just above the water surface (Hawley 1988), whereas *Culex* mosquitoes oviposit directly on the water surface (Subra 1981). The arrangement of treatments within each cage was randomized. Because three different species/strains (*Ae. albopictus*, wild *Cx. quinquefasciatus*, lab *Cx. quinquefasciatus*; hereafter, strains) were used across three different pairwise chemical concentration pairings (hereafter, combinations), I was unable to divide experimental units evenly among strains within a single run of the experiment while maintaining a balanced design with respect to combinations; therefore, I divided the 24 experimental

units among the three strains in a 6-6-12 arrangement and ran the experiment three times, with each strain receiving 12 experimental units in one run of the experiment, and six units in the other two runs. Each combination was replicated two times per strain when six experimental units were used, and four times per strain when 12 units were used. This produced eight replicates per strain of each combination after three runs of the experiment.

Ten gravid female *Ae. albopictus* or 20 gravid *Cx. quinquefasciatus* were released into each cage at the center of the west facing side of the cage. I used a higher number of *Cx. quinquefasciatus* in order to increase the number of observations per replicate, as a single egg raft (attributable to one female) was considered one observation. Individual eggs were considered independent observations for *Ae. albopictus*, as this species lays eggs singly and exhibits skip oviposition (unlike *Cx. quinquefasciatus*). Only one strain was released into each cage. *Aedes albopictus* was released into cages at 0800 h, and Cx. *quinquefasciatus* was released into cages at 1700 h on the same day. Mosquitoes were released at different times so as to allow both species time to acclimate to their surroundings before their peak oviposition times (afternoon for *Ae. albopictus*, dusk for *Cx. quinquefasciatus*). Egg papers and *Cx. quinquefasciatus* egg rafts were collected at 0800 h the next morning. For *Cx. quinquefasciatus*, I quantified the number of egg rafts laid in each tire. I quantified individual eggs laid in each *Ae. albopictus* tire.

Survival Experiment

After eggs were collected from all tires, hand pumps were used to collect a 500 mL aliquot of water from each tire; pumps were moved around the circumference of the tire while removing water in order to mix the water and obtain a representative water

sample. Water samples were stored in airtight Nalgene bottles at room temperature until needed.

Eggs and tire water were taken back to the lab, at which time *Ae. albopictus* eggs were counted. Egg rafts of *Cx. quinquefasciatus* were transferred to plastic cups containing RO water, and newly hatched larvae were added to cups containing tire water the next day; *Ae. albopictus* eggs were stored in an incubator (Percival Scientific, Inc., Perry, IA, USA) at 24 °C and ~85 % relative humidity for four days after oviposition to allow ample time for egg counting and embryogenesis, and the eggs were then placed in a solution of 0.33 g Nutrient Broth (Difco™, BD, Sparks, MD, USA) per 750 mL deionized water for hatching.

One hundred mL of water from each tire was added to a 100 mL plastic cup. For consistency, only larvae from eggs deposited in the preferred tire from each replicate were used, as some tires received 100 percent of the eggs deposited. Within 24 hours of hatching, 10 larvae were introduced to both cups corresponding to the respective tire pairing from which the larvae originated, and the cups were stored in an incubator set to 27 °C on a 14:10 hr day:night cycle (approximate photoperiod [www.fcc.gov] and mean temperature [www.weather.com] for June-August in Hattiesburg, MS). Larvae were fed ground Purina® Puppy Chow® and brewers yeast (Acros Organics, Morris Plains, NJ, USA) using an eight-day schedule (Table 1). Water levels within cups were maintained at 100 mL on a daily basis using RO water. Pupae were removed each day, transferred to glass shell vials, and stored in an incubator with the same settings described for larval rearing. The experiment ended 45 days after larvae were introduced to cups, and any larvae that had not pupated were considered mortalities. Individuals surviving to adulthood where quantified for each cup. Three runs of the oviposition bioassay and

subsequent survival experiment were conducted in May-August 2011; gravid females were released into enclosures on May 31, June 14, and June 29.

Analyses

To compare oviposition responses of each species among pollution treatments, pairings of treatments for each species were analyzed separately using analysis of variance (ANOVA) to test for effects of pollution concentration on number of eggs (*Ae. albopictus*) or egg rafts (*Cx. quinquefasciatus*) allocated to each treatment; blocks for experimental run and cage (i.e., replicate, nested within run) were included to account for variation due to time and paired samples, respectively. No transformations were used, as raw data met assumptions of normality and homogeneity of variances.

To examine associations of oviposition response with larval survival, I calculated oviposition preference indices (O) and larval survival indices (S) for each experimental unit. The term *preference* is used to refer to oviposition response, although responses may be involuntary. Preference was calculated as,

$$O = \frac{O_H - O_L}{O_H + O_L}$$

where O_H is the number of eggs or egg rafts deposited in the preferred tire (i.e., the tire that received more eggs/rafts), and O_L is the number eggs or egg rafts deposited in the non-preferred tire. Values of O can range from 0 to 1, with 0 indicating no preference between tires, and 1 indicating complete preference for one tire over the other. An index measuring relative suitability of the preferred oviposition site for larval survival was calculated as,

$$S = \frac{S_{OH} - S_{OL}}{S_{OH} + S_{OL}}$$

where S_{OH} is the number of larvae that survived to adulthood in water from the preferred oviposition tire, and S_{OL} is the number of larvae that survived in water from the non-preferred tire. Values of *S* can range from -1 to 1, with positive values indicating that the preferred habitat is more suitable, negative values indicating that the non-preferred habitat is more suitable, and 0 values indicating that both habitats are equally suitable. *S* does not measure the overall suitability of a habitat; rather, it is a measurement of the degree to which one habitat is more suitable than the alternative habitat.

Separate statistical analyses were conducted for each strain. Values of *O* and *S* were analyzed using analysis of covariance (ANCOVA), with pollution combination as a factor, *O* as a covariate, and *S* as the response variable. Data were pooled across runs, as preliminary ANCOVA indicated that slopes and intercepts of the regression lines for each run did not differ for any strain. All statistics were conducted using JMP® Version 8 (SAS Institute Inc., Cary, NC).

Results

Field Bioassays

Oviposition responses of both *Ae. albopictus* and wild *Cx. quinquefasciatus* significantly differed between at least one pollution concentration and water controls, and no effects of run or cage were found for any strain (Table 3). *Aedes albopictus* laid significantly more eggs in the high concentration than in the control, but oviposition responses did not differ significantly in other concentration pairings (Table 4).

Wild *Cx. quinquefasciatus* deposited significantly higher numbers of egg rafts in both pollution concentrations than in water controls, but the number of egg rafts did not differ between low and high pollution concentrations (Table 4). The lab strain deposited a significantly higher number of eggs rafts in the high concentration than in the low

concentration, but differences between either pollution concentration and controls were not significant (Table 4).

Table 3

Results of ANOVA within each pollution concentration pairing on number of Ae. albopictus *eggs and* Cx. quinquefasciatus *(lab and wild) egg rafts deposited in each pollution concentration. Significant effects are shown in bold.*

Effect Tests	Control vs. Low			Control vs. High			Low vs. High		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Ae. albopictus									
Run	2,5	2.8109	0.1520	2,5	1.9162	0.2411	2,5	0.5975	0.5852
Cage	5,7	1.2083	0.3947	5,7	2.8972	0.0990	5,7	0.3603	0.8606
Pollution	1,7	0.4119	0.5415	1,7	16.6590	**0.0047**	1,7	0.2264	0.6487
Cx. quinquefasciatus (wild)									
Run	2,5	0.4325	0.6711	2,5	1.8243	0.2541	2,5	3.3235	0.1207
Cage	5,7	1.1850	0.4035	5,7	0.1121	0.9858	5,7	0.2324	0.9363
Pollution	1,7	7.2000	**0.0314**	1,7	6.8182	**0.0349**	1,7	0.1094	0.7505
Cx. quinquefasciatus (lab)									
Run	2,5	1.0067	0.4292	2,5	0.2531	0.7858	2,5	0.5898	0.5889
Cage	5,7	0.2562	0.9235	5,7	0.0986	0.9893	5,7	0.1855	0.9590
Pollution	1,7	0.8826	0.3788	1,7	2.5409	0.1550	1,7	9.1755	**0.0191**

Note. For all analyses, run and cage are included as random effects; cage is nested within run.

Table 4

Least-squared mean (± 1 SE) number of Ae. albopictus *eggs or* Cx. quinquefasciatus *egg rafts deposited in each pollution concentration within each pollution concentration pairing. Bold pairs are significantly different (determined by ANOVA).*

	Ae. albopictus		*Cx. quinquefasciatus* (wild)		*Cx. quinquefasciatus* (lab)	
	Mean	± SE	Mean	± SE	Mean	± SE
Control	108.4	± 17.1	**1.7**	**± 0.8**	6.0	± 1.8
Low	123.5	± 17.1	**4.7**	**± 0.8**	8.4	± 1.8
Control	**114.3**	**± 17.3**	**2.1**	**± 1.0**	3.5	± 2.3
High	**211.7**	**± 17.3**	**5.8**	**± 1.0**	8.6	± 2.3
Low	209.4	± 50.6	3.7	± 1.1	**3.5**	**± 1.6**
High	176.2	± 50.6	4.2	± 1.1	**10.1**	**± 1.6**

Larval Survival

For all strains, no significant relationship was found between oviposition preference and relative survivability of the larval habitat when all treatments combinations were pooled, and the slopes and intercepts of regression lines did not significantly differ among pollution concentration pairings for any strain (Tables 5 and 6). Additionally, no significant associations for wild or lab *Cx. quinquefasciatus* were found within any treatment pairing (Table 6). Oviposition preference of *Ae. albopictus* had a significant positive association with survivability in the control vs. high concentration pairing, and a negative association that approached significance in the control vs. low pairing (Table 6; Figure 1).

Discussion

Results of the oviposition experiment supported my hypothesis that polluted water would influence oviposition responses of both species, but the results did not support my prediction that *Ae. albopictus* would avoid polluted water. Both species displayed

Table 5

Results of ANCOVA on relative habitat suitability for each mosquito strain with oviposition preference as a covariate and pollution concentration combination as a factor.

	F	df	*P*
Ae. albopictus			
Preference	0.6561	1,13	0.4325
Combination	0.2684	2,13	0.7687
Preference x combination	3.0894	2,13	0.0799
Cx. quinquefasciatus (wild)			
Preference	1.4690	1,12	0.2488
Combination	0.1514	2,12	0.8611
Preference x combination	0.3153	2,12	0.7354
Cx. quinquefasciatus (lab)			
Preference	0.2839	1,15	0.6020
Combination	0.0605	2,15	0.9415
Preference x combination	0.1961	2,15	0.8240

Table 6

ANCOVA estimates describing slopes and intercepts for survival index as a function of oviposition preference for Ae. albopictus *and* Cx. quinquefasciatus *(lab and wild). Estimates within each pollution concentration pairing are given below the main model. Significant effects are shown in bold.*

		Regression Formulas				
	n	Slope (SE)	*P*	Intercept (SE)	*P*	r^2
Ae. albopictus	19	0.322 (0.398)	0.433	-0.283 (0.136)	0.059	0.363
Control vs. Low	7	-1.218 (0.606)	0.066	-0.073 (0.108)	0.511	
Control vs. High	5	1.501 (0.610)	**0.029**	0.066 (0.105)	0.541	
Low vs. High	7	-0.284 (0.458)	0.546	0.007 (0.097)	0.942	
Cx. quinquefasciatus (wild)	18	-0.598 (0.494)	0.249	0.328 (0.245)	0.204	0.221
Control vs. Low	7	-0.065 (0.557)	0.909	0.037 (0.162)	0.822	
Control vs. High	4	-0.376 (0.895)	0.682	0.050 (0.195)	0.803	
Low vs. High	7	0.441 (0.593)	0.472	-0.087 (0.160)	0.597	
Cx. quinquefasciatus (lab)	21	0.273 (0.513)	0.602	-0.079 (0.339)	0.819	0.079
Control vs. Low	7	0.280 (0.707)	0.698	-0.070 (0.202)	0.735	
Control vs. High	6	-0.490 (0.783)	0.541	0.055 (0.220)	0.808	
Low vs. High	8	0.210 (0.683)	0.763	0.015 (0.184)	0.935	

increased preference for polluted water in at least one concentration pairing. Wild *Cx. quinquefasciatus* laid more eggs in both concentrations of polluted water than in water controls (Table 4), which is consistent with previous findings under laboratory conditions (Du and Millar 1999). *Aedes albopictus* laid significantly more eggs in high concentration compared water controls (Table 4). Past work indicates *Ae. albopictus* does not discriminate between the five-chemical blend (skatole, *p*-cresol, indole, phenol, and 4-ethylphenol) originally isolated by Millar et al. (1992) and water controls (Allan and Kline 1995). When tested individually, the five compounds at various concentrations have ranged from repellant (or deterrent) to slightly attractive (or stimulatory) to ovipositing *Ae. albopictus*, with only skatole and *p*-cresol eliciting some degree of increased oviposition response (Allan and Kline 1995, Trexler et al. 2003). However, Allan and Kline (1995) reported that *Ae. albopictus* showed a greater response to

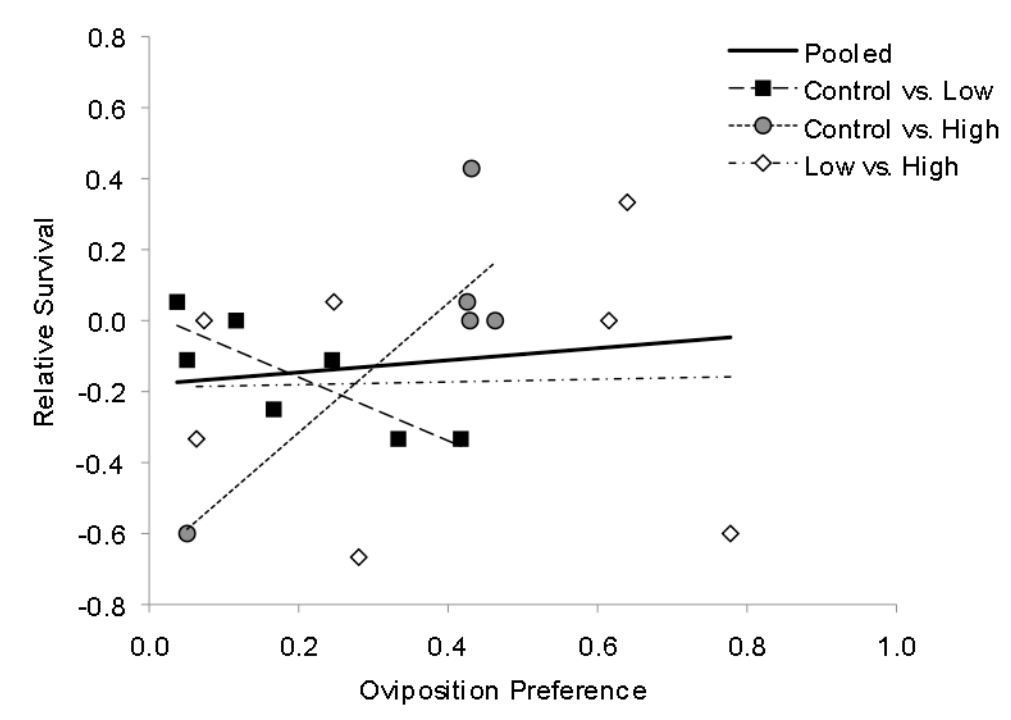

Figure 1. Regression lines for relative survivability of larval habitat as a function of oviposition preference for *Ae. albopictus*. The solid line represents all pollution concentration combinations pooled, and the dashed lines represent relationships within treatment combinations. Positive y-axis values indicate higher survival in water from the preferred oviposition tire, negative values indicate higher survival in water from the non-preferred tire, and zero values indicate equal survival.

Bermuda grass infusions (which the blend is intended to mimic) than to the five-chemical blend, suggesting that the chemicals responsible for *Ae. albopictus* attraction to grass infusions were not fully accounted for. The presence of additional compounds (nonanal, 2-undecanone, 2-tridecanone, naphthalene, and dimethyl trisulfide) in the 10-chemical blend may more closely resemble the attractive or stimulatory aspects of Bermuda grass infusions to gravid *Ae. albopictus*. *Aedes albopictus* did not discriminate between water controls and low concentration, or between low and high concentration, suggesting that lower magnitudes of difference between concentrations of these chemicals do not lead to differential oviposition responses.

My results indicate that the 10 chemical blend used to mimic pollution may enhance surveillance and extermination efforts for these species. Both the chemical blend and Bermuda grass infusions appear to elicit oviposition responses from *Cx. quinquefasciatus* through both olfactory and tactile cues, with the low concentration blend eliciting the greatest olfactory response (Du and Millar 1999), indicating potential for use of the blend at low concentration to attract blood-fed *Cx. quinquefasciatus* to gravid traps; previous trials have shown that the five-chemical blend (Millar et al. 1992) is effective for attracting gravid *Cx. quinquefasciatus* to these traps (Beehler et al. 1994). Use of the 10-chemical blend may further enhance trapping efficiency. The blend may be less effective in gravid traps for *Ae. albopictus*, as oviposition responses of this species to infusions appear to be due to tactile cues rather than olfactory responses (Trexler et al. 1998). However, the blend may enhance the effectiveness of ovitraps and lethal ovitraps for surveying and exterminating *Ae. albopictus*.

Comparisons of lab and wild strains of *Cx. quinquefasciatus* indicated that the lab strain did not show an increased preference for polluted water over clean water, while the wild strain preferred to oviposit in polluted water (Table 4). This demonstrates that the use of laboratory acclimated mosquito strains in oviposition bioassays may lead to different conclusions than would be drawn from wild strains. In this case, the lab strain of *Cx. quinquefasciatus* I used may have been selected for laying eggs in clean water, which possibly diluted over time its selectiveness for oviposition substrates. Therefore, when it is impractical to use wild mosquitoes in controlled oviposition bioassays, it is advisable to corroborate the results with field surveys (e.g., Beehler et al. 1994, Allan et al. 2005) before assuming that the observed effects (or lack thereof) are applicable to wild populations.

The results of the survival experiment did not support my hypothesis that oviposition preference would have different associations with larval survival for the two species. I found no association between oviposition preference and larval survival for *Cx. quinquefasciatus*. Associations for *Ae. albopictus* were weak and varied by pollution concentration combination (Figure 1). The chemicals used do not appear to affect larval survival or performance of either species at either concentration (see Chapter III). Despite the fact that both the high and low concentrations and the control are equally survivable, not all treatments received equal oviposition responses. If oviposition preferences of these species reflect larval habitat quality, then the preference of both species for polluted water may reflect resource availability rather than toxicity. Tires in my experiment were not supplemented with resources, but the presence of this combination of chemicals in the wild is associated with the presence of decomposing organic matter harboring microorganisms, the primary food source of larval mosquitoes (Merritt et al. 1992). The use of organic infusions containing different amounts detritus rather than a synthetic chemical blend may have produced more informative results.

The relationship between oviposition preference and offspring performance is seldom compared directly for mosquitoes. Existing studies suggest that ovipositing mosquitoes may show a preference for habitats where their offspring will receive sufficient nourishment, either through avoidance of competition or choice of optimal detritus type. *Aedes triseriatus* seems to avoid ovipositing in containers with high larval densities (Edgerly et al. 1998). Additionally, this species shows an oviposition preference for deciduous over evergreen forest habitats, and larval performance is better in deciduous forests at high larval densities (Ellis 2008). *Aedes albopictus* prefers to oviposit in oak leaf infusions as opposed to fern, grape, or coffee leaves, and infusions

containing oak leaves are associated with superior larval performance (Reiskind et al. 2009). Because relationships between oviposition preferences and offspring performance may be density dependent (Ellis 2008), future work could examine the effects of resource level, larval density, and their interaction on mosquito preference-performance relationships.

In summary, this work demonstrates that the blend of 10 chemicals identified by Du and Millar (1999) is effective at eliciting increased oviposition responses from *Ae. albopictus* and *Cx. quinquefasciatus* in tires under field conditions. Future work is needed to assess its effectiveness as bait in gravid traps and ovitraps in the field. Additionally, I did not find a clear relationship between oviposition preferences and larval survival in this blend, as the blend affected oviposition responses but not larval survival. Little is known about the relationship between mosquito oviposition preferences and larval survival, and further work is needed to test optimal oviposition hypotheses for mosquito taxa.

CHAPTER III

EFFECTS OF DETRITAL RESOURCE LEVELS AND CHEMICAL POLLUTION ON INTERSPECIFIC COMPETITION

Introduction

Cues associated with aquatic environments can affect the susceptibility of an environment to colonization (see Chapter II). However, abiotic (e.g., temperatures, chemical properties) and biotic (e.g., predation, competition) factors within the aquatic environment may affect an organism's survival and performance once that environment has been colonized (Macan 1961). For instance, when interspecific competition is asymmetrical, competitive exclusion (local extinction) or reduction of the weaker species are expected to occur (Lawton and Hassell 1981, Connell 1983, Lounibos 2007). The consequences of resource competition among larval mosquitoes have important ecological and medical implications. In addition to directly affecting populations of disease vectoring mosquitoes, stress from competition in the larval stage can indirectly affect susceptibility to infection by diseases in the adult stage (Alto et al. 2005, 2008b).

Artificial containers, including tires, constitute important mosquito breeding habitats in residential areas, especially in low income neighborhoods (Chambers et al. 1986). *Aedes albopictus* and *Culex quinquefasciatus* are often the most abundant species of their respective genera found in tires within their ranges (e.g., Chambers et al. 1986, Sprenger and Wuithiranyagool 1986, Lopes et al. 2004). Despite the fact that both species are common in these habitats and are medically important, virtually nothing is known about their interspecific interactions. In a tire study in Brazil, larval *Cx. quinquefasciatus* abundance in tires declined with increasing distance from urban areas, a pattern concomitant with an increase in *Ae. albopictus* abundance (Lopes et al. 2004); the

authors suggested that the observed pattern may have been due to competition, but this hypothesis has never been tested. In the southern U.S., *Cx. quinquefasciatus* has been found to be second in abundance to *Ae. albopictus* in tires in both urban (Sprenger and Wuithiranyagool 1986) and rural (Yee et al., in prep) areas. Other factors besides competition may also explain abundance patterns in the field. For instance, *Ae. albopictus* is a container specialist and may utilize tires to a greater degree than *Cx. quinquefasciatus*, which also utilizes non-container habitats. Nevertheless, understanding the nature of interspecific interactions between these species in the larval stage is important to the understanding of disease transmission patterns, especially in situations where tires represent the majority of available mosquito breeding habitats.

In the larval stage, *Ae. albopictus* has been shown to be a superior resource competitor to several native or established species. Most notably, *Ae. albopictus* has replaced the Yellow Fever mosquito (*Aedes aegypti* L.) as the dominant container species in the eastern U.S., and *Ae. aegypti* populations have become locally extinct with the exception of a few urban populations in the south (O'Meara et al. 1995, Braks et al. 2003). The decline of *Ae. aegypti* prompted numerous investigations of the mechanism of displacement, including larval competition. Subsequent findings revealed that *Ae. albopictus* is a superior resource competitor to *Ae. aegypti* both in the lab (Murrell and Juliano 2008) and in the field (Juliano 1998), and that displacement of *Ae. aegypti* by *Ae. albopictus* is likely not due to differential infection rates by parasites (Juliano 1998). *Aedes albopictus* is also a superior competitor to the eastern tree hole mosquito (*Ae. triseriatus* Say) (Yee et al. 2007); the spread of *Ae. albopictus* is associated with a decline but not a replacement of *Ae. triseriatus* in urban and suburban container habitats (Lounibos et al. 2001). In addition to other *Aedes*, *Ae. albopictus* has been shown to be a

superior resource competitor to the northern house mosquito (*Culex pipiens* L.) (Carrieri et al. 2003, Costanzo et al. 2005a). The competitive superiority of *Ae. albopictus* to *Cx. pipiens* (Carrieri et al. 2003, Costanzo et al. 2005a) suggests that *Ae. albopictus* is likely superior to *Cx. quinquefasciatus*; *Cx. quinquefasciatus* and *Cx. pipiens* are sometimes regarded as subspecies of *Cx. pipiens* (i.e., *Cx. pipiens quinquefasciatus* and *Cx. p. pipiens*) (Vinogradova 2000), but it cannot necessarily be assumed that ecological traits of *Cx. pipiens* apply to *Cx. quinquefasciatus*, as the ecologies of these two species have not been compared.

Resource competition between *Ae. albopictus* and other mosquito species may be condition-specific, such that the competitive advantage of a species may be nullified or reversed under a different set of conditions (Dunson and Travis 1991, Chesson 2000). For example, dry conditions negatively impact the competitive advantage of larval *Ae. albopictus* over *Ae. aegypti* (Costanzo et al. 2005b), and use of artificial diets (e.g., liver powder) confers the competitive advantage to *Ae. aegypti* (Barrera 1996, Juliano 1998). Additionally, certain ratios of plant and animal detritus alleviate resource competition between *Ae. albopictus* and *Ae. triseriatus* (Yee et al. 2007), as well as greater susceptibility of *Ae. albopictus* to predation by the dipteran predators *Toxorhynchites rutilus* Coquillett and *Corethrella appendiculata* Grabham (Griswold and Lounibos 2005b, 2006). The competitive advantage of *Ae. albopictus* over *Cx. pipiens* decreases when rapidly decomposing detritus is present (Costanzo et al. 2011). If *Ae. albopictus* is indeed a superior resource competitor to *Cx. quinquefasciatus*, the presence of organic pollutants, to which *Cx. quinquefasciatus* is presumably more tolerant, may serve to nullify the competitive advantage of *Ae. albopictus* by detrimentally affecting larval performance.

In this chapter, my objectives were to determine if interspecific resource competition occurs between *Ae. albopictus* and *Cx. quinquefasciatus*, and if the effects of interspecific interactions between these species are context specific (i.e., affected by chemicals associated with organic pollution). I hypothesized that, 1) interspecific competition will occur between *Ae. albopictus* and *Cx. quinquefasciatus* when resources are limited, and 2) effects of interspecific competition will differ in polluted water as opposed to reverse osmosis filtered (RO) water. Based on current knowledge, I predicted that, 1) *Ae. albopictus* will be a superior resource competitor to *Cx. quinquefasciatus*, and 2) the competitive advantage of *Ae. albopictus* will be reduced in polluted water, as *Ae. albopictus* is ostensibly less pollution tolerant than *Cx. quinquefasciatus*.

Methods

Resource Levels

Experimental microcosms consisted of 100 mL plastic beakers filled with 99 mL of reverse osmosis (RO) water and 1 mL of microorganism inoculum; inoculum was water collected from field tires containing mosquito larvae and detritus in Hattiesburg, MS. Microcosms were housed in an incubator (27 °C on a 14:10 hour day:night cycle) in plastic trays (24 microcosms per tray). Microcosms were assigned to trays such that each factor level combination (see below) was equally represented in each tray. Microcosms were arranged randomly within trays, and tray positions were rotated within the incubator every 24 hours to control for effects of location within the incubator.

Resources consisted of senescent live oak (*Quercus virginiana*) leaves (LO) and insect carcasses (IC) present in three different quantities at a constant 5:1 (LO:IC) ratio, as mosquitoes require less animal detritus than plant detritus to obtain similar growth rates, adult mass, survivorship, and population growth rates (Yee and Juliano 2006). The

three quantities of LO and IC (respectively) used were low (0.05 g, 0.01 g), medium (0.25 g, 0.05 g), and high (0.50 g, 0.10 g). Leaves were collected from the University of Southern Mississippi's (USM) Lake Thoreau Environmental Center (hereafter, LTEC), located approximately five miles west of the USM campus in Hattiesburg, MS. Insect carcasses consisted of fruit flies (*Drosophila melanogaster* Meigen; obtained from colonies within the Department of Biological Sciences, USM) and freeze-dried crickets (*Acheta domesticus* L.; Fluker Laboratories, Baton Rouge, LA, USA) present in a 4:1 (fly:cricket) ratio. Flies were freeze-killed and all detritus was oven dried for 48 h at 80 °C to kill any pre-existing microorganisms prior to the start of the experiment. Water, inoculum, and detritus were added to beakers and stored in the incubators for three days prior to the introduction of mosquito larvae to allow time for microorganism population growth.

Eggs of both species were simultaneously hatched in a solution of 0.33 g Nutrient Broth (Difco™, BD, Sparks, MD, USA) per 750 mL deionized water, and larvae were added to microcosms simultaneously within 24 h of hatching. *Aedes albopictus* larvae were the progeny of field collected specimens (F_1); I was unable to generate F_1 *Cx. quinquefasciatus* for this experiment, so lab *Cx. quinquefasciatus* larvae were used. Eight different density combinations of low (5) or high (10) numbers of mosquitoes (*Ae. albopictus* : *Cx. quinquefasciatus*) were used: 0:5, 0:10, 5:0, 10:0, 5:5, 5:10, 10:5, 10:10. Each resource level (3) was replicated evenly across the eight density combinations for a total of 24 resource x density combinations; each combination was replicated ten times for a total of 240 experimental units. Water levels in microcosms were refilled to 100 mL with RO water prior to the introduction of mosquito larvae, and maintained at 100 mL thereafter.

The experiment was ended 45 days after larvae were added (ample time for well-fed larvae to complete development at 27 °C) (Gerberg et al. 1994). Mosquito larvae that did not pupate by day 45 were considered mortalities. Pupae were removed from microcosms each day and transferred to glass shell vials. Sex, species, date of pupation, and date of emergence were recorded for each newly eclosed adult, and adults were freeze killed and dried for 48 hours at 50 °C. After drying, dry mass was measured to the nearest 0.0001 g using a XP2U ultra-microbalance (Mettler-Toledo Inc., Columbus, OH, USA). At the conclusion of the experiment, survivorship (the percentage of initial larvae surviving to adulthood), mean development time (number of days from hatching to pupation), mean adult dry mass, and a composite index of mosquito population performance were calculated for each species in each experimental unit. The performance index (λ') is an estimate of finite rate of increase [$\lambda = \exp(r)$], where r is the per capita rate of population change (dN/N dt) (Smith and Smith 2006). Values of λ' are commonly used to estimate the effects of competition on population performance for *Aedes* species (e.g., Juliano 1998, Lounibos et al. 2002, Yee et al. 2007) and have also been used for *Culex* species (Costanzo et al. 2011). The estimated finite rate of increase is calculated as:

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

where r' is an estimate of r derived by Livdahl and Sugihara (1984), N_0 is the initial number of females in a cohort (assumed to be 50%), D is the time from eclosing to first oviposition [assumed to be 5 days for both species (Subra 1981, Hawley 1988)], A_x is the

number of females eclosing on day x, w_x is the mean mass of females eclosing on day x, and $f(w_x)$ is a function that estimates fecundity from female mass based on regressions in the literature. For *Ae. albopictus*, $f(w_x) = 19.5 + 152.7w_x$ (Lounibos et al. 2002). Because regressions directly relating female mass to fecundity were not available for *Cx. quinquefasciatus*, a function relating wing length (l) to fecundity [$f(l) = -123.88 + 90.31l$] (McCann et al. 2009) was modified using regressions relating female wing length to female mass; these regressions, solved for wing length, were $l = [(w + 0.162)/0.021]^{1/3}$ for wild *Cx. quinquefasciatus*, and $l = [(w + .130)/.018]^{1/3}$ for *Cx. quinquefasciatus* after two years of laboratory colonization (Nasci 1990). The wing length regressions were substituted into the fecundity function to give the modified functions $f(w) = -123.88 + 90.31*[(w + .162)/.021]^{1/3}$ and $f(w) = -123.88 + 90.31*[(w + .130)/.018]^{1/3}$ relating mass to fecundity for wild and colonized *Cx. quinquefasciatus*, respectively. Because the regressions of wing length with mass are significantly different between wild and colonized female *Cx. quinquefasciatus* (Nasci 1990), the colonized function was used for lab *Cx. quinquefasciatus*. In the pollution experiment (see below), the wild function was used for colonies that had been in the lab for < 3 generations.

Pollution

A second experiment was conducted to determine the effects of chemicals associated with organic pollution on survivorship, development, and interspecific interactions of *Ae. albopictus* and *Cx. quinquefasciatus*. The same setup and procedure from the previous competition experiment were used for this experiment, with the following changes: 1) in addition to 99 mL RO water and 1 mL of inoculum, each microcosm received 100 µL of an appropriately concentrated stock solution to produce the desired chemical concentrations (i.e., low or high; Table 2) of the chemical blend

described in Chapter II when added to 100 mL of water; the control was 100 µL of diethyl ether (Du and Millar 1999), 2) the amount of detritus used in this experiment was the medium detritus level used in the previous experiment across all treatments, as competitive asymmetry appeared to be strongest at this detritus level, and 3) both lab and F_2 *Culex quinquefasciatus* larvae were used in this experiment; this was done to assess possible effects of lab acclimation on competitive outcomes, and to allow for comparable results between the two competition experiments, as only lab *Cx. quinquefasciatus* were used in the first experiment.

Each pollution concentration (3) and density (8) combination was replicated ten times for a total of 240 experimental units. Within each pollution-density combination that contained *Cx. quinquefasciatus*, seven replicates contained F_2 *Cx. quinquefasciatus* larvae, and three replicates contained lab *Cx. quinquefasciatus* larvae (I was unable to generate enough lab larvae to use five cups per strain; no cups contained mixed strains). Water levels in microcosms were refilled to 100 mL with RO water prior to the introduction of mosquito larvae, and thereafter as needed.

Analyses

Before conducting parametric tests, I tested each dataset for normality and homogeneity of variances (SAS Institute, Inc. 2004); transformations were used when necessary to meet assumptions. For the resource level experiment, *Cx. quinquefasciatus* development time and mass for both sexes were inverse transformed (1/x), and *Ae. albopictus* female mass was square-root transformed ($\sqrt{x}$). For the pollution experiment, *Cx. quinquefasciatus* mass data for both sexes were log transformed (ln(x)), and survivorship and development time data were power transformed ($[x + 1]^2$ for survivorship; $x^{-2.8}$ for male development time; $x^{-2.3}$ for female development time); *Aedes*

albopictus female development time data were log transformed (ln(x)). Mass data for both sexes were not transformed as the raw data met parametric assumptions. All other data sets (including λ' for both species in both experiments) did not meet parametric assumptions, and no transformation eliminated this problem. All means and standard errors presented in subsequent sections are back-transformed if the original dataset was transformed; otherwise, the raw means are presented.

For both competition experiments, analysis of variance (ANOVA) was used (for data sets that met parametric assumptions) to test for effects of treatment (i.e., resource level or pollution concentration), larval density combination, and a treatment x density interaction on dependent variables for both mosquito species. For analyses of *Cx. quinquefasciatus* survivorship, development time, and adult mass in the pollution experiment, strain was included as a block to account for variation due to lab acclimation; *Cx. quinquefasciatus* strain was not included as a block in analyses of *Ae. albopictus*, as preliminary analyses indicated that *Ae. albopictus* survivorship, development time, and mass in mixed species treatments were not affected by *Cx. quinquefasciatus* strain (results not shown). To elucidate the effects of *Cx. quinquefasciatus* lab acclimation on competition with *Ae. albopictus*, ANOVA was used to test for effects of strain, density combination, and a strain x density interaction on *Cx. quinquefasciatus* survivorship, development time for each sex, and adult dry mass for each sex in the pollution experiment. When an ANOVA indicated significant factor effects or interactions, Tukey's Honestly Significant Difference (HSD) test was used to test for pairwise differences.

When parametric assumptions were not met, Kruskal-Wallis tests were used to test for differences in dependent variables among treatments and density combinations;

when Kruskal-Wallis tests indicated significant differences, Dunn's test for nonparametric multiple comparisons was used to reveal pairwise differences (Zar 2010). Because I could not directly test for an interaction using Kruskal-Wallis tests, I tested for differences among treatment levels within each density combination, and I tested for differences among density combinations within each treatment level. When multiple Kruskal-Wallis tests were used for the same dependent variable, the α level (set at 0.05) was adjusted using sequential Bonferroni correction (Rice 1989) to reduce the likelihood of committing a Type I error due to multiple comparisons. All ANOVAs and Kruskal-Wallis tests were conducted using JMP® Version 8 (SAS Institute Inc., Cary, NC).

Results

Survival: Resource Levels

Survivorship of *Cx. quinquefasciatus* was negatively affected by *Ae. albopictus* in limited resources. No larval *Cx. quinquefasciatus* survived to adulthood at the low resource level except in the lowest density; therefore, the low resource level was excluded from all analyses of *Cx. quinquefasciatus* in this experiment. Survivorship of *Cx. quinquefasciatus* was significantly higher in high resources in all but the lowest density combination (Table 7; Fig 2a). Survivorship differed among larval density combinations within the medium resource level (Table 7), with significantly lower survivorship when *Ae. albopictus* density was high (Figure 2a). Survivorship in high resources was not affected by *Ae. albopictus* density (Table 7).

Survivorship of *Ae. albopictus* was not affected by *Cx. quinquefasciatus* in high and medium resources, but differences were found within the low resource level (Table 7). In low resources, *Ae. albopictus* survivorship significantly declined when both intra- and interspecific density increased simultaneously, but not when *Cx. quinquefasciatus*

Table 7

Kruskal-Wallis test results on Ae. albopictus *and* Cx. quinquefasciatus *survivorship and estimated population growth (λ') differences among resource levels within each density combination and among density combinations within each resource level. Significance at sequential Bonferroni adjusted significance levels is shown in bold type.*

Factor	Survivorship			λ'		
	X^2	df	*P*	X^2	df	*P*
Cx. quinquefasciatus						
[a]Resource (A0:C5)	2.4429	1	0.1181	0.0079	1	0.9292
[a]Resource (A5:C5)	7.2068	1	**0.0073**	1.5267	1	0.2166
[a]Resource (A10:C5)	13.5034	1	**0.0002**	12.1784	1	**0.0005**
[a]Resource (A0:C10)	7.5476	1	**0.0060**	4.5106	1	0.0337
[a]Resource (A5:C10)	8.1856	1	**0.0042**	7.8799	1	**0.0050**
[a]Resource (A10:C10)	9.2208	1	**0.0024**	8.6133	1	**0.0033**
[b]Density (Low)	nt			nt		
[b]Density (Medium)	31.6395	5	**<0.0001**	22.9391	5	**0.0003**
[b]Density (High)	12.1891	5	0.0323	6.8047	5	0.2356
Ae. albopictus						
[a]Resource (A5:C0)	6.3633	2	0.0415	6.7773	2	0.0338
[a]Resource (A5:C5)	8.2059	2	0.0165	8.7838	2	**0.0124**
[a]Resource (A5:C10)	1.5046	2	0.4713	7.0001	2	0.0302
[a]Resource (A10:C0)	9.0634	2	0.0108	12.5513	2	**0.0019**
[a]Resource (A10:C5)	17.9120	2	**0.0001**	20.3121	2	**<0.0001**
[a]Resource (A10:C10)	20.0099	2	**<0.0001**	15.2431	2	**0.0005**
[b]Density (Low)	24.3455	5	**0.0002**	22.9629	5	**0.0003**
[b]Density (Medium)	9.8768	5	0.0788	20.6825	5	**0.0009**
[b]Density (High)	4.7697	5	0.4446	0.6136	5	0.9874

[a]Tests are for differences among resource levels within a single larval density combination (noted in parentheses).
[b]Tests are for differences among larval density combinations within a single resource level (noted in parentheses).

density alone increased (Figure 2b). When differences among resource levels occurred, fewer individuals survived in low versus medium and high resources (Figure 2b).

Survival: Pollution

Survivorship of both species was generally unaffected by pollution, and effects of density combinations were similar to those in the resource experiment. For *Cx. quinquefasciatus*, ANOVA indicated effects of pollution concentration ($F = 3.2013$; df = 2, 157; $P = 0.0434$), density ($F = 35.9128$; df = 5, 157; $P < 0.0001$), the pollution x density interaction ($F = 2.2420$; df = 10, 157; $P = 0.0180$), and *Cx. quinquefasciatus*

strain (F = 9.6278; df = 1, 157; P = 0.0023). There were no density combinations where *Cx. quinquefasciatus* survivorship in pollution differed significantly from controls (Figure 3a). Survivorship in all pollution concentrations significantly declined when *Ae. albopictus* density increased from absent to high in the high *Cx. quinquefasciatus* density (Figure 3a). *Aedes albopictus* survivorship did not differ among pollution concentrations at any density, and did not differ among density combinations at any pollution concentration (Table 8; Figure 3b).

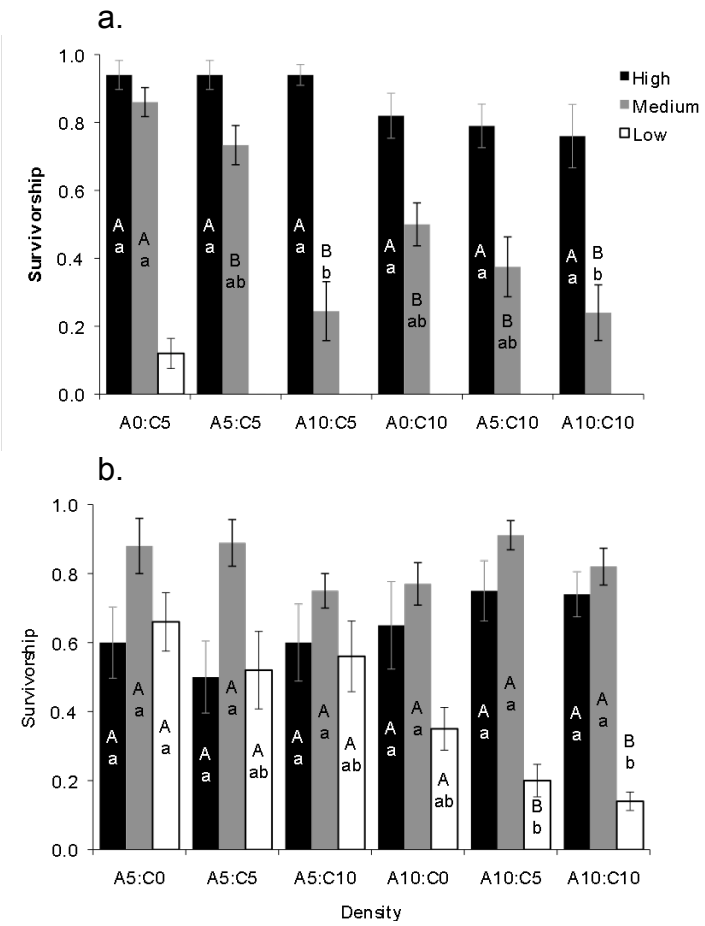

Figure 2. Mean (± 1 SE) survivorship across *Ae. albopictus*:*Cx. quinquefasciatus* (A:C) density combinations by resouce level for (a) *Cx. quinquefasciatus*, and (b) *Ae. albopictus*. Different uppercase letters indicate significant pairwise differences between resource levels within a density combination. Different lowercase letters indicate differences between density combinations within a resource level. *Culex quinquefasciatus* survivorship in low resources is presented but was excluded from all analyses.

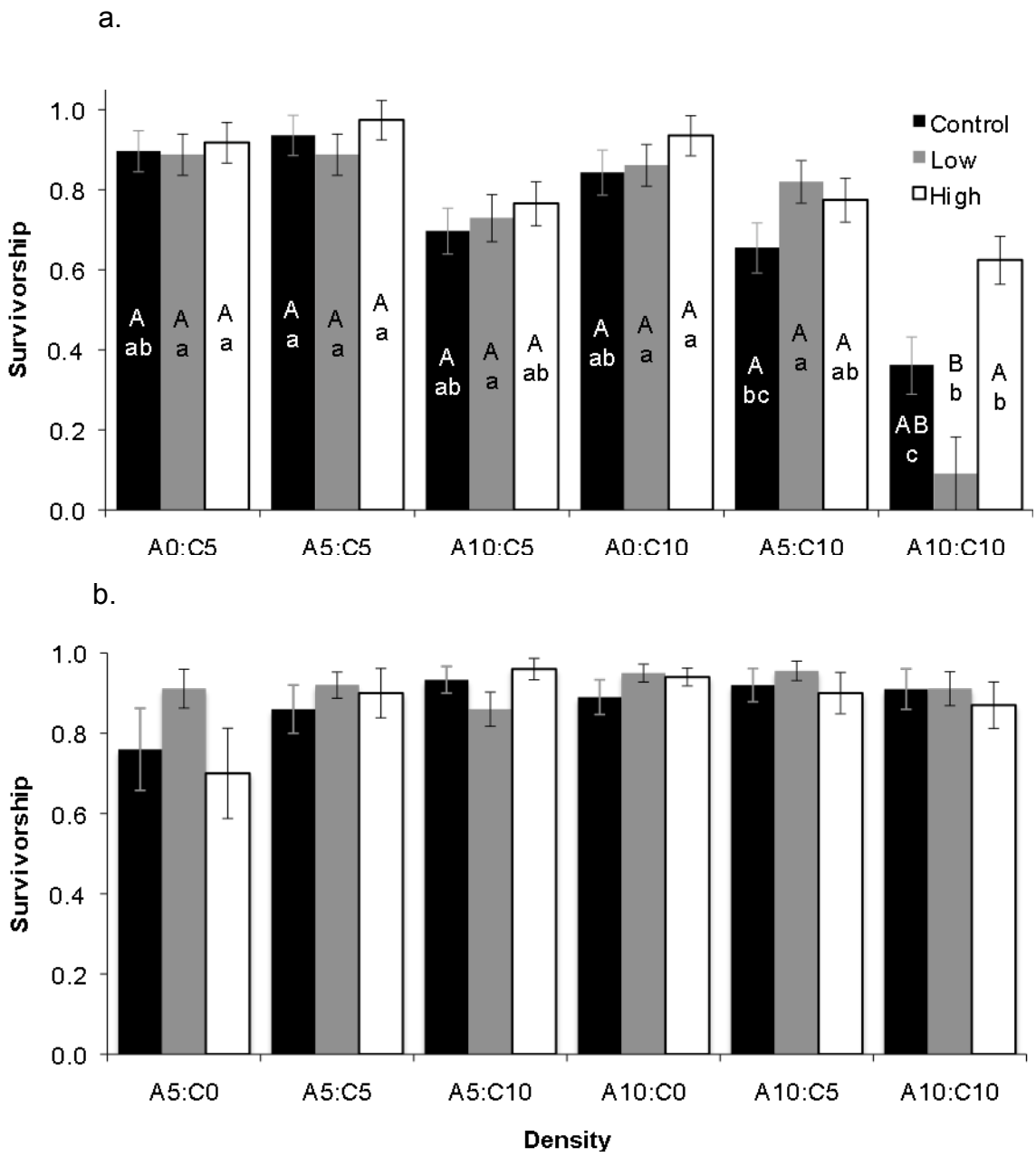

Figure 3. Mean (± 1 SE) survivorship across *Ae. albopictus*:*Cx. quinquefasciatus* (A:C) density combinations by pollution concentration for (a) *Cx. quinquefasciatus*, and (b) *Ae. albopictus*. Different uppercase letters indicate significant pairwise differences between pollution concentrations within a density combination. Different lowercase letters indicate differences between density combinations within a pollution concentration. No significant differences were found for *Ae. albopictus.*

Development Time and Mass: Resource Levels

There were no clear trends for *Cx. quinquefasciatus* development times, but lower resources and higher densities negatively affected adult mass. For development time,

Table 8

Kruskal-Wallis test results on Ae. albopictus *and* Cx. quinquefasciatus *survivorship and estimated population growth (λ') differences among pollution concentrations within each density combination and among density combinations within each pollution concentration. Significance at sequential Bonferroni adjusted significance levels is shown in bold type.*

Factor	Survivorship			λ'		
	X^2	df	*P*	X^2	df	*P*
Ae. albopictus						
[a]Pollution (A5:C0)	1.7767	2	0.4113	0.4314	2	0.8060
[a]Pollution (A5:C5)	0.7181	2	0.6983	1.6046	2	0.4483
[a]Pollution (A5:C10)	3.7928	2	0.1501	3.2089	2	0.2010
[a]Pollution (A10:C0)	1.0690	2	0.5860	1.3239	2	0.5159
[a]Pollution (A10:C5)	0.3114	2	0.8558	0.2359	2	0.8888
[a]Pollution (A10:C10)	0.2867	2	0.8664	6.6759	2	0.0355
[b]Density (Control)	3.2581	5	0.6603	11.1504	5	0.0485
[b]Density (Low)	3.6811	5	0.5962	7.0237	5	0.2189
[b]Density (High)	3.9901	5	0.5508	6.6650	5	0.2468
Cx. quinquefasciatus						
[a]Pollution (A0:C5)				0.0335	2	0.9834
[a]Pollution (A5:C5)				2.9961	2	0.2236
[a]Pollution (A10:C5)				0.6838	2	0.7104
[a]Pollution (A0:C10)				2.8824	2	0.2366
[a]Pollution (A5:C10)				7.2218	2	0.0270
[a]Pollution (A10:C10)				6.3589	2	0.0416
[b]Density (Control)				35.7225	5	**<0.0001**
[b]Density (Low)				30.4275	5	**<0.0001**
[b]Density (High)				19.3746	5	**0.0016**

Note. *Culex quinquefasciatus* survivorship was analyzed using ANOVA and is omitted from the table.
[a]Tests are for differences among pollution concentrations within a single larval density combination (noted in parentheses).
[b]Tests are for differences among larval density combinations within a single pollution concentration (noted in parentheses).

ANOVAs for both males and females indicated no main effect of resource level, but there were effects of density and the resource x density interaction (Table 9). Male and female development time did not significantly differ between resource levels within any density (Figure 4a and 4b). Significant pairwise differences between density combinations were found for development time of both sexes (Figure 4a and 4b), but no pattern for either sex was observed concomitant with increasing *Ae. albopictus* density. Adult mass of both sexes was significantly affected by resource level, density, and their interaction (Table 9).

Table 9

Results of two-way ANOVA (resource level and density combination) on transformed values for development time and adult mass of Cx. quinquefasciatus *males and females. Significant effects are shown in bold type.*

Effect	Male			Female		
	df	*F*	*P*	df	*F*	*P*
Development Time						
Resource	1, 92	3.5603	0.0623	1, 82	1.6421	0.2036
Density	5, 92	7.1799	**<0.0001**	5, 82	2.8887	**0.0188**
Resource x density	5, 92	2.7256	**0.0242**	5, 82	3.5845	**0.0056**
Mass						
Resource	1, 89	129.8803	**<0.0001**	1, 73	64.7833	**<0.0001**
Density	5, 89	7.2198	**<0.0001**	5, 73	9.4135	**<0.0001**
Resource x density	5, 89	2.8657	**0.0191**	5, 73	3.0627	**0.0145**

In general, both sexes were significantly heavier in high resources than in medium resources (Figure 4c and 4d). In the medium resource level, significant declines in male mass were reflective of increased intraspecific density rather than *Ae. albopictus* density (Figure 4c), whereas female mass was negatively affected by both increased intraspecific density and *Ae. albopictus* density (Figure 4d). *Aedes albopictus* development time did not differ among most treatments, but mass was negatively affected by *Cx. quinquefasciatus*. Development time of both sexes did not differ among resource levels at most densities (Table 10), but development time was slower in low resources where differences occurred (Figure 5a and 5b). Development time differed among density combinations for males within the medium resource level (Table 10), but there was no trend with respect to intra- or interspecific densities (Figure 5a). Female development time did not differ among density combinations within any resource level (Table 10). Male mass significantly differed among resource levels at all density combinations, and among density combinations within all resource levels (Table 10). For female mass, ANOVA indicated significant effects of resource level ($F = 433.1607$; df = 2, 118; $P <$

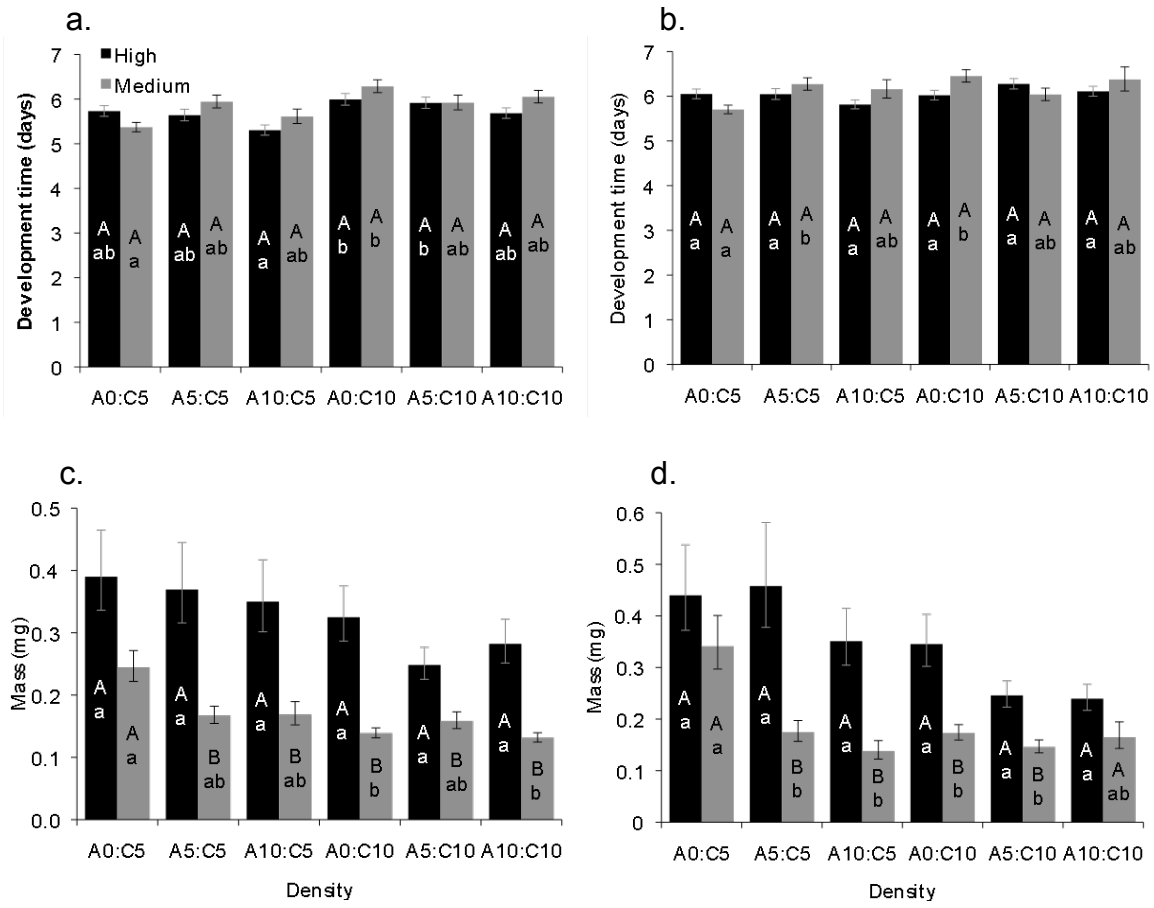

Figure 4. Mean (± 1 SE) *Cx. quinquefasciatus* development time of (a) males and (b) females, and adult mass of (c) males and (d) females across *Ae. albopictus*:*Cx. quinquefasciatus* (A:C) density combination by resource level treatments. Different uppercase letters indicate significant pairwise differences between resource levels within a density combination. Different lowercase letters indicate differences between density combinations within a resource level.

0.0001), density ($F = 25.7078$; df = 5, 118; $P < 0.0001$), and their interaction ($F =$ 4.0766, df = 10, 118; $P < 0.0001$). In general, both sexes became smaller as resource levels decreased (Figure 5c and 5d). In high and medium resources, mass of both sexes was generally lower in the presence of *Cx. quinquefasciatus* (Figure 5c and 5d). Significant decreases in mass of both sexes within low resources reflected simultaneous increases in both intra- and interspecific density (Figure 5c and 5d).

Table 10

Kruskal-Wallis test results on Ae. albopictus *development time and adult male mass differences among resource levels within each density combination and among density combinations within each resource level. Significance at sequential Bonferroni adjusted significance levels is shown in bold type.*

Factor	Male			Female		
	X^2	df	*P*	X^2	df	*P*
Development Time						
[a]Resource (A5:C0)	9.1488	2	0.0103	5.0962	2	0.0782
[a]Resource (A5:C5)	1.4434	2	0.4859	3.1655	2	0.2054
[a]Resource (A5:C10)	3.9273	2	0.1403	14.6991	2	**0.0006**
[a]Resource (A10:C0)	9.8310	2	0.0073	3.5841	2	0.1666
[a]Resource (A10:C5)	14.5802	2	**0.0007**	8.5976	2	0.0136
[a]Resource (A10:C10)	6.6461	2	0.0360	5.6769	2	0.0585
[b]Density (Low)	3.1505	5	0.6768	1.4970	5	0.9134
[b]Density (Medium)	18.9161	5	**0.0020**	4.4747	5	0.4833
[b]Density (High)	9.9215	5	0.0775	7.1442	5	0.2101
Mass						
[a]Resource (A5:C0)	20.1961	2	**<0.0001**			
[a]Resource (A5:C5)	11.2851	2	**0.0035**			
[a]Resource (A5:C10)	16.7702	2	**0.0002**			
[a]Resource (A10:C0)	20.0123	2	**<0.0001**			
[a]Resource (A10:C5)	19.6966	2	**<0.0001**			
[a]Resource (A10:C10)	23.9540	2	**<0.0001**			
[b]Density (Low)	16.9192	5	**0.0047**			
[b]Density (Medium)	33.9552	5	**<0.0001**			
[b]Density (High)	19.8159	5	**0.0014**			

[a]Tests are for differences among resource levels within a single larval density combination (noted in parentheses).
[b]Tests are for differences among larval density combinations within a single resource level (noted in parentheses).

Development Time and Mass: Pollution

Culex quinquefasciatus development time and mass were not affected by pollution, but there were adverse effects of *Ae. albopictus* on both traits. For *Cx. quinquefasciatus*, ANOVA for male development time indicated a significant effect of density, but no significant effects of pollution concentration or pollution x density interaction (Table 11). For female development time, ANOVA indicated no main effect of pollution, but significant effects of density and pollution x density interaction (Table

11). No pairwise differences between pollution levels were found within any density. Both sexes developed significantly more slowly when *Ae. albopictus* density increased, with the exception of females in the high pollution concentration (Figure 6a and 6b). For both male and female mass, ANOVA indicated significant effects of pollution and density, but no effect of pollution x density interaction (Table 11). Mass of both sexes declined with increasing *Ae. albopictus* density (Figure 6c and 6d). Males were

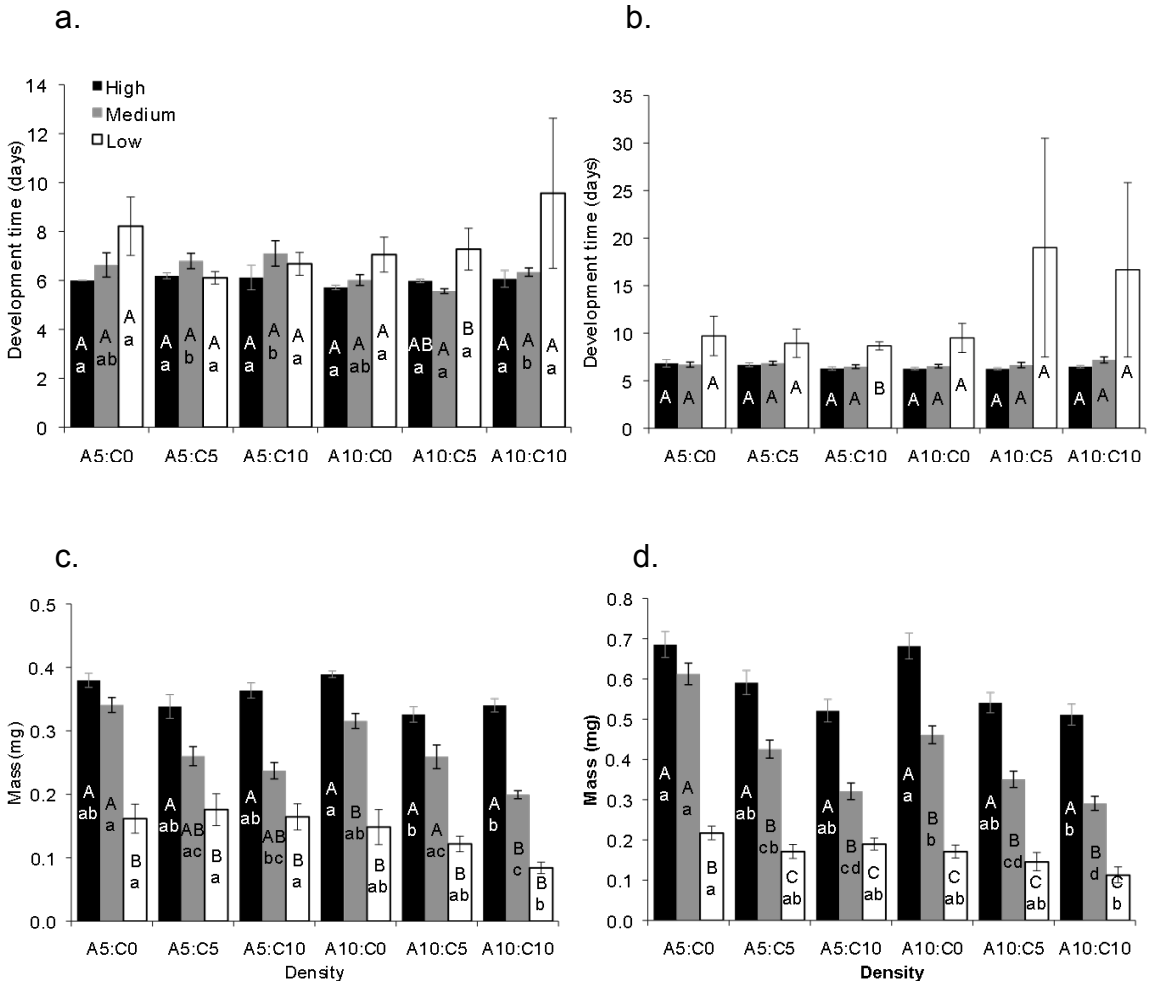

Figure 5. Mean (± 1 SE) *Ae. albopictus* development time of (a) males and (b) females, and adult mass of (c) males and (d) females across *Ae. albopictus*:*Cx. quinquefasciatus* (A:C) density combination by resource level treatments. Different uppercase letters indicate significant pairwise differences between resource levels within a density combination. Different lowercase letters indicate differences between density combinations within a resource level.

significantly heavier in high pollution (ls mean ± SE = 0.2383 + 0.0054, - 0.0053) than in the control (0.2183 + 0.0050, - 0.0049) and low pollution (0.2198 + 0.0060, - 0.0059). Females were significantly heavier high pollution (0.3266 + 0.0092, - 0.0090) than in the control (0.2830 + 0.0083, -0.0081), but mass in low pollution (0.2979 + 0.0105, - 0.0102) did not differ from other concentrations.

Aedes albopictus development time was not affected by pollution or density, but mass was negatively affected by the density of *Cx. quinquefasciatus*. For male development time, ANOVA indicated a significant pollution x density interaction (Table

Table 11

Results of two-way ANOVA (pollution and density combination) on transformed (except Ae. albopictus *male and female mass) values for development time and adult dry mass of* Ae. albopictus *and* Cx. quinquefasciatus *males and females in experimental microcosms. Significant effects are shown in bold type.*

Effect	Male			Female		
	df	*F*	*P*	df	*F*	*P*
Development Time						
Cx. quinquefasciatus						
Pollution	2, 144	1.2600	0.2868	2, 136	0.9245	0.3992
Density	5, 144	14.1976	**<0.0001**	5, 136	24.8909	**<0.0001**
Pollution x Density	10, 144	1.7971	0.0660	10, 136	2.6076	**0.0063**
Strain	1, 144	11.5660	**0.0009**	1, 136	37.9158	**<0.0001**
Ae. albopictus						
Pollution	2, 150	1.4503	0.2378			
Density	5, 150	0.5066	0.7709			
Pollution x Density	10, 150	2.2652	**0.0170**			
Mass						
Cx. quinquefasciatus						
Pollution	2, 142	4.9287	**0.0085**	2, 136	6.8575	**0.0015**
Density	5, 142	76.9999	**<0.0001**	5, 136	61.5169	**<0.0001**
Pollution x Density	10, 142	1.8560	0.0562	10, 136	1.7696	0.0718
Strain	1, 142	8.5810	**0.0040**	1, 136	6.8403	**0.0099**
Ae. albopictus						
Pollution	2, 150	6.4238	**0.0021**	2, 152	3.1269	**0.0467**
Density	5, 150	21.7686	**<0.0001**	5, 152	55.6586	**<0.0001**
Pollution x Density	10, 150	2.1076	**0.0271**	10, 152	2.0212	**0.0347**

Note. For analyses of *Cx. quinquefasciatus*, strain is included as a block. *Aedes albopictus* female development time was analyzed using Kruskal-Wallis tests and is omitted from the table.

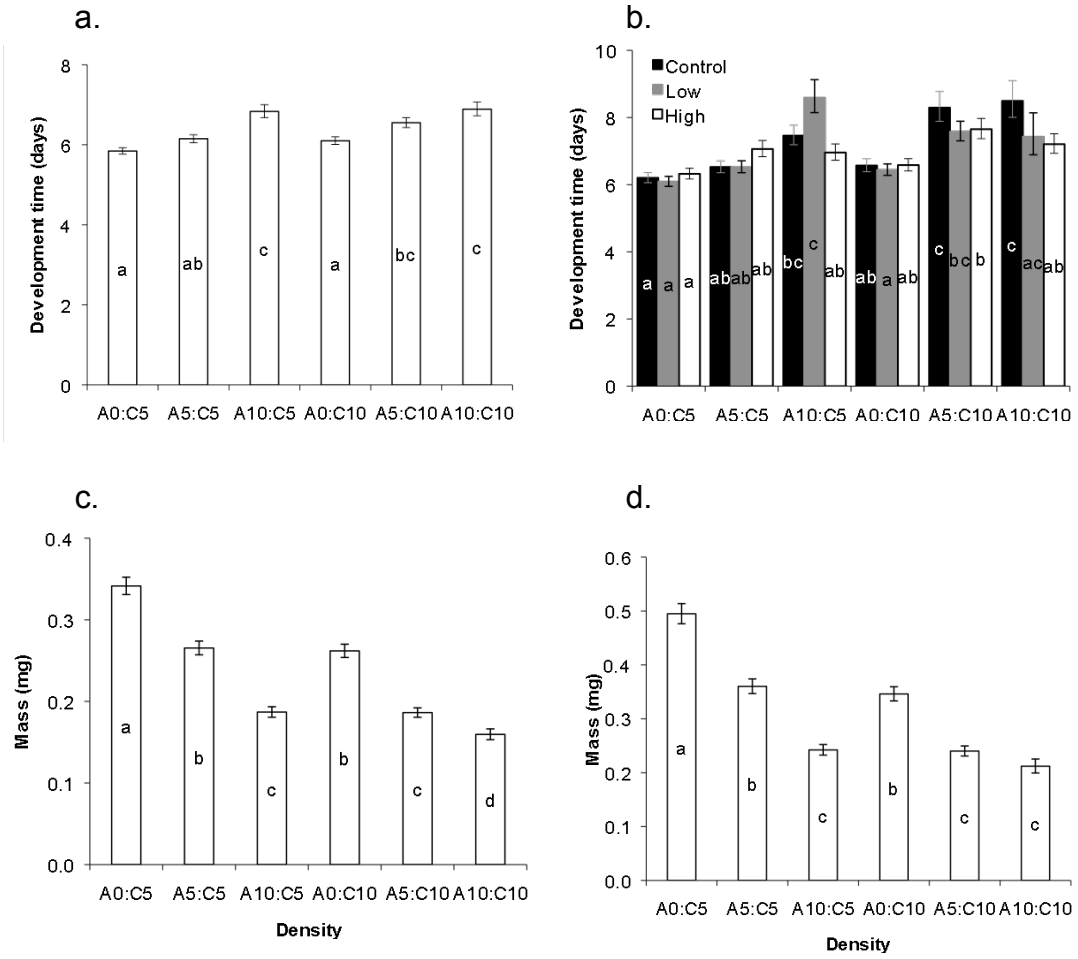

Figure 6. Mean (± 1 SE) *Cx. quinquefasciatus* development time of (a) males and (b) females, and adult mass of (c) males and (d) females across *Ae. albopictus*:*Cx. quinquefasciatus* (A:C) density combinations. Means for female development time (b) are separated by pollution concentration to show the pollution x density interaction; means for all other variables (a, c, d) are pooled across pollution concentrations. Different lowercase letters indicate significant pairwise differences between density combinations; lowercase letters for female development time (b) indicate significant differences among density combinations within pollution concentrations.

11), but post-hoc analysis revealed no pairwise differences between any treatments. Kruskal-Wallis tests indicated that female development time did not significantly differ among pollution levels within any density, or among density combinations within any pollution level (Tables 12 and 13). For adult mass of both sexes, ANOVA indicated significant effects of pollution, density, and their interaction (Table 11). Neither pollution level differed from the control for either sex in any density combination (Figure

Table 12

Kruskal-Wallis test results on Ae. albopictus *female development time differences among resource levels within each density combination and among density combinations within each resource level.*

Factor	X^2	df	*P*
[a]Pollution (A5:C0)	2.9990	2	0.2232
[a]Pollution (A5:C5)	0.2024	2	0.9038
[a]Pollution (A5:C10)	0.2258	2	0.8932
[a]Pollution (A10:C0)	2.3095	2	0.3151
[a]Pollution (A10:C5)	1.7202	2	0.4231
[a]Pollution (A10:C10)	1.7841	2	0.4098
[b]Density (Low)	6.7357	5	0.2411
[b]Density (Medium)	6.6172	5	0.2507
[b]Density (High)	6.1203	5	0.2947

[a]Tests are for differences among pollution concentrations within a single larval density combination (noted in parentheses).
[b]Tests are for differences among larval density combinations within a single pollution concentration (noted in parentheses).

7). Decreases in male mass concomitant with increasing *Cx. quinquefasciatus* density were found only within the low pollution concentration at high intraspecific density (Figure 7a). Female mass decreased when *Cx. quinquefasciatus* density increased from low to high, except in low pollution at low intraspecific density and high pollution at high intraspecific density (Figure 7b).

Population Growth: Resource Levels

Population growth of *Cx. quinquefasciatus* was negatively affected by *Ae. albopictus* under limited resources. Values for *Cx. quinquefasciatus* λ' were significantly lower in medium compared to high resources in density combinations where *Ae. albopictus* was present, and within the medium resource level when *Ae. albopictus* density was high (Table 7; Fig 8a). No *Cx. quinquefasciatus* females survived to adulthood in low resource treatments. Mean values of λ' indicated positive population growth (i.e., $\lambda' > 1$) in all density combinations in high resources, and in medium

resources in the absence of *Ae. albopictus*; mean λ' in medium resources with *Ae. albopictus* present indicated negative population growth (i.e., λ' < 1; Figure 8a).

Aedes albopictus performed best in medium resources; effects of density varied within each resource level, but negative effects of high density were found only in low resources. Values of λ' differed among resource levels at four larval density combinations (Table 7), with significantly greater values generally occurring in high and

Table 13

Mean (± SE) Ae. albopictus *development time of males and females across pollution concentration by* Ae. albopictus*:*Cx. quinquefasciatus *(A:C) density combination treatments.*

	Control		Low		High	
	Mean	± SE	Mean	± SE	Mean	± SE
Male						
A5:C0	5.6978	+0.1477 - 0.1440	5.9918	+0.1463 - 0.1428	5.7793	+0.1733 - 0.1683
A5:C5	5.9900	+0.1387 - 0.1356	5.4796	+0.1269 - 0.1240	5.8813	+0.1436 - 0.1402
A5:C10	5.9439	+0.1452 - 0.1417	5.8649	+0.1358 - 0.1327	5.5092	+0.1345 - 0.1313
A10:C0	5.8188	+0.1347 - 0.1317	5.7294	+0.1327 - 0.1297	6.0294	+0.1396 - 0.1364
A10:C5	5.7954	+0.1342 - 0.1312	5.7589	+0.1406 - 0.1373	6.1832	+0.1432 - 0.1399
A10:C10	5.8150	+0.1346 - 0.1316	5.6421	+0.1378 - 0.1345	5.8701	+0.1359 - 0.1328
Female						
A5:C0	6.1296	± 0.0668	6.0185	± 0.0705	6.3000	± 0.1548
A5:C5	6.3000	± 0.1356	6.3704	± 0.1614	6.2191	± 0.1393
A5:C10	6.2813	± 0.1856	6.5833	± 0.3721	6.2083	± 0.0778
A10:C0	6.1767	± 0.1300	6.2491	± 0.1225	6.4760	± 0.1429
A10:C5	6.3433	± 0.1239	6.4093	± 0.2080	6.4298	± 0.0721
A10:C10	6.7433	± 0.3078	6.5278	± 0.1571	6.3052	± 0.0866

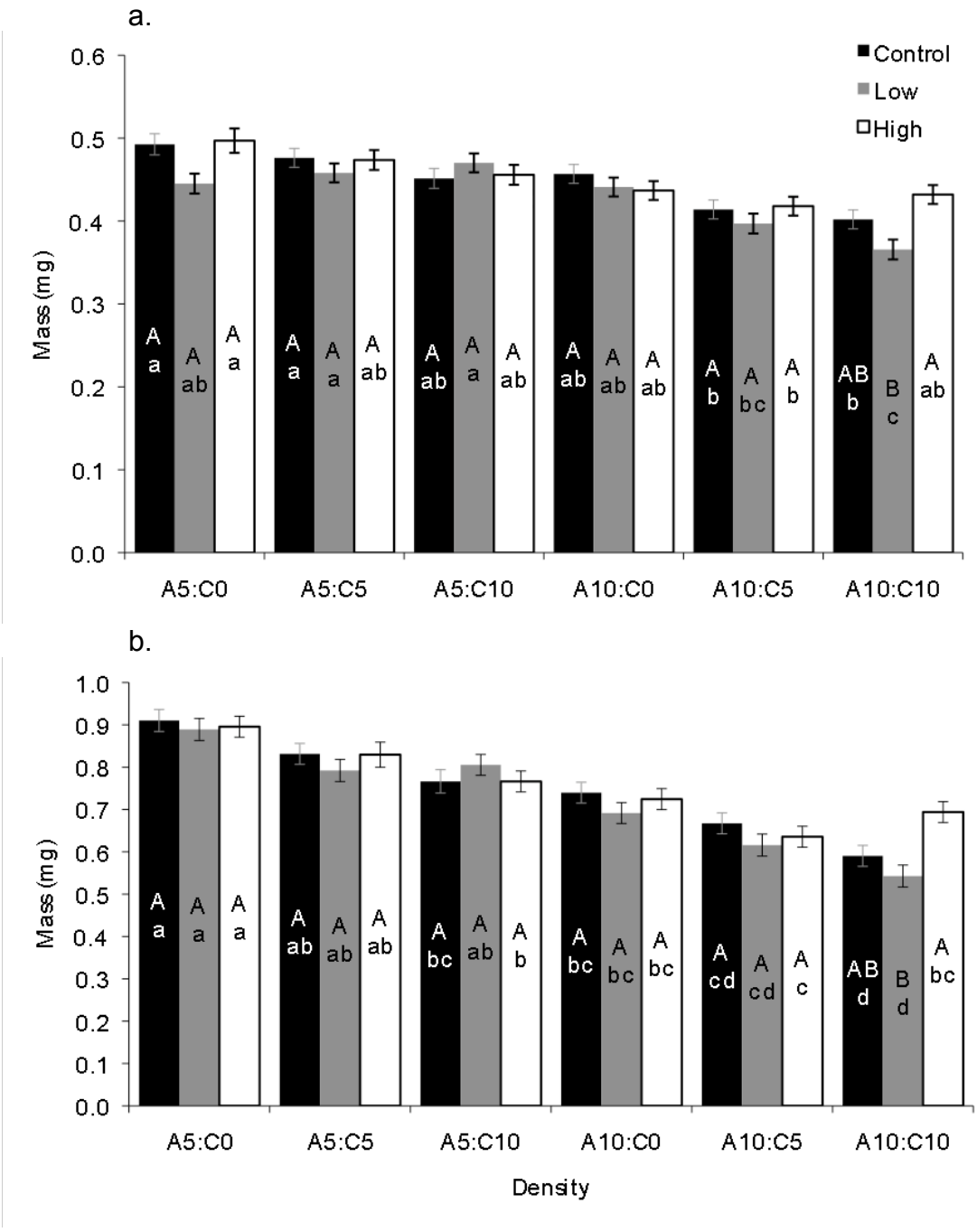

Figure 7. Mean (± 1 SE) *Ae. albopictus* adult mass of (a) males and (b) females *Ae. albopictus*:*Cx. quinquefasciatus* (A:C) density combinations by pollution concentraion. Different uppercase letters indicate significant pairwise differences between pollution concentrations within a density combination. Different lowercase letters indicate differences between density combinations within a pollution concentration.

medium resources than in low resources (Figure 8b). Differences among density combinations were found in medium and low resources (Table 7), but significant

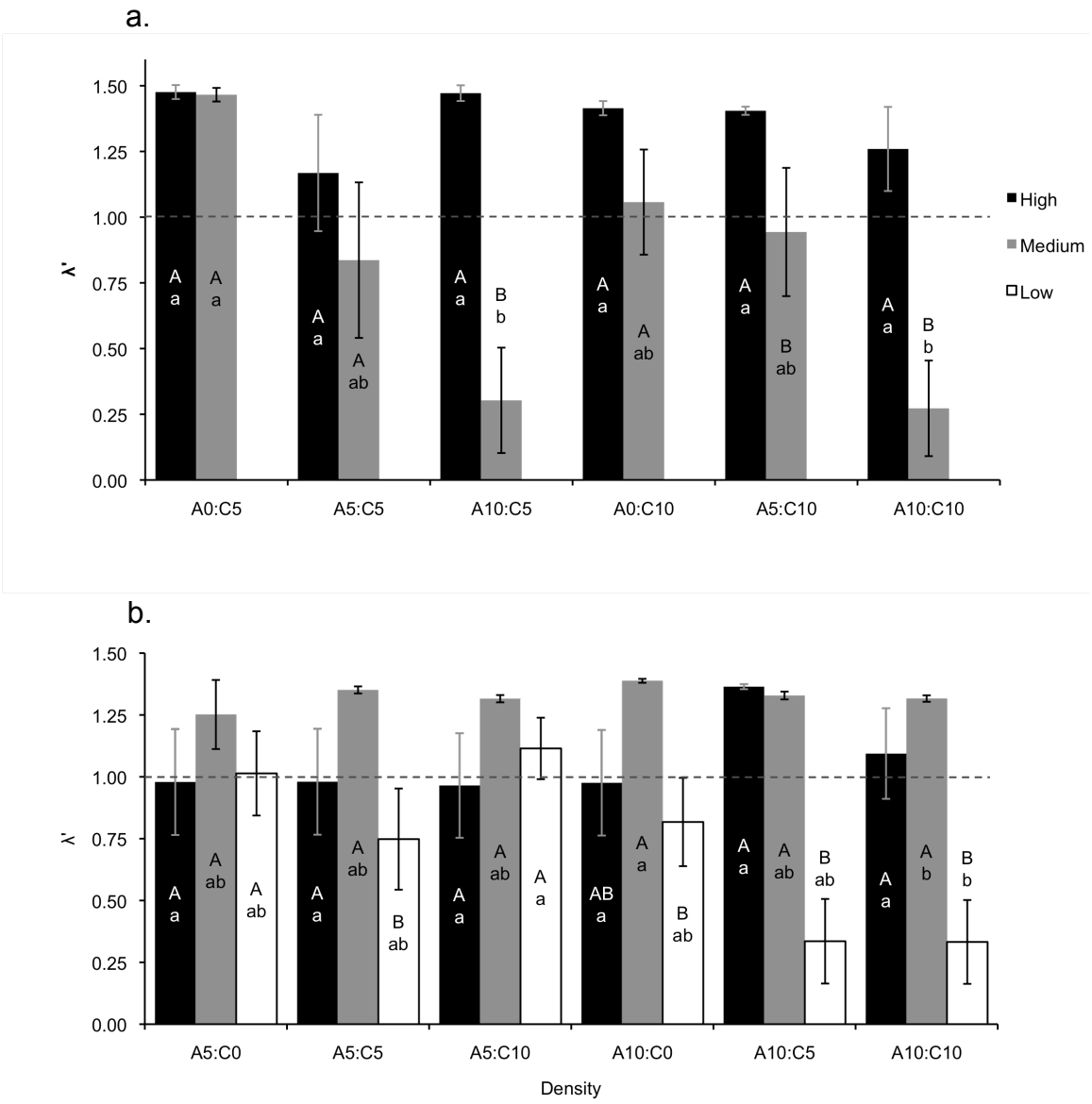

Figure 8. Mean (± 1 SE) estimated population growth (λ') across *Ae. albopictus*:*Cx. quinquefasciatus* (A:C) density combinations by resource level for (a) *Cx. quinquefasciatus*, and (b) *Ae. albopictus*. The dashed line at λ' = 1 indicates population growth equal to zero. Different uppercase letters indicate significant pairwise differences between resource levels within a density combination. Different lowercase letters indicate differences between density combinations within a resource level.

pairwise differences were slight in the medium resource level, and were attributable to intraspecific density rather than *Cx. quinquefasciatus* in the low resource level (Figure 8b). Mean λ' values indicated population growth in all density combinations in medium resources (Figure 8b). In high resources, mean λ' values indicated slight population

decline except when intraspecific density was high and *Cx. quinquefasciatus* was present (Figure 8b). Population decline also was indicated in low resources, with the exception of two density combinations in the low intraspecific density (Figure 8b).

Population Growth: Pollution

Culex quinquefasciatus population growth was not affected by pollution, and trends for density were similar but less pronounced than in the resource experiment.

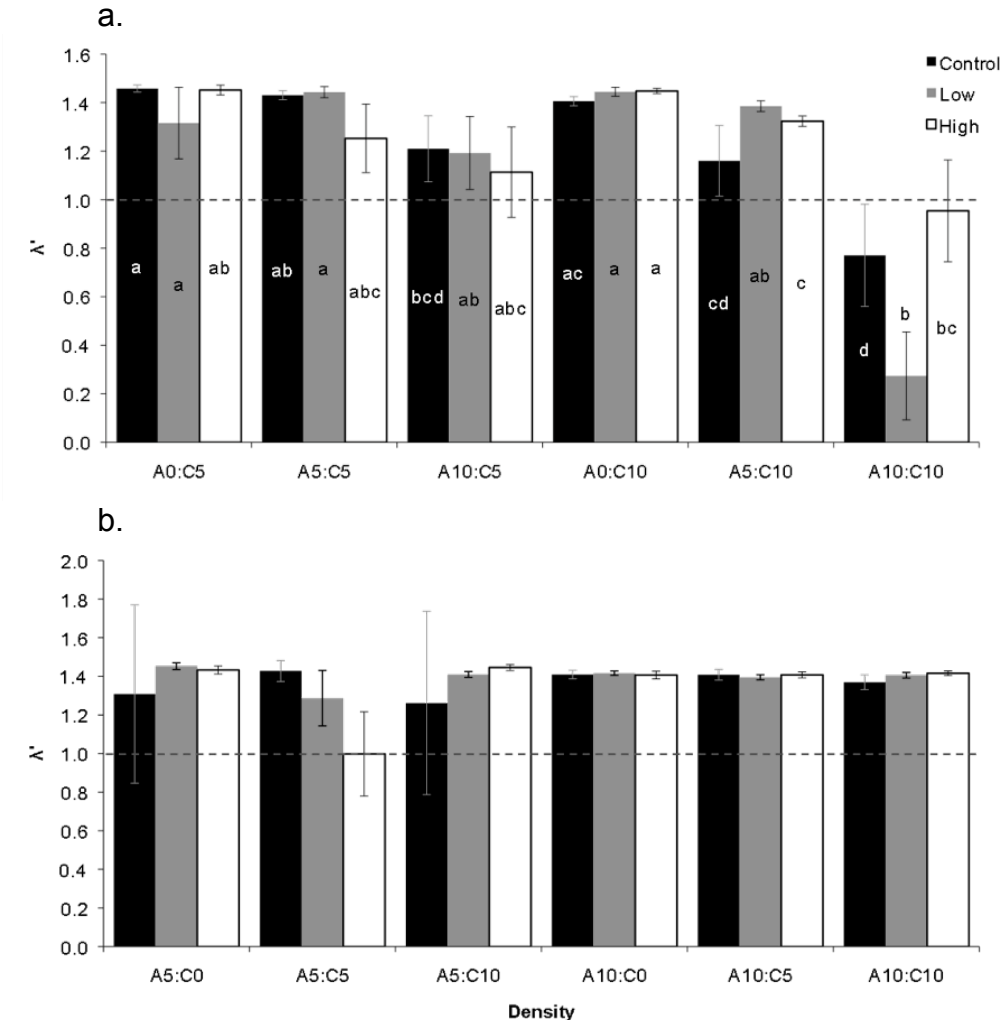

Figure 9. Mean (± 1 SE) estimated population growth (λ') across *Ae. albopictus*:*Cx. quinquefasciatus* (A:C) density combinations by pollution concentration for (a) *Cx. quinquefasciatus*, and (b) *Ae. albopictus*. The dashed line at λ' = 1 indicates population growth equal to zero. Different lowercase letters indicate significant pairwise differences between density combinations within pollution concentrations. No significant differences were found for *Ae. albopictus*.

Values of λ' differed among density combinations in all pollution concentrations, but did not differ among pollution concentrations in any density combination (Table 8). In all pollution concentrations at high intraspecific density, λ' was significantly lower when *Ae. albopictus* density increased from absent to high (Figure 9a). At low intraspecific density, λ' decreased significantly with *Ae. albopictus* density only in the control (Figure 9a). Mean values of λ' indicated positive population growth in all but the highest density combination (Figure 9a).

For *Ae. albopictus*, there were no differences in λ' among pollution concentrations at any density, or among density combinations at any pollution

Table 14

Results of two-way ANOVA (density combination and strain) on transformed values for Cx. quinquefasciatus *survivorship, and development time (days) and mass (mg) for males (m) and females (f). Significant effects are shown in bold type.*

Effect	df	*F*	*P*
Survivorship			
Density	5, 164	21.9399	**<0.0001**
Strain	1, 164	10.5274	**0.0014**
Density x strain	5, 164	1.6030	0.1620
Development Time (m)			
Density	5, 151	9.6309	**<0.0001**
Strain	1, 151	12.9564	**0.0004**
Density x strain	5, 151	1.6069	0.1615
Development time (f)			
Density	5, 143	16.6996	**<0.0001**
Strain	1, 143	36.6042	**<0.0001**
Density x strain	5, 143	1.1386	0.3428
Mass (m)			
Density	5, 149	49.0756	**<0.0001**
Strain	1, 149	10.4604	**0.0015**
Density x strain	5, 149	1.6425	0.1522
Mass (f)			
Density	5, 134	43.4058	**<0.0001**
Strain	1, 134	9.3531	**0.0027**
Density x strain	5, 134	1.1086	0.3585

concentration (Table 8). Mean values of λ' indicated positive population growth in every treatment combination except for high pollution when both species were present at low densities (Figure 9b).

Culex quinquefasciatus *Laboratory Acclimation*

Analysis indicated a significant effect of strain for *Cx. quinquefasciatus* survivorship, development time, and mass of both sexes; there was no significant density x strain interaction for any of these dependent variables (Table 14). Specifically, the lab

Table 15

Back-transformed least squared means (± SE) for wild and lab Cx. quinquefasciatus *survivorship, and development time (days) and mass (mg) for males (m) and females (f).*

	Wild		Lab	
	Mean	± SE	Mean	± SE
Survivorship	0.7279	+0.0164 - 0.0165	0.8244	+0.0242 - 0.0246
Development Time (m)	6.5250	+0.0610 - 0.0589	6.1532	+0.0810 - 0.0772
Development time (f)	7.4008	+0.0888 - 0.0855	6.6210	+0.0910 - 0.0870
Mass (m)	0.2170	+0.0034 - 0.0033	0.2392	+0.0063 - 0.0061
Mass (f)	0.2903	+0.0061 - 0.0060	0.3254	+0.0102 - 0.0099

strain produced higher survivorship, faster development times for both sexes, and larger adults of both sexes than the wild strain (Table 15).

Discussion

The results of the resource level experiment supported my hypothesis that resource competition occurs between *Ae. albopictus* and *Cx. quinquefasciatus*, and the results supported my prediction that *Ae. albopictus* is a superior resource competitor to

Cx. quinquefasciatus. Competitive asymmetry was produced when resources were limited (i.e., medium or low): *Culex quinquefasciatus* survival and population growth were lower in the presence of high numbers of *Ae. albopictus*, but *Ae. albopictus* was less affected by *Cx. quinquefasciatus* density within the same resource levels. In medium resources, *Cx. quinquefasciatus* experienced population decline in the presence of *Ae. albopictus* (Figure 8a), but *Ae. albopictus* maintained population growth within all density combinations (Figure 8b). Moreover, *Cx. quinquefasciatus* went extinct in low resources after one generation, as no females emerged from that resource level; *Ae. albopictus* experienced population decline in most density combinations, but it maintained population growth at one mixed-species density (A5:C10; Figure 8). Therefore, *Ae. albopictus* appears to be capable of competitively reducing or excluding *Cx. quinquefasciatus* in containers with limited resources.

The observed asymmetry is possibly due to the differing foraging strategies of the two species and the decay rates of the detritus used. Mosquitoes perform better in rapidly decaying detritus that supports high microorganism productivity (Dieng et al. 2002, Murrell and Juliano 2008), but species differ in their ability to exploit slowly decaying detritus. *Aedes albopictus* appears to better able to exploit slowly decaying resources (e.g., oak and elm leaves) than competitors (e.g., *Ae. aegypti*, *Ae. triseriatus*, and *Cx. pipiens*) (Barrera 1996, Yee et al. 2007, Murrell and Juliano 2008, Costanzo et al. 2011). This is possibly due to the superior ability of *Ae. albopictus* to harvest resources and efficiently convert them to biomass (Carrieri et al. 2003, Yee et al. 2004a). Additionally, *Ae. albopictus* allocates more time to browsing detrital surfaces for microorganisms than its competitors (Yee et al. 2004a, b), which may serve as an advantage when microorganism productivity is low. In addition, insect carcasses decay more rapidly and

support higher bacterial productivity than oak leaves (Murrell and Juliano 2008). *Aedes albopictus* can exploit both resource types (Yee et al. 2007), but *Cx. quinquefasciatus* may be less able to exploit leaves, as evidenced by its congener *Cx. pipiens* (Costanzo et al. 2011). Further studies are needed to determine how foraging behavior, efficiency of resource assimilation, and overall competitive outcomes between *Ae. albopictus* and *Cx. quinquefasciatus* compare in different resource environments.

Aedes albopictus survivorship and population growth were generally unaffected by *Cx. quinquefasciatus* density, but competition from *Cx. quinquefasciatus* had clear effects on *Ae. albopictus* adult mass. In medium resources, and in high resources at high intraspecific density, *Ae. albopictus* adults of both sexes were smaller when *Cx. quinquefasciatus* was present (Figure 5c and 5d). Although the presence of *Cx. quinquefasciatus* does not appear to affect population performance of *Ae. albopictus* at these resource levels, its effects on *Ae. albopictus* adult mass may have important implications for disease transmission patterns, as smaller females stressed by competition are more prone to arbovirus infection (Alto et al. 2005, 2008a). Thus, competition appears to be highly asymmetrical between these species, but subtle effects of *Cx. quinquefasciatus* competition on *Ae. albopictus* may still have consequences for disease dynamics.

Significant differences in development time among density combinations were found within resource levels for both species, but these differences did not appear to be associated with heterospecific densities. *Culex quinquefasciatus* exhibit rapid development in the presence of interspecific competition for space and resources from *Culex tarsalis* Coquillett (Smith et al. 1995). Although not statically significant, the trend for faster development times in mixed species treatments was apparent under high

resources for *Cx. quinquefasciatus* males (Figure 4a), and for females at low intraspecific density (Figure 4b). *Culex quinquefasciatus* appears to escape crowding (i.e., competition for space) via rapid development, but it may be unable to use this tactic to escape from resource competition, as limited resources may be insufficient to support rapid development (Harbison et al. 2009). Further studies are needed to see if *Cx. quinquefasciatus* uses rapid development when resources are sufficient to support it to escape from spatial competition with *Ae. albopictus*. This result may have implications for disease transmission, as rapid development to escape competition leads to reduced body size (Smith et al. 1995), which in turn may affect arbovirus infection rates (Alto et al. 2005, 2008a).

For *Ae. albopictus*, I observed that survivorship and population performance appeared to have opposite associations with increasing density in low and high resources. When grown alone in low density, survivorship in low and high resources was intermediate and similar, but this diverged in high densities, with survivorship being different in high (positive association) and low (negative association) resources with increased density (Figure 2b). This trend was also observed for population growth, where negative population growth ($\lambda' < 1$) was observed in high resources except when intraspecific density was high and *Cx. quinquefasciatus* was present (Figure 8b). In contrast, *Cx. quinquefasciatus* attained positive population growth in all high resource treatments regardless of density (Figure 8a). The observed pattern may have been due to the increased amount of insect detritus in high resources, which putrefies the water and may be toxic to *Ae. albopictus* larvae in high amounts (Murrell and Juliano 2008); *Culex quinquefasciatus* is less likely to be affected by this, as it is highly tolerant to organic pollution (Subra 1981). High intra- and interspecific densities may serve to facilitate *Ae.*

albopictus performance in the presence of high amounts of rapidly decomposing detritus (e.g., grasses, invertebrate carcasses) via increased control of microbial communities (Kaufman et al. 1999).

The results of the pollution experiment did not support my hypothesis that pollution would affect interspecific competition between *Ae. albopictus* and *Cx. quinquefasciatus*. The addition of chemicals associated with detrital decay and animal excrement did not alter the outcome of competition. With the exception of *Cx. quinquefasciatus* mass, there were no cases in which any of the variables measured for either species differed between the control and either concentration of the chemical blend. This suggests that the chemicals either were not responsible for the negative effect of high detritus on *Ae. albopictus*, or that the concentrations used were insufficient to affect the performance of either species. The concentrations of the chemicals present in the blend are based on the amounts present in headspace extracts above water containing decomposing grass (Du and Millar 1999), and therefore may not reflect the amounts present in the water itself. Further studies of the chemicals released into the water column by detrital decomposition and their concentrations at various detritus levels and water volumes are needed to assess what effects, if any, these chemicals have on mosquito survival and interspecific interactions at concentrations reflective of those in the field.

Although no significant effects of pollution were found, the effect of *Ae. albopictus* density on *Cx. quinquefasciatus* survivorship in the pollution experiment was less pronounced than in the resource experiment. Under the medium resource level in the resource experiment, *Cx. quinquefasciatus* survivorship was negatively affected by *Ae. albopictus* regardless of *Cx. quinquefasciatus* density (Figure 2a), whereas in the

pollution experiment, which used the same resource amount, this effect was only significant when *Cx. quinquefasciatus* density was high (Figure 3a). The differences in survivorship between the two experiments were substantial enough to alter estimated population growth; in the resource experiment, competitive reduction of *Cx. quinquefasciatus* was projected in the presence of *Ae. albopictus* in medium resources in all mixed-species density combinations (Figure 8a), but in the pollution experiment, *Cx. quinquefasciatus* seems capable of co-existing with *Ae. albopictus* at this resource level at lower densities (Figure 9a). Despite that the magnitude of the competitive effect of *Ae. albopictus* on *Cx. quinquefasciatus* differed in the two experiments, the overall conclusion that competition between *Cx. quinquefasciatus* and *Ae. albopictus* is asymmetrical with *Ae. albopictus* the superior competitor holds true.

I found that while there were effects of lab acclimation on *Cx. quinquefasciatus* life history traits, that these effects did not interact with larval density, indicating *Ae. albopictus* competition has the same negative effect on wild and lab *Cx. quinquefasciatus*. Therefore, results of the resource level experiment, which used only lab *Cx. quinquefasciatus*, should be applicable to wild *Cx. quinquefasciatus* with the caveat that wild survivorship and mass would likely be have been lower, and wild development times would likely have been longer.

This is the first study to investigate larval interactions between *Ae. albopictus* and *Cx. quinquefasciatus*. I demonstrated that *Ae. albopictus* is a superior resource competitor and appears to be capable of competitively reducing or excluding *Cx. quinquefasciatus* from an individual container after one generation under limited resources. Because the competitive advantage of *Ae. albopictus* over other mosquito species is often context-dependent (e.g., Barrera 1996, Costanzo et al. 2005b, Griswold

and Lounibos 2005a), more studies are needed to understand the effects of extraneous factors (e.g., predation, weather patterns, resource types) on competition between *Ae. albopictus* and *Cx. quinquefasciatus*. Additionally, *Cx. quinquefasciatus* lays its eggs on the water surface, and the eggs hatch after one day (Subra 1981), whereas *Ae. albopictus* lays the majority of its eggs on container walls above the water surface, and the eggs do not hatch until the water level rises sufficiently to submerge them (Hawley 1988). Therefore, egg hatching times of these species are not necessarily synchronous and may vary due to rainfall patterns, meaning that interspecific competition between *Aedes* and *Culex* in the field is likely to occur between different larval instars. Future work could test the effects of non-synchronous egg hatching on competitive outcomes between these species. Although it is unlikely that *Ae. albopictus* will displace *Cx. quinquefasciatus* on a regional scale, as *Cx. quinquefasciatus* also utilizes non-container habitats (Subra 1981), interspecific competition between these species clearly has the potential to affect vector population dynamics, especially when containers represent the majority of available mosquito breeding habitats.

APPENDIX

IACUC PROTOCOL

The University of
Southern Mississippi

Institutional Animal Care and Use Committee

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

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION

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

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

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

Jodie M. Jawor, Ph.D.
IACUC Chair

9/30/11
DATE

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