

INTERACTIONS OF INVASIVE SPECIES IN MOSQUITO CONTAINER COMMUNITIES  
IN VIRGINIA

By

JENNIFER S. ARMISTEAD

A THESIS PRESENTED TO THE GRADUATE SCHOOL  
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2007

© 2007 Jennifer S. Armistead

To my husband Paul, whose love, friendship, patience and encouragement are heaven-sent, and to my father Gary, for showing me that in all things nature there is something marvelous.

## ACKNOWLEDGMENTS

I would like to thank my advisor, L. P. Lounibos, for sharing his expertise and providing direction for my research, as well as for his confidence, patience, and flexibility in working with me from a distance. I am grateful to my committee members, G. F. O'Meara for his insight in working with *Ochlerotatus atropalpus*, and J.R. Arias for sharing his knowledge and ingenuity and for graciously allowing me to use his equipment and laboratory space. I would like to thank R. Escher, N. Nishimura, and M. Reiskind for their technical assistance during my time at the Florida Medical Entomology Laboratory, and at the Fairfax County Department of Health in Virginia I am appreciative of J. Frescholtz and J. van der Voort for their assistance in the field, and A. Joye for his help with GIS. I am grateful to L. McCuiston for providing the *O. atropalpus* and *O. japonicus* eggs used in this research. I would also like to thank B. Harrison and J. Scott for engaging in discussions with me regarding *O. japonicus*.

## TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS .....	4
ABSTRACT.....	10
CHAPTER	
1 INTRODUCTION AND LITERATURE REVIEW .....	12
Invasion Biology.....	12
<i>Ochlerotatus japonicus</i> .....	15
2 FIELD ASSESSMENT OF INTERSPECIFIC INTERACTIONS AMONG INVASIVE AND NATIVE CONTAINER-INHABITING MOSQUITOES .....	19
Introduction.....	19
Materials and Methods .....	21
Oviposition Traps .....	21
Natural and Artificial Containers .....	23
Adult Surveillance .....	24
Data Analysis.....	24
Oviposition Traps .....	24
Natural and Artificial Containers .....	25
Adult Surveillance .....	27
Results.....	28
Oviposition Traps .....	28
Natural and Artificial Containers .....	29
Interspecific Associations .....	30
Habitat Comparisons .....	31
<i>Aedes albopictus</i> and <i>Ochlerotatus japonicus</i> .....	31
<i>Ochlerotatus japonicus</i> and <i>Ochlerotatus triseriatus</i> .....	34
<i>Ochlerotatus atropalpus</i> and <i>Ochlerotatus japonicus</i> .....	34
Adult Surveillance .....	35
Discussion.....	36
3 INTERSPECIFIC COMPETITION BETWEEN <i>AEDES ALBOPICTUS</i> AND <i>OCHLOEROTATUS JAPONICUS</i> .....	66
Introduction.....	66
Materials and Methods .....	69
Data Analysis.....	71
Population Growth Correlates .....	71
Composite Index of Population Performance.....	72
Results.....	74

Survivorship to Adulthood .....	74
Developmental Time .....	74
Female Wing Length .....	75
Estimated Finite Rate of Increase ( $\lambda'$ ).....	75
Discussion.....	75
<b>4 INTERSPECIFIC COMPETITION BETWEEN <i>OCHLEROTATUS ATROPALPUS</i></b> <b>AND <i>OCHLEROTATUS JAPONICUS</i> .....</b>	<b>85</b>
Introduction.....	85
Materials and Methods .....	87
Data Analysis.....	89
Population Growth Correlates .....	89
Composite Index of Population Performance.....	89
Results.....	92
Survivorship to Adulthood .....	92
Developmental Time .....	92
Female Wing Length .....	93
Estimated Finite Rate of Increase ( $\lambda'$ ).....	93
Discussion.....	93
<b>5 CONCLUSIONS .....</b>	<b>105</b>
<b>APPENDIX</b>	
<b>A DESCRIPTIVE STATISTICS AND INFORMATION: CHAPTER 2.....</b>	<b>109</b>
<b>B HABITAT COMPARISONS: RANK ORDERS OF MOSQUITO SPECIES .....</b>	<b>110</b>
<b>LIST OF REFERENCES.....</b>	<b>111</b>
<b>BIOGRAPHICAL SKETCH .....</b>	<b>124</b>

## LIST OF TABLES

<u>Table</u>	<u>page</u>
2-1 Frequency of occurrence of <i>A. albopictus</i> , <i>O. hendersoni</i> , and <i>O. triseriatus</i> in ovitraps set at three sites and two heights in Fairfax, Virginia in 2006.....	44
2-2 Frequency of occurrence, by month, of <i>A. albopictus</i> , <i>O. hendersoni</i> , and <i>O. triseriatus</i> in ovitraps in Fairfax County, Virginia in 2006.....	45
2-3 Monthly abundance (mean number of eggs per trap) of <i>A. albopictus</i> , <i>O. hendersoni</i> , and <i>O. triseriatus</i> collected in ovitraps.....	46
2-4 Coefficients of association ( $C_8$ ) for the most abundant species in 191 artificial and natural container samples from Fairfax County, Virginia in 2006.....	47
2-5 Intra- and interspecific mean crowding of the most abundant mosquito species collected from artificial and natural containers in Fairfax County, Virginia in 2006.....	48
2-6 Species rank abundances compared for different container habitats sampled in Fairfax County, Virginia from May – September 2006.....	49
2-7 Number of larvae and average instar number for <i>A. albopictus</i> and <i>O. japonicus</i> collected in Fairfax County, Virginia by month, May – September 2006.....	50
2-8 Tests for significant heterogeneity of monthly instar distributions of <i>A. albopictus</i> and <i>O. japonicus</i> from June – August 2006, based on log-rank statistics.....	51
2-9 Least square means ( $\pm$ SE) for intraspecific crowding (transformed by $\log_{10}(x + 1)$ ) among larvae of <i>A. albopictus</i> and <i>O. japonicus</i> June – August 2006.....	52
2-10 Frequencies of collection of <i>A. albopictus</i> , <i>O. japonicus</i> , and <i>O. triseriatus</i> adult females in CO <sub>2</sub> -baited CDC light traps and gravid traps, 2004 – 2006.....	53
3-1 Means ( $\pm$ SE) of population growth correlates for <i>A. albopictus</i> and <i>O. japonicus</i> .....	79
4-1 Means ( $\pm$ SE) of population growth correlates for <i>O. japonicus</i> and <i>O. atropalpus</i> .....	99
A-1 Descriptive statistics and information for mosquito species collected in a survey of natural and artificial container habitats in Fairfax County, Virginia in 2006.....	109
B-1 Rank orders of immature mosquito abundances used for habitat comparisons of rock pools, tires, small and large artificial containers.....	110

## LIST OF FIGURES

<u>Figure</u>	<u>page</u>
2-1 Map of Fairfax County, Virginia showing locations of study areas that were sampled or censused repeatedly. ....	54
2-2 Proportion of mosquito-positive containers containing <i>A. albopictus</i> , <i>O. japonicus</i> , or both <i>A. albopictus</i> and <i>O. japonicus</i> May – September 2006.....	55
2-3 Monthly abundance (mean number of mosquitoes collected per container) of <i>A. albopictus</i> and <i>O. japonicus</i> ( $\pm$ SE) from 91 mosquito-positive artificial containers .....	56
2-4 Seasonal occurrences (proportion of species-positive containers per month) of <i>A. albopictus</i> and <i>O. japonicus</i> collected from artificial containers in 2006 .....	57
2-5 Monthly instar distributions of <i>A. albopictus</i> from May – September 2006 in Fairfax, Virginia. ....	58
2-6 Monthly instar distributions of <i>O. japonicus</i> from May – September 2006 in Fairfax, Virginia. ....	60
2-7 Interspecific mean crowding of <i>A. albopictus</i> by <i>O. japonicus</i> and <i>O. japonicus</i> by <i>A. albopictus</i> .....	60
2-8 Intraspecific mean crowding (density of conspecifics encountered per unit resource, $a$ ) of <i>A. albopictus</i> and <i>O. japonicus</i> by month.....	61
2-9 Metamorphic success of <i>A. albopictus</i> and <i>O. japonicus</i> collected from containers in which the two species co-occurred. ....	62
2-10 Mean weekly abundance of <i>A. albopictus</i> collected in (A) CO <sub>2</sub> -baited light traps and (B) gravid traps over time, from 2004 through 2006, in Fairfax County, Virginia. ....	63
2-11 Mean weekly abundance of <i>O. japonicus</i> collected in (A) CO <sub>2</sub> -baited light traps and (B) gravid traps over time, from 2004 through 2006, in Fairfax County, Virginia. ....	64
2-12 Mean weekly abundance of <i>O. triseriatus</i> collected in (A) CO <sub>2</sub> -baited light traps and (B) gravid traps over time, from 2004 through 2006, in Fairfax County, Virginia. ....	65
3-1 Mean survivorship (proportion of the original number of larvae surviving to adulthood) of <i>A. albopictus</i> and <i>O. japonicus</i> ( $\pm$ SE) .....	80
3-2 Means of median time to adulthood for female <i>A. albopictus</i> and <i>O. japonicus</i> ( $\pm$ SE).....	81
3-3 Means of median time to adulthood for male <i>A. albopictus</i> and <i>O. japonicus</i> ( $\pm$ SE).....	82
3-4 Means of median wing lengths of <i>A. albopictus</i> and <i>O. japonicus</i> adult females ( $\pm$ SE) .....	83

3-5 Mean estimates of population performance ( $\lambda'$ , an estimate of the finite rate of increase for the cohort) for female <i>A. albopictus</i> and <i>O. japonicus</i> adults ( $\pm$ SE) .....	84
4-1 Mean survivorship (proportion of the original number of larvae surviving to adulthood) of <i>O. japonicus</i> and <i>O. atropalpus</i> ( $\pm$ SE) .....	100
4-2 Means of median time to adulthood for female <i>O. japonicus</i> and <i>O. atropalpus</i> ( $\pm$ SE).....	101
4-3 Means of median time to adulthood for male <i>O. japonicus</i> and <i>O. atropalpus</i> ( $\pm$ SE).....	102
4-4 Means of median wing lengths of <i>O. japonicus</i> and <i>O. atropalpus</i> adult females ( $\pm$ SE) .....	103
4-5 Mean estimates of population performance ( $\lambda'$ , an estimate of the finite rate of increase for the cohort) for female <i>O. japonicus</i> and <i>O. atropalpus</i> adults ( $\pm$ SE) .....	104

ABSTRACT OF THESIS PRESENTED TO THE GRADUATE SCHOOL  
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

INTERACTIONS OF INVASIVE SPECIES IN MOSQUITO CONTAINER COMMUNITIES  
IN VIRGINIA

By

Jennifer S. Armistead

May 2007

Chair: L. Philip Lounibos

Major: Entomology and Nematology

The success of an invasive species to become established in a new region often depends on its interactions with ecologically similar resident species. Introductions of disease vectors, particularly mosquitoes, are of significant importance as their invasions may have ecological and epidemiological consequences. Interactions of a recent invasive mosquito with resident species in containers in Virginia were evaluated through field surveys and controlled experiments.

In my study, sampling of larvae from natural and artificial containers, trapping of adults, and ovitraps were used to confirm and quantify co-occurrences and potential interactions of *Ochlerotatus japonicus* with resident mosquitoes in these habitats. Frequent and abundant occurrences of *O. japonicus* in rock pools were associated with the possible decline and displacement of native *O. atropalpus*. Laboratory evaluation of the effects of larval resource competition on the population performance of these two species suggest that interspecific competition is probable and likely to favor the success of *O. japonicus* over *O. atropalpus*. Autogenous reproduction of *O. atropalpus*, which requires a lengthened period of larval

development to obtain nutrient reserves for egg development, may disadvantage it in larval competition in conditions of limited resources.

In my study, field collections of *O. japonicus* from artificial containers inhabited by larvae of resident mosquitoes demonstrated their coexistence in these habitats. A field experiment that measured inter- and intraspecific effects of larval density on the population performance of *A. albopictus* and *O. japonicus* indicated the former species to be a superior competitor. However, the ability of *O. japonicus* to perform equally well in the presence or absence of *A. albopictus* suggests these two species will be able to coexist in artificial container habitats in nature. High larval densities, intraspecific mean crowding, and superior population performance of *A. albopictus* under inter- rather than intraspecific conditions suggest that intraspecific competition may be most important in regulating population growth of this species in container habitats.

Interactions of *O. japonicus* with resident container-inhabiting mosquitoes appear to be influenced by species-specific differences in seasonality. The ability of *O. japonicus* to overwinter as larvae allows it to resume development earlier in the spring than both *A. albopictus* and *O. atropalpus*. Consequent co-occurrence of older *O. japonicus* with early hatchlings of *A. albopictus* and *O. atropalpus* may favor the more recent invader during competition.

Infrequent collections of *O. japonicus* in ovitraps suggest that use of this technique may be improved in alternative macrohabitats where this species is most abundant. While trapping of adults over a three-year period indicated no changes in the frequency or abundance of *A. albopictus*, significant population declines of both *O. japonicus* and *O. triseriatus* were observed over this period. Continued monitoring of all life stages of these species over several years will be necessary to observe any significant population changes or identify ecological processes at work since the invasion of *O. japonicus*.

## CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

#### **Invasion Biology**

Biological invasions are occurring at an alarming frequency worldwide, the impacts of which threaten biodiversity, ecosystem functioning, resource availability, national economies, and human health (Ruesink et al. 1995, Simberloff 1996). Nonindigenous organisms, including invertebrates, vertebrates, plants, bacteria, and fungi, are spreading into new regions at unprecedented rates, becoming established in all but the most remote areas of the planet. Historically, some of the most important biological invasions have involved dispersal of disease vectors, particularly mosquitoes (Diptera: Culicidae), a trend that has continued at an increasing rate over the past century (reviewed by Lounibos 2002, Juliano and Lounibos 2005). The most recent successful invasions of mosquitoes have resulted from human transport of immature stages. The most notable invasions and range expansions in the United States have been by mosquitoes that occupy container habitats (Lounibos 2002), which is the topic of my study.

The patterns and processes related to the introduction, establishment, spread, and impact of non-native species is the focus of invasion biology (Williamson 1996). The term invasive refers to an introduced species that has increased and spread (Daehler 2001, Richardson et al. 2000), with the potential to impact native species and ecosystems, or human activities (Juliano and Lounibos 2005). Common characteristics of invasive species are thought to include an *r*-selected life history strategy, range expansion, high rate of population increase, ability to compete for resources and habitat with native species, repeated introductions, and associations with humans (reviewed by Sakai et al. 2001). The three most successful species of invasive mosquitoes, *Aedes aegypti*, *A. albopictus* and the *Culex pipiens* Complex, seem to have been favored by propagule (or invasion) frequency and previous invasion success (Lounibos 2002). A retrospective review

of life history characteristics of successful invasive mosquitoes by Juliano and Lounibos (2005) revealed that desiccation-resistant eggs and occupying human-dominated habitats are significantly associated with invasive status, while larval habitat, autogeny, and diapause are not; however data are absent for many species.

Invasive organisms may impact native species through biotic interactions, such as predation, parasitism and competition, as well as ecosystems by affecting ecological processes, such as water and nutrient cycling (Williamson 1996). Invasions may also have genetic and evolutionary consequences, resulting in short-term changes, such as hybridization or introgression (Rhymer and Simberloff 1996), or long-term changes from genetic drift or natural selection (Williamson 1996, Sakai et al. 2001). Multiple processes will likely act in a single invasion system, and they may either facilitate invasion success and spread, which is often indicated by the decline or elimination of ecologically similar species (Juliano 1998), or act as barriers to invasion (Rosen et al. 1976, O'Meara et al. 1989).

Many ecological processes associated with invasive species have been shown to play important roles in structuring the mosquito communities of container habitats, including interspecific competition, predation, and parasitism. Interspecific competition may be defined as any negative – negative effect between two species or a negative – zero effect where one species affects another but is not reciprocally affected. Interspecific competition is unavoidable unless the invader is filling a vacant niche by exploiting a previously unoccupied habitat or unused resource (Williamson 1996). Interspecific competition among mosquitoes encompasses resource competition (Ho et al. 1989, Livdahl and Willey 1991, Daugherty et al. 2000, Juliano et al. 2004, Costanzo et al. 2005), chemical interference (Sunahara and Mogi 2002, Bédhomme et al. 2005), mating interference (Ribiero and Spielman 1986, Nasci et al. 1989), and hatching inhibition

(Edgerly et al. 1993). Predation is a prominent feature of many container habitats utilized by mosquitoes. Differences in behavioral responses to water-borne cues from predation (Kesavaraju and Juliano 2004) are thought to result in selective predation on invasive (Grill and Juliano 1996, Griswold and Lounibos 2005a,b) or native (Lounibos et al. 2001) species. Intraguild predation, in which older immature stages prey upon newly hatched larvae of conspecifics or heterospecifics, is also known to occur in mosquito communities (Edgerly et al. 1999, Koenraadt and Takken 2003, Koenraadt et al. 2004). This type of predation is often facilitated by differences in seasonality, in which one species hatches earlier in the year than the other (Teng and Apperson 2000). Apparent competition caused by shared gregarine parasites has been investigated in interactions of invasive mosquitoes with multiple resident species with varying results (Juliano 1998, Aliabadi and Juliano 2002).

In addition to effects on resident species or ecosystems, invasive mosquitoes are of particular concern because they may also impact human or vertebrate animal health. While interactions with resident species usually occur among aquatic larvae, it is the terrestrial adult phase of mosquitoes that is most likely to impact human health. Invasive mosquitoes in the New World have been associated with human disease outbreaks of yellow fever (Tabachnick 1991), dengue (Gubler 1997), and malaria (Soper and Wilson 1943, Lounibos 2002), and may be associated with the transmission of Eastern equine, LaCrosse, and West Nile encephalitis viruses (reviewed by Lounibos 2002, Juliano and Lounibos 2005). Human health may be affected by invasive mosquitoes in three ways (Juliano and Lounibos 2005): simultaneous introduction of a novel vector and novel pathogen (Tabachnick 1991, Gubler 1997), acquisition of a native pathogen by a novel vector (Soper and Wilson 1943, Gubler 1997, Lounibos 2002), or independent introductions of a novel vector and a novel pathogen (Ross 1911, Kramer and

Bernard 2001). Simultaneous or independent introductions of novel vectors and novel pathogens may create new public health threats due to the high susceptibility of the host population, whereas if a novel vector becomes involved in an existing disease transmission cycle, it alters the nature of an existing public health threat by changing the transmission rate due to differences in vector efficiency (Juliano and Lounibos 2005).

### ***Ochlerotatus japonicus***

In 1998, *Ochlerotatus japonicus*, a container-inhabiting mosquito native to eastern Asia (Tanaka et al. 1979), was detected independently in light trap collections in New York and New Jersey (Peyton et al. 1999) and human biting collections in Connecticut (Andreadis et al. 2001). The introduction of *O. japonicus* is presumed to have occurred via tire shipments (Peyton et al. 1999), a mechanism which accounted for multiple interceptions of larvae of this species in New Zealand (Heardon et al. 1999, Laird et al. 1994). Since its arrival in 1998, *O. japonicus* has spread throughout the eastern United States with reports from Alabama (Qualls and Mullen 2006), Georgia and South Carolina (Reeves and Korecki 2004), Indiana (Young et al. 2004), Maine (Foss and Dearborn 2001), Maryland (Sardelis and Turell 2001), Missouri (Gallitano et al. 2006), Ohio and Pennsylvania (Fonseca et al. 2001), Tennessee (Caldwell et al. 2005), Virginia (Harrison et al. 2002), and Vermont (Graham and Turmel 2001). On the West coast of the United States, *O. japonicus* is only known to occur in Washington state (Roppo et al. 2004). Examination of patterns of genetic diversity using random amplified polymorphic DNA and sequences of mtDNA from populations spanning the range of *O. japonicus* in Japan and the United States revealed distinct genetic signatures in U.S. populations, suggesting multiple introductions from Japan (Fonseca et al. 2001). In addition to the United States, *O. japonicus* has become established in Canada (Savignac et al. 2002, Thielman and Hunter 2006) Belgium (Widdel et al. 2005), and France (Schaffner et al. 2003).

*Ochlerotatus japonicus* was previously known as *Aedes japonicus* until revisions of that genus by Reinert (2000), based on differences in the primary characteristics of male and female genitalia, elevated the subgenus *Ochlerotatus* to generic level. *Ochlerotatus japonicus* is a member of the *Chrysolineatus* subgroup of the subgenus *Finlaya*. *Ochlerotatus japonicus* sensu lato includes four morphologically similar subspecies that occur throughout most of Japan, Taiwan, Korea, eastern China, and Russia (Tanaka et al. 1979): *O. japonicus amamiensis* (Tanaka, Mizusawa and Saugstad 1979), *O. japonicus japonicus* (Theobald 1901), *O. japonicus shintienensis* (Tsai and Lien 1950), and *O. japonicus yaeyamensis* (Tanaka, Mizusawa and Saugstad 1979). Only the subspecies *O. japonicus japonicus*, which is common in Palearctic Japan and Korea, has been detected outside its native range (Fonseca et al. 2001); henceforth in this thesis, *O. japonicus japonicus* will be referred to simply as *O. japonicus*.

In both the native range of this species and the United States, *O. japonicus* larvae inhabit a wide variety of natural (treeholes, rock pools) and artificial containers (buckets, tires, birdbaths), with rock pools being the preferred habitat (Tanaka et al. 1979, Andreadis et al. 2001, Scott 2003). Surveys have revealed that the species may occur in temporary ground water sources as well, although this is uncommon (LaCasse and Yamaguti 1950, Andreadis et al. 2001).

*Ochlerotatus japonicus* larvae have been detected in containers with varying levels of sun exposure and water temperature (Oliver et al. 2003). Adults of this species have been shown to feed on avian and mammalian hosts in the laboratory (Miyagi 1971), although bloodmeal analyses of wild-caught females have all been mammalian in origin (Scott 2003, Apperson et al. 2002). *Ochlerotatus japonicus* will bite humans that encroach on its habitat (Knight 1968), but are often protracted in their approach (B. Harrison personal communication).

The introduction of *O. japonicus* into the United States is of considerable interest because of the vector potential of this species as well as the ecological consequences that may result from its invasion. *Ochlerotatus japonicus* has demonstrated the ability to transmit Japanese encephalitis (Sucharit et al. 1989, Takashima and Rosen 1989) and Getah (Takashima and Hashimoto 1985) viruses in the laboratory, although it is not considered an important vector of either virus in its native range, having only been implicated in the transmission of Japanese encephalitis virus in Far East Asia (Chagin and Kondratiev 1943). *Ochlerotatus japonicus* is also a competent laboratory vector of eastern equine encephalitis (Sardelis et al 2002a), LaCrosse (Sardelis et al. 2002b), St. Louis encephalitis (Sardelis et al. 2003), and West Nile (Sardelis and Turell 2001, Turell et al. 2001) viruses. However, in the United States only the latter has been recovered from wild-caught females of this species (Werner 2001, White et al. 2001, Scott 2003). This combination of a separately introduced novel vector and novel pathogen could become epidemiologically significant in North America.

*Ochlerotatus japonicus* is known to co-occur with numerous resident species in natural and artificial containers in North America (Andreadis et al. 2001, Scott et al. 2001a, Oliver et al. 2003, Thielman and Hunter 2006). This species has most frequently been recovered from containers inhabited by *A. albopictus*, *C. pipiens* (Andreadis et al. 2001, Gallitano et al. 2006), *Culex restuans* (Scott et al. 2001a), *O. atropalpus*, and *O. triseriatus* (Andreadis et al. 2001, Oliver et al. 2003), although it has also been known to occur with *Anopheles punctipennis* (Scott et al. 2001a), *Anopheles quadrimaculatis* (Thielman and Hunter 2006), and *Culex territans* (Oliver et al. 2003). However, there is currently no information concerning the nature of the interactions of *O. japonicus* with these species or the ecological processes that have been

operating during its invasion. Therefore, a study of the interactions of *O. japonicus* with resident species in natural and artificial container communities was proposed.

The primary objective of my study was to confirm and assess the interactions of *O. japonicus* in mosquito container communities and make predictions concerning the ecological processes that may be operating as a result of the invasion of this species. This was accomplished through a field survey of all life stages conducted areas in which this species is known to occur in various types of mosquito container communities. As rock pools are the species' preferred habitat, it is predicted that *O. japonicus* will most likely interact with and impact the native rock pool mosquito, *O. atropalpus*. Furthermore, the tendency of *O. japonicus* to inhabit artificial containers suggests that this species will also likely encounter and interact with the Eastern treehole mosquito, *O. triseriatus*, and its closely related sibling species, *O. hendersoni*, as well the now resident invader *A. albopictus*. Because severe crowding and limiting resources are frequent in container habitats (Kitching 2000), it is predicted that interspecific competition via depletion of shared resources will be important in the interactions of the larvae of *O. japonicus* and *A. albopictus* in artificial containers, and *O. atropalpus* in rock pools. Therefore, the effects of interspecific resource competition on the growth, survivorship, and reproductive success of these species were measured in a series of field and laboratory experiments, in order to determine the impact of these larval conditions on the overall population growth of these species.

## CHAPTER 2

### FIELD ASSESMENT OF INTERSPECIFIC INTERACTIONS AMONG INVASIVE AND NATIVE CONTAINER-INHABITING MOSQUITOES

#### **Introduction**

Naturally occurring throughout Japan, Korea, Taiwan, and China (Tanaka et al. 1979), *Ochlerotatus japonicus* is the most recently recognized mosquito species to invade the United States, which is thought to have occurred via the international transport of used tires (Peyton et al. 1999, Lounibos 2002). Initially collected in the summer of 1998 in Connecticut (as reported by Andreadis et al. 2001), New York and New Jersey (Peyton et al. 1999), *O. japonicus* has since become established throughout the northeastern United States (reviewed by Scott 2003), and spread south to Georgia (Reeves and Korecki 2004), north into Canada (Savignac et al. 2002, Thielman and Hunter 2006), and west to Missouri (Gallitano et al. 2006). The spread of *O. japonicus* on the west coast has so far been limited to Washington State (Roppo et al. 2004). Distinct genetic profiles of *O. japonicus* collected from various sites in Japan and throughout its range in the United States suggest multiple introductions of the species in the U.S. (Fonseca et al. 2001).

Although common and widely distributed within its native range in eastern Asia (LaCasse and Yamaguti 1950, Tanaka et al 1979), *O. japonicus* attracted little scientific attention until its discovery in North America, where its potential involvement in the transmission of West Nile virus (Sardelis and Turell 2001) and other endemic arboviruses (Sardelis et al. 2002ab, 2003) has stimulated significant scientific interest. Knowledge of the biology and surveillance methods for the collection of *O. japonicus* has since improved remarkably. Collection records from both Japan (Sasa et al. 1947) and the United States (Andreadis et al. 2001) indicate that adults are rarely taken in light traps, carbon dioxide (CO<sub>2</sub>) – baited traps, or biting collections. The use of

infusion-baited gravid traps, typically intended to attract mosquitoes of the genus *Culex* (Reiter 1983), has provided the most consistent and greatest numbers of *O. japonicus* adults (Scott et al. 2001b). As a container-inhabiting mosquito, oviposition traps are a logical sampling method with many potential applications, including detection in new areas, routine population surveillance, and species distribution studies. However this technique has been employed with varying success in collecting *O. japonicus* (Andreadis et al. 2001). Modification of the traditional oviposition trap using blocks of expanded polystyrene as an oviposition substrate in a variety of aquatic habitats has been indicated as a suitable alternative (Scott and Crans 2003).

*Ochlerotatus japonicus* appears to be most easily and consistently collected in large numbers in the larval stage. Within its native range in Asia, *O. japonicus* larvae are found in a wide variety of natural and artificial containers, with rock pools being the preferred oviposition substrate (LaCasse and Yamaguti 1950, Tanaka et al. 1979). In the United States, *O. japonicus* larvae are commonly found in artificial (e.g. automobile tires, bird baths, gutters, flower pots) and natural (tree holes and rock pools) containers where they feed on organic detritus and microorganisms, often in close association with human dwellings (Andreadis et al. 2001). Larvae may be found in shaded or sunny locations (Scott 2003) in clean, clear water containing decaying leaf litter and algae (Scott et al. 2001a).

The likelihood of an invasive species colonizing a new region depends on its ability to adapt to new environmental conditions and compete with resident species that occupy a similar ecological niche. The indigenous mosquito species most likely to be affected by *O. japonicus* in the United States include the rock hole mosquito, *O. atropalpus*, the Eastern treehole mosquito, *O. triseriatus* and its closely related sibling species, *O. hendersoni*, as well as the now resident invader *Aedes albopictus*. Frequent and abundant collections of *O. japonicus* larvae co-occurring

with both *O. atropalpus* in rock pools and *A. albopictus* in artificial containers, provide a natural setting for interspecific larval resource competition (Andreadis et al. 2001) which some speculate may limit its invasion success (Juliano and Lounibos 2005). However at present, no field research has been published to confirm this conjecture following the establishment of *O. japonicus* in the United States.

To better understand the ecological niche of *O. japonicus* with respect to the aforementioned resident container-inhabiting species, a broad-based survey of all life stages was conducted in Fairfax County, Virginia (latitude 38°50' N, longitude 77°7' W), a suburb of Washington, D.C., where all of these species are known to co-occur. Sampling of natural and artificial larval habitats was used to confirm and quantify co-occurrences and potential interactions of *O. japonicus* with resident mosquitoes in these habitats. Routine trapping of adults over a three-year period was used to assess changes in frequency or abundance of the species of interest. Ovitrap were employed to better understand the seasonality of egg-laying behavior of these species as well as their tendencies to oviposit at ground level versus in the canopy, or in residential areas versus disturbed or undisturbed forests. In particular, the current chapter focuses on the potential interactions of *O. japonicus* with *A. albopictus* in artificial containers and *O. atropalpus* in rock pools in an effort to make predictions regarding the competitive outcomes resulting from the co-occurrence of these species.

## **Materials and Methods**

### **Oviposition Traps**

To monitor the distribution, abundance, and oviposition of *O. japonicus* and resident container-inhabiting mosquitoes in Fairfax County, VA, standardized collections of eggs employed the use of ovitraps. These dark, water-filled receptacles containing a removable oviposition substrate have been commonly used for these purposes (e.g., Kitron et. al. 1989,

Lounibos et al. 2001). Ovitrap were constructed from black polypropylene cups (400 ml capacity) filled with 200 ml of distilled water and approximately 1 g of dried pin oak leaves (*Quercus palustris*), one of the most common trees in the study area. An oviposition stick, constructed from a single tongue depressor covered with 76 lb. seed germination paper (Anchor Paper, St. Paul, MN), served as an oviposition substrate (Steinly et al. 1991). A hole, 2.5 cm in diameter, was drilled 2.5 cm from the top of each cup to allow for suspension and drainage of the ovitrap.

Ovitrap were placed within or on the edge of three separate sites in Fairfax County, VA (Figure 2-1) from May through September 2006. The study sites included a high-density residential development, an undisturbed forested streambed, and a rural forest disturbed from frequent dumping of trash. Ovitrap were secured to trees using bundling twine at two heights, five at ground level and five at approximately 3m, that were pre-selected randomly at each site using a numbered 10 m by 10 m grid overlay of aerial photographs of each site. Vertical stratification was employed to increase the likelihood of encountering native treehole mosquitoes, namely *O. hendersoni* and *O. triseriatus*, and to assess how frequently *O. japonicus* mosquitoes seek oviposition sites above ground-level. Ovitrap were set at each site simultaneously for a period of five days each month, after which they were retrieved and the oviposition stick of each was stored separately in an individual plastic bag for transport to the laboratory.

In the laboratory, each oviposition stick was examined using a dissecting microscope, and the number of eggs present on each was counted. Seed germination papers were carefully removed from the oviposition sticks and allowed to dry for seven days at room temperature to ensure embryonation, after which they were placed in the bottom portion of a plastic tray (21 cm

high x 12 cm diameter) and flooded with 400 ml of tap water. Non-natural food, comprised of ground rabbit chow and brewer's yeast in a 3:1 ratio, was added to each tray upon flooding every other day to ensure larval development. All mosquitoes were identified in the fourth larval instar using keys (Slaff and Apperson 1989, Darsie and Ward 2005), and mortality at other immature stages was noted. Because intermittent hatching is a common occurrence among the eggs of *Aedes* and *Ochlerotatus* mosquitoes, those eggs that did not hatch after being submerged for a period of seven days were dried on the oviposition substrate for an additional seven days before reflooding. Those eggs that did not hatch after three cycles of drying and flooding were bleached and examined microscopically to assess their viability (Trpis 1970). Unhatched eggs were considered viable if the embryo was segmented, possessed ocelli and an eggbreaker (Christophers 1960).

### **Natural and Artificial Containers**

To assess the potential for interactions in nature among the larvae of container-inhabiting mosquitoes in Fairfax, Virginia, a field survey of such habitats was conducted from May through September 2006 throughout the area (Figure 2-1). Although a few tree holes and ornamental bromeliad plants were sampled, rock pools were the only natural containers sampled in large numbers during the study. Only a single river system, the Potomac River and associated tributaries, were sampled for rock pool mosquitoes. Containers were only sampled once, and the entire aquatic contents of each were removed using a turkey baster or siphon, measured, and concentrated by filtering through a fine mesh (small enough to retain first instars) sieve. Samples were transported to the laboratory in 18-ounce (532.32 ml) plastic Whirl-pak bags. Those containers containing water but no mosquitoes were noted. The geocoordinates of each site were obtained with a global positioning system, and the degree of sun exposure (none, partial, or full) and container type were recorded.

In the laboratory, the mosquitoes from each container were examined separately in a 34.3 x 24.4 cm plastic tray. Fourth instars were identified to species and counted. All other mosquito immatures were counted, sorted by stage and placed in separate 21 x 12 cm cylindrical growth chambers (BioQuip Products, Inc., Rancho Dominguez, CA ) containing 400 ml of tap water and food, consisting of three parts ground rabbit chow and one part brewer's yeast. Any predatory species present, such as *Toxorhynchites rutilus*, were removed and stored separately. Mosquitoes collected from the field as pupae were identified upon emergence as adults while all others were identified as fourth instars. Mortality at all immature stages was noted.

### **Adult Surveillance**

Adult mosquitoes were collected with carbon dioxide (CO<sub>2</sub>)-baited CDC light traps and gravid traps set at 70 pre-selected trapping sites located throughout Fairfax County (Figure 2-1) from May through October over a three-year period from 2004 to 2006. One of each type of trap was set weekly at each site. Traps were set in the early afternoon and retrieved the following morning. All mosquitoes collected were sorted, counted and identified to the species level using keys and a dissecting microscope.

### **Data Analysis**

#### **Oviposition Traps**

*G*-tests (Sokal and Rohlf 1981) were used to test for heterogeneity among the frequencies of mosquito species collected at each site. Pairwise comparisons between species were conducted as necessary with additional *G*-tests using the Bonferroni adjustment to correct for experimentwise error. *G*-tests were also used to compare the frequency of occurrence of each individual species by height. Again, pairwise comparisons between sites were conducted for each species with *G*-tests using the Bonferroni adjustment to correct for experimentwise error. Due to non-normal distributions not remediable by standard transformations, Friedman's two-way

analysis of variance and Cochran's  $Q$  test were used to compare mean abundance per trap and frequency of occurrence, respectively, for each individual species among months.

### **Natural and Artificial Containers**

$G$ -tests (Sokal and Rohlf 1981) were used to detect significant differences in frequency of occurrence of each individual mosquito species, including *A. albopictus*, *O. japonicus*, *Culex pipiens*, *C. restuans*, *O. triseriatus*, and *O. hendersoni*, by container type, fluid volume, and exposure to the sun. The coefficient of association ( $C_8$ ), which ranges from +1 to -1, as described by Hurlbert (1969) was used to quantify co-occurrences of the six most abundant species collected during the container habitat survey. This metric quantifies frequencies of co-occurrence based on species presence-absence data. Positive associations between species may reflect a common habitat preference or interspecific attraction, while negative associations may result from different habitat preferences or interspecific repulsion. Statistical significances of  $C_8$  values were assessed with a corrected  $\chi^2$  formula (Pielou 1977) for approximating a discrete distribution. Fisher's exact test was applied in those cases with cell totals less than or equal to five. Only samples from which one or more larvae or pupae were identified were included in calculations.

To quantify frequencies of encounter among mosquitoes in containers as a function of habitat size, Lloyd's (1967) indices of mean intra- and interspecific crowding were used per unit resource (Rathcke 1976), in this case container fluid volume. The mean crowding of one species upon itself and each of the other species per unit resource was calculated. Interspecific mean crowding of species  $x$  by  $y$  per unit resource  $a$  was quantified as  $\sum(x_i y_i / a_i) / \sum x_i$ , and intraspecific mean crowding by the same formula but substituting  $(x_i - 1)$  for  $y_i$ . Mean inter- and intraspecific crowding were calculated per month to assess the potential for larval competition between and

among *O. japonicus* and *A. albopictus*. A two-way repeated measures-analysis of variance, with species, month, and interactions as effects was used to detect significant differences in interspecific mean crowding between the two species over time. Only those months in which the two species co-occurred more than five times were considered. One-way repeated measures-ANOVAs were used to detect differences in intraspecific crowding among months for each species. To compare differences in intraspecific crowding among *O. japonicus* larvae in artificial containers and rock pools, a two-way repeated measures ANOVA was used with container type, month, and interaction as effects. Interspecific crowding data were  $\log_{10}$  transformed and intraspecific crowding data were  $\log_{10}(x + 1)$  transformed to meet assumptions of normality and homoscedacity. All analyses were performed with PROC MIXED in SAS (SAS 1989).

The large number of samples from both artificial and natural containers permitted a comparison of the mosquito community structure of these habitats. Kendall's coefficient of rank correlation, tau ( $\tau$ ), was used to estimate the degree of similarity of rank order abundance of mosquito species in containers in Fairfax County, Virginia (Ghent 1963). The ranked abundances of species in small vs. large artificial containers, and in rock pools versus tires and artificial containers were compared. Only those species occurring at least once in both habitats were considered in the comparisons.

Other analyses focused on potential interactions between *A. albopictus* and *O. japonicus*. Instar distributions were compared using a Kolmogorov-Smirnov two-sample test, and the average instar number for each species was calculated per month. Heterogeneity of instar distributions of *O. japonicus* and *A. albopictus* for the months of June, July, and August was assessed with the life table procedure (PROC LIFETEST) in SAS (SAS 1989). The significance

of heterogeneities was assessed with Wilcoxon and Log-rank tests, which were then used to construct  $z$ -statistics for post-hoc multiple comparisons among groups (Fox 1993).

Metamorphic success of *O. japonicus* and *A. albopictus* was calculated per month as Williams' means (Williams 1937, Haddow 1960), denoted by  $M_w$ , where  $\log_{10}(M_w+1) = [\sum \log_{10}(n_i+1)]/N$ , where  $n_i$  is the number of pupae per total number of immatures in a series of  $N$  container samples.  $M_w$  is obtained by subtracting one from the antilog quantity  $[\sum \log(n+1)]/N$ . Williams' mean is frequently used as a measure of central tendency in entomological collections with many zero values (Haddow 1960, Lounibos 1981, Lounibos et al. 2001). Metamorphic success of the two species was compared using a two-way repeated measures ANOVA with species, month, and interaction as effects. Only those months in which the two species co-occurred more than five times were considered. One-way repeated measures-ANOVAs were used to detect differences in metamorphic success among months for each species. Metamorphic success of *O. japonicus* in rock pool and artificial containers was also compared using a two-way repeated measures-ANOVA, with container type, month, and interaction as effects. All metamorphic success data were arcsine square root transformed, to best meet assumptions of normality and homogeneity of variance. Analyses were performed with PROC MIXED in SAS (SAS 1989)

### **Adult Surveillance**

The frequency of collection for *A. albopictus*, *O. japonicus*, and *O. triseriatus* adult females was calculated per week for 2004 through 2006. Frequencies of collection were analyzed for each species using two-way repeated measures ANOVAs, with year, trap type, and interactions as effects. Weekly abundances of adult female *A. albopictus*, *O. japonicus*, and *O. triseriatus* collected in CO<sub>2</sub>-baited CDC light traps and gravid traps were calculated as arithmetic

means (average number collected per trap night) as well as Williams' means, denoted as  $\log_{10}(M_w+1) = [\sum \log_{10}(n_i+1)]/N$ , where  $n_i$  is the number of individuals collected in each of  $N$  trap nights. To meet assumptions of normality and homoscedacity, a  $\log_{10}(x+1)$  transformation was applied to Williams' means before analyzing trends in abundance over time, from 2004 through 2006, for each species by linear regression. All analyses were performed with SPSS (SPSS 2002).

## Results

### Oviposition Traps

*Ochlerotatus japonicus* was detected only once in a single ovitrap set at ground level at the disturbed forest site in June, and was not included in any subsequent analyses. *Aedes albopictus*, *O. hendersoni*, and *O. triseriatus* were the only other species collected. The frequencies of collection were homogeneous among all three species at the undisturbed forest site ( $G = 2.282$ ,  $p = 0.319$ ,  $df = 2$ ), but were heterogeneous at both the residential ( $G = 21.06$ ,  $p < 0.001$ ,  $df = 2$ ) and disturbed forest sites ( $G = 25.575$ ,  $p < 0.001$ ,  $df = 2$ ), however *A. albopictus* was the only species detected at the former site (Table 2-1). Only *A. albopictus* and *O. triseriatus* were collected at the disturbed forest site, but pairwise comparisons did not indicate a significant difference in the frequency of occurrence between these two species (Table 2-1). The frequencies of occurrence among all three species in ovitraps placed above ground were homogeneous ( $G = 0.59$ ,  $p = 0.744$ ,  $df = 2$ ), but were heterogeneous at ground level ( $G = 30.11$ ,  $p < 0.001$ ,  $df = 2$ ). Pairwise comparisons indicated that *A. albopictus* was collected more frequently in ovitraps at ground level than either *O. hendersoni* or *O. triseriatus* (Table 2-1).

$G$ -tests for heterogeneity for the presence of *A. albopictus* indicated no significant difference in the frequency of collection of this species by site ( $G = 3.206$ ,  $p = 0.201$ ,  $df = 2$ ). The frequency of occurrence of *O. triseriatus* ( $G = 12.468$ ,  $p = 0.002$ ,  $df = 2$ ) and *O. hendersoni*

( $G = 7.284$ ,  $p = 0.026$ ,  $df = 2$ ) was heterogeneous among sites, however upon exclusion of those sites where these species were never detected (residential and disturbed forest, respectively), these tests were not significant. The frequency of occurrence of *A. albopictus* was significantly greater in ovitraps at ground level than above ground ( $G = 22.55$ ,  $p < 0.001$ ,  $df = 1$ ). There was no significant difference in the frequency of occurrence of *O. triseriatus* ( $G = 0.445$ ,  $p = 0.505$ ,  $df = 1$ ) or *O. hendersoni* ( $G = 0.002$ ,  $p = 0.966$ ) by ovitrap height.

The frequency of collection of *A. albopictus* was significantly different among months ( $Q = 25.217$ ,  $p < 0.001$ ,  $df = 4$ ), with the highest frequencies of collection occurring in July and August (Table 2-2). There was a significant difference among months for the frequency of collection of *O. hendersoni* ( $Q = 24.8$ ,  $p < 0.001$ ,  $df = 4$ ); a comparison of only the two months in which this species was collected was also significant ( $Q = 6.0$ ,  $p = 0.008$ ,  $df = 1$ ). The frequency of collection of *O. triseriatus* was significantly different among months ( $Q = 26.4$ ,  $p < 0.001$ ,  $df = 4$ ), with the highest frequency of collection occurring in July (Table 2-2). Mean abundances of these three species followed trends similar to their monthly egg frequencies (Table 2-3).

### **Natural and Artificial Containers**

Overall, 191 containers (131 artificial and 61 natural) were sampled for the presence of larvae and pupae during the study period, of which 134 were positive for mosquitoes. These included rock pools, tires, flowerpot saucers, tarpaulins, drainpipes, French drains, birdbaths, cemetery vases, trashcans, drums, and other small miscellaneous artificial containers. The following 10 species were collected, in order of frequency of occurrence: *A. albopictus*, *O. japonicus*, *Culex pipiens*, *C. restuans*, *O. triseriatus*, *O. hendersoni*, *Anopheles punctipennis*, *T. rutilus*, *O. atropalpus*, and *Orthopodomyia signifera*. Descriptive statistics and collection information for each of these species are provided in Appendix A. *Ochlerotatus atropalpus* was

collected from only four containers, all of which were rock pools. *Anopheles punctipennis* was collected only from rock pools, while *T. rutilus* and *O. signifera* were collected only from artificial containers. These three rare species have not been included in any subsequent analyses because they were collected in very few samples.

*Ochlerotatus japonicus* was the only species to occur more frequently in rock pools than artificial containers ( $G = 5.98, p = 0.015, df = 1$ ) while *A. albopictus* ( $G = 139.15, p < 0.001, df = 1$ ), *C. pipiens* ( $G = 25.6, p < 0.001, df = 1$ ), *C. restuans* ( $G = 14.73, p < 0.001, df = 1$ ), *O. hendersoni* ( $G = 24.95, p < 0.001, df = 1$ ), and *O. triseriatus* ( $G = 29.4, p < 0.001, df = 1$ ) were collected significantly more frequently from artificial containers. *Aedes albopictus* ( $G = 27.63, p < 0.001, df = 1$ ), *C. restuans* ( $G = 8.16, p = 0.004, df = 1$ ), *O. japonicus* ( $G = 83.65, p < 0.001, df = 1$ ), and *O. triseriatus* ( $G = 10.03, p < 0.001, df = 1$ ) were collected more frequently from containers that were at least partially shaded, while there was no significant difference for *C. pipiens* ( $G = 0.39, p = 0.84, df = 1$ ), *O. atropalpus* ( $G = 2.09, p = 0.15, df = 1$ ), or *O. hendersoni* ( $G = 3.29, p = 0.070, df = 1$ ).

The mean fluid volume ( $\pm$ SE) of containers sampled was 2.42 l, with a median of 0.3 l, and range of 0.01 – 120 l. *Aedes albopictus* ( $G = 35.37, p < 0.001, df = 1$ ), *O. japonicus* ( $G = 7.99, p = 0.005, df = 1$ ), and *O. triseriatus* ( $G = 4.61, p = 0.032, df = 1$ ) were sampled more frequently from containers containing less than 1 l of water, while there was no significant difference for *O. hendersoni* ( $G = 0.22, p = 0.637, df = 1$ ), *C. pipiens* ( $G = 3.19, p = 0.07, df = 1$ ), or *C. restuans* ( $G = 2.01, p = 0.156, df = 1$ ).

### **Interspecific Associations**

Using the  $C_8$  index of association, the 15 pairings from containers sampled in Fairfax County, Virginia revealed seven positive, six negative, and two zero associations (Table 2-4). Four of the positive associations were significant and those included pairings between *C. pipiens*

and *C. restuans*, *A. albopictus* and *O. triseriatus*, *A. albopictus* and *O. hendersoni*, and *O. hendersoni* and *O. triseriatus*. There were only two significant negative associations, that of *O. japonicus* and *O. triseriatus*, and *O. japonicus* and *A. albopictus*.  $C_8$  indices were recalculated excluding rock pools, to determine if this container type contributed disproportionately to those associations involving species commonly found in rock pools. However, this exclusion did not have any affect on the magnitude or direction of the associations.

Mean intra- and interspecific crowding was calculated for the six most abundant mosquito species collected from 191 containers censused from May through September (Table 2-5). *Aedes albopictus* and *C. restuans* both encountered a higher density of conspecifics than any other mosquito species. *O. japonicus*, *O. triseriatus*, and *C. pipiens* encountered a higher density of *A. albopictus* than any other species or themselves, while *O. hendersoni* encountered a higher density of *O. triseriatus* than any other species or themselves.

### **Habitat Comparisons**

Kendall's coefficient of rank correlation tau ( $\tau$ ) was positive in all three comparisons of rank abundance (Table 2-6), but only the comparison of small and large artificial containers was significant. These results indicate that while the rank order of abundances of mosquito species occurring in rock pools and artificial containers are similar, they are not significantly similar. *Ochlerotatus japonicus* was the most abundant species found in rock pools, while *A. albopictus* was the most abundant species found in all four subclasses of artificial containers considered for this analysis (see Appendix B). Although only collected from rock pools, *O. atropalpus* ranked fifth in abundance in this habitat (see Appendix B).

### ***Aedes albopictus* and *Ochlerotatus japonicus***

*Aedes albopictus* and *O. japonicus* occurred either alone or together in 97.75% of those containers sampled from May through September that were positive for mosquito immatures.

*Aedes albopictus* was collected from approximately half of these containers (50.75%) in the absence of *O. japonicus*, which was collected from 26% of these containers in the absence of *A. albopictus*. The two species occurred together in 21% of these containers. Co-occurrences of these two species occurred more frequently in medium-to-large sized ( $G = 3.67, p = 0.05, df = 1$ ), shaded ( $G = 29.56, p < 0.001, df = 1$ ) artificial containers ( $G = 17.65, p < 0.001, df = 1$ ) containing less than 1 l of water ( $G = 9.23, p = 0.002, df = 1$ ). *Aedes albopictus* was most frequently sampled in the absence of *O. japonicus* in September, while the highest proportion of containers positive for only *O. japonicus* were sampled in July (Figure 2-2). The two species were found together most frequently in June (Figure 2-2). *Aedes albopictus* was consistently present in greater abundance than *O. japonicus* in artificial containers in all months except May (Figure 2-3). *Ochlerotatus japonicus* was most frequently collected from artificial containers in July while *A. albopictus* was most frequent in August (Figure 2-4).

The average instar number, or age, of *A. albopictus* larvae was 2.77, ranging among months from 2.67 to 3.65, while that of *O. japonicus* was 3.86, ranging from 3.17 to 4.19 (Table 2-7). A comparison of the overall instar distributions of the two species from May through September by Kolmogorov-Smirnov two-sample test indicated the two were significantly different ( $D = 18.130, p < 0.001$ ). Significant heterogeneity among monthly instar distributions was detected from a log-rank test for both *A. albopictus* ( $\chi^2 = 13.46, p < 0.001, df = 2$ ) and *O. japonicus* ( $\chi^2 = 24.22, p < 0.001, df = 2$ ). Paired comparisons showed that the significant heterogeneity of instar distributions of *A. albopictus* was largely attributable to that of July, which was significantly different from June and August (Table 2-8). Paired comparisons for instar distributions of *O. japonicus* indicated highly significant differences among all months (Table 2-8), however graphical comparisons revealed no obvious differences (Figure 2-6. It

should be noted that pupae comprised the greatest proportion of *O. japonicus* sampled in each of the months considered (Figure 2-6).

Investigation of the temporal dynamics of co-occurrences of *A. albopictus* and *O. japonicus* indicated that interspecific mean crowding was not significantly affected by species ( $F_{1,24} = 0.46, p = 0.561$ ) or month ( $F_{2,22} = 0.39, p = 0.8839$ ). Mean crowding of *O. japonicus* by *A. albopictus* was always more than that of *A. albopictus* by *O. japonicus*, and was highest for both species in August (Figure 2-7). Intraspecific mean crowding was significantly different among months for both *A. albopictus* ( $F_{2,30} = 76.51, p < 0.001$ ) and *O. japonicus* ( $F_{2,29} = 9.36, p < 0.001$ ). While intraspecific mean crowding of *O. japonicus* was greatest in July, *A. albopictus* encountered the greatest density of conspecifics per unit resource the following month in August (Figure 2-8), pairwise comparisons of least square means indicated that intraspecific competition was lower for both species in June than July or August (Table 2-9). Because *O. japonicus* was collected frequently in high densities from both artificial containers and rock pools, intraspecific mean crowding for this species was calculated for each habitat as 33.36 and 109.48, respectively. However, analysis by two-way repeated measures-ANOVA did not indicate that intraspecific crowding of this species was effected by container type ( $F_{1,23} = 1.77, p = 0.1962$ ) or month ( $F_{1,18} = 0.15, p = 0.6989$ ).

In containers where the two species co-occurred, the metamorphic successes of *A. albopictus* and *O. japonicus*, measured as Williams Mean ( $M_w$ ) number of pupae collected per total number of immatures, were found to be 0.1234 and 0.1133, respectively. However, metamorphic success of was not effected by species ( $F_{1,24} = 0.33, p = 0.5721$ ) or month ( $F_{2,22} = 0.24, p = 0.7871$ ). Metamorphic success was greatest for *O. japonicus* in June, while there appeared to be no difference among months for that of *A. albopictus* (Figure 2-9). Repeated

measures-ANOVAs indicated that there were no significant differences in the metamorphic success of *A. albopictus* ( $F_{2,30} = 0.33, p = 0.5620$ ) or *O. japonicus* ( $F_{2,29} = 1.63, p = 0.2135$ ) among months. While the metamorphic success of *O. japonicus* was higher in rock pools (0.1479) than artificial containers (0.1047), this difference was not found to be significant among container type ( $F_{1,26} = 2.09, p = 0.1599$ ) or month ( $F_{1,25} = 1.15, p = 0.2930$ ).

#### ***Ochlerotatus japonicus* and *Ochlerotatus triseriatus***

*Ochlerotatus triseriatus* was only collected from 15 containers during the course of the census, most of which (73.7%) were sampled in August. Because *O. triseriatus* occurred with *O. japonicus* in only five of these containers, analysis of the potential for interactions between these species was rather limited. In addition to the significant negative association of these species as indicated by the  $C_8$  index (Table 2-4), it was found that *O. triseriatus* encountered more conspecifics (34.47) than *O. japonicus* (19.21) than conspecifics per unit resource, however this was much less than the interspecific mean crowding of *O. triseriatus* by *A. albopictus* (136.7). *Ochlerotatus japonicus* encountered only 12.85 *O. triseriatus* compared to 139.3 *A. albopictus*, and 127.56 conspecifics per unit resource (Table 2-5). Finally, the metamorphic success of *O. triseriatus* was found to be 0.59, while that of *O. japonicus* was only 0.01 in containers in which the two species co-occurred.

#### ***Ochlerotatus atropalpus* and *Ochlerotatus japonicus***

Because *O. atropalpus* was collected on only four occasions, and only twice with *O. japonicus*, an assessment of the potential for interactions of these species was limited. While *O. atropalpus* was recovered exclusively from rock pools exposed fully to the sun, *O. japonicus* was only collected from those that were at least partially shaded. A non-significant coefficient of association ( $C_8$ ) of zero was calculated for the two species when all container types were considered. When only rock pools were included, the coefficient decreased slightly to -0.04, but

was still non-significant. These findings are not surprising considering the limited collections of *O. atropalpus* from any container habitat. No effort was made to determine the metamorphic success or interspecific mean crowding of these two species because they only co-occurred twice.

### **Adult Surveillance**

The frequency of collection of *A. albopictus* was affected by trap type ( $F_{1,19} = 69.439$ ,  $p < 0.001$ ) but not by year ( $F_{2,38} = 1.984$ ,  $p = 0.151$ ), with a higher frequency of collection of this species in CO<sub>2</sub>-baited CDC light traps than gravid traps in all years (Table 2-10). The frequency of collection of *O. japonicus* was affected by both trap type ( $F_{1,22} = 75.034$ ,  $p < 0.001$ ) and year ( $F_{2,44} = 71.504$ ,  $p < 0.001$ ), with a higher frequency of collection of this species in gravid traps (Table 2-10). Pairwise comparisons indicated significantly different frequencies of collection of *O. japonicus* among all years, with a decline in frequency of collection in both CO<sub>2</sub>-baited CDC light traps and gravid traps with each subsequent year from 2004 to 2006 (Table 2-10). The frequency of collection of *O. triseriatus* was also affected by both trap type ( $F_{1,22} = 9.245$ ,  $p < 0.001$ ) and year ( $F_{2,44} = 67.027$ ,  $p < 0.001$ ), with a higher frequency of collection of this species in CO<sub>2</sub>-baited CDC light traps in all years (Table 2-10). Pairwise comparisons indicated that *O. triseriatus* was collected more frequently in 2004 than 2006 in CO<sub>2</sub>-baited CDC light traps, and more frequently in 2004 than in 2005 or 2006 in gravid traps (Table 2-10).

Linear regression analysis of the weekly abundance, given as the log<sub>10</sub> Williams mean ( $M_w$ ) number of adult females collected per trap + 1, of *A. albopictus*, as collected in both CO<sub>2</sub>-baited CDC light traps and gravid traps, over time did not indicate any significant change in abundance of this species from 2004 through 2006 (Figure 2-10). However, this time series analysis indicated that the mean abundances of both *O. japonicus* (Figure 2-11) and *O. triseriatus* (Figure 2-12) declined significantly over this period of time.

## Discussion

The results of this preliminary assessment demonstrate the coexistence of *O. japonicus* with resident container-inhabiting mosquitoes in Fairfax County, Virginia, facilitated by what appears to be species-specific differences in habitat preference and seasonality. The negative associations of *O. japonicus* immatures with *A. albopictus* and *O. triseriatus* seem largely due to an apparent divergence in container preference, with *O. japonicus* occurring predominately in rock pools rather than artificial containers. Furthermore, the limited collection of *O. triseriatus* from artificial containers suggests that perhaps this species prefers an alternative habitat, such as treeholes. Findings from the oviposition survey were similar. A wide distribution and high abundance of *A. albopictus* across macrohabitats was observed throughout the study period, with this species occurring more frequently at residential and disturbed habitats than other resident container-inhabiting mosquitoes, a trend similar to that which has been observed in Florida (Lounibos et al. 2001). Spatial and temporal distributions of *O. hendersoni* and *O. triseriatus* were not consistent with those observed in other areas, but this may be due to the collection effort of this study. *Ochlerotatus triseriatus* is known to seek out oviposition sites at ground level (Scholl and DeFoliart 1977) while *O. hendersoni* prefers to oviposit above ground level (Sinsko and Grimstad 1977, Clark and Craig 1985), even up to 9 m (Beier et al. 1982). However, these results suggest that there is no significant difference in the frequency of oviposition of either species at ground level or above ground. While the period of oviposition activity of *O. triseriatus* typically extends beyond that of *O. hendersoni* into late summer (Scholl and DeFoliart 1977), the results of this study suggest a similar seasonality for these two species.

While *O. japonicus* was only detected once throughout the oviposition study, possibly implying a univoltine population of this species in Fairfax County, Virginia, oviposition by and hatching of viable eggs from wild-caught females of this species collected from May through

October in previous years (personal observation, unpublished data) suggests this is an artifact. While these findings are likely a reflection of inappropriate macrohabitat placement of ovitraps, it should be noted that others have had limited success with this collection technique for *O. japonicus* in the past (Andreadis et al. 2001). Ovitrap would perhaps have been more useful in collecting *O. japonicus* in different macrohabitats, particularly near rock pools where this species is most abundant.

Findings from the survey of artificial containers suggest that interspecific competition may be occurring in these habitats where *O. japonicus* coexists with resident mosquito species in the presence of limited resources; however this study suggests that the invasion of *O. japonicus* may not result in competition in artificial containers that leads to displacement. This is supported by the abundances and co-occurrences of *O. japonicus* with *A. albopictus* and *O. triseriatus*, the two aedine mosquitoes this species is most likely to encounter in artificial containers in Fairfax County, Virginia. Metamorphic success of *O. japonicus* was not significantly different from that of *A. albopictus*, nor were there any significant differences in interspecific crowding of the two species among months. However, *A. albopictus* encountered a higher density of conspecifics than *O. japonicus* per unit resource, suggesting that intra- rather than interspecific interactions may be more important in regulating the population growth of this species in artificial containers. Similarly, there appeared to be greater crowding of *O. japonicus* by conspecifics than by *A. albopictus* (Figures 2-7, 2-8). Furthermore, intraspecific crowding and metamorphic success of *O. japonicus* in artificial containers and rock pools, where it is often the only species present, were similar. While these findings suggests that intraspecific competition may be just as important as interspecific competition for this species, it is possible that there has been selection among *O. japonicus* since arriving in this area to avoid competition in artificial containers with

*A. albopictus*; however the plausibility of such selection is currently unknown due to the paucity of data regarding seasonal patterns and abundance of *O. japonicus* since its arrival in northern Virginia. Such speculation may be supported further by differences in the seasonal abundances and frequencies of occurrence of the two species in artificial containers.

The success of *O. japonicus* in artificial containers in relation to *A. albopictus* seems to be facilitated by differences in seasonality and instar distributions. *Ochlerotatus japonicus* was collected most frequently and in greater abundance early in the season in contrast to *A. albopictus*, which was most active in later months. Activity of *O. japonicus* during this time also happened to coincide with the greatest metamorphic success of this species, and was the only time when the interspecific mean crowding of *O. japonicus* on *A. albopictus* was higher than that of *A. albopictus* on *O. japonicus*. The different instar distributions of *A. albopictus* (Figure 2-5) and *O. japonicus* (Figure 2-6) promoted the early season metamorphic success of the latter species in artificial containers, with the presence of older instars of *O. japonicus* in May, June, and July perhaps giving this species a head start over *A. albopictus*. This suggests the capacity of this species to overwinter in such habitats in the larval stage, an observation that has been made by others in the species native range (LaCasse and Yamaguti 1950), and the United States in New Jersey (Scott 2003) and North Carolina (B. Harrison personal communication). However, no data regarding the induction or termination of diapause in *O. japonicus* are currently available. Furthermore, the disparity in the age of *O. japonicus* and *A. albopictus* larvae, with the average instar of *O. japonicus* consistently higher than that of *A. albopictus* throughout all months of the study, may provide a competitive advantage to *O. japonicus* in containers with *A. albopictus*, allowing the former species to persist later into the season despite the high abundance of *A. albopictus*. The importance of cohort structure in density-dependent intraspecific

competition has been documented for *O. triseriatus*, whose early hatching larvae experienced higher survivorship, faster development time, and higher per capita growth rate, than cohorts that hatched later (Livdahl 1982, Edgerly and Livdahl 1992). Such an advantage may be due to the size-efficiency of the early cohort (Brooks and Dodson 1965), who as large filter feeders are more efficient and can exploit a wider range of food particles than smaller competitors, or even be cannibalistic (Koenekoop and Livdahl 1986), however evidence of the latter may have been an artifact of simple experimental conditions (Edgerly and Livdahl 1992). Furthermore, egg – larva interactions between larvae of early hatching cohorts and eggs of later cohorts are a known form of interference competition among container-inhabiting mosquitoes, in which the presence of feeding older larvae delayed hatching (Edgerly et al. 1993). The impact of interspecific larval resource competition, particularly among different instars, on the population growth of these two species needs to be explored experimentally to reveal the complexities of community dynamics.

This study revealed somewhat different findings for interactions between *O. japonicus* and *O. triseriatus*. In contrast to *A. albopictus*, *O. triseriatus* had five times the metamorphic success of *O. japonicus* in containers in which these species co-occurred, suggesting that this species is superior in larval resource utilization. However, the abundance and of *O. triseriatus* and co-occurrences with *O. japonicus* were low in these collections. Although outside the scope of this study, interspecific interactions with *O. triseriatus* may be important for *O. japonicus* in treeholes, as these species have been collected from such habitats in Connecticut (Andreadis et al. 2001), and should be considered in future research. Experimental studies should be viewed in the context of field observations of the frequency of interspecific interactions, seasonal distributions, and overwintering behaviors, as these life history traits may ultimately influence the community structure of treeholes in which these species may coexist. *Aedes albopictus* is

known to be superior to *O. triseriatus* larvae in competition (Livdahl and Willey 1991), which coupled with the high interspecific mean crowding of *O. triseriatus* by *A. albopictus* demonstrated in this study, suggests that interactions between these two species may be most important in determining the success of *O. triseriatus* populations in artificial containers in Fairfax County, Virginia. It would be of great value to compare these findings with that from similar studies conducted in areas where *A. albopictus* does not occur, as well as in eastern Asia where the native ranges of these species overlap; however such data are currently unavailable. The only comparable survey, conducted by Andreadis et al. (2001) in Connecticut shortly after the invasion of *O. japonicus*, indicated that this species ranked fourth in overall abundance (9.4%) in a tire survey, in which it was severely outnumbered by *O. triseriatus*. The potential for larval resource competition between these two species in areas where *A. albopictus* does not occur seems likely, and results of this study suggest that *O. triseriatus* will likely be the superior competitor under such conditions.

Broad-based surveillance of adult females of *A. albopictus*, *O. japonicus*, and *O. triseriatus* with CO<sub>2</sub>-baited CDC light traps and gravid traps provided a limited perspective of the population trends of these species over time in Fairfax, Virginia. The adult population of *A. albopictus* did not alter in frequency or abundance during the three year surveillance period, which leads one to speculate that perhaps this species has been unaffected by the introduction of *O. japonicus* in the area. On the contrary, the adult populations of both *O. japonicus* and *O. triseriatus* declined during the surveillance period, although this trend seemed to be somewhat more severe for the former species. While these observations are likely attributable to annual variations in environmental conditions (i.e. temperature and rainfall), they reflect the local decline, or possibly the eventual extinction, of *O. japonicus* populations following the

introduction and initial expansion of this species throughout the area. This phenomenon in which an invading species reaches a peak of density and then declines is often referred to as boom-and-bust (Williamson 1996). Selective pressures from predation, competition, or lack of availability of accessible resources may promote this type of invasion trend (Williamson 1996). Additional years of consistent surveillance will be required to fully appreciate these trends with respect to the invasion success of *O. japonicus*.

The successful establishment of *O. japonicus* in Fairfax County, Virginia appears to be associated with a population decline and potential displacement of *O. atropalpus* in local rock pools. The limited collection of this native rock pool mosquito while surveying these habitats is cause for concern as this species was once abundant throughout the area; in fact the type-form given by Coquillett (1902) was from nearby Plummer's Island in Montgomery County, Maryland. A similar pattern of decline for *O. atropalpus* has been observed in rock pool communities of Connecticut (Andreadis et al. 2001) and North Carolina (B. Harrison personal communication), and the absence of this species in rock pools has been noted in New Jersey (Scott et al. 2001a), however *O. atropalpus* is more common in tires than in rock pools in this area. It is important to note two major flooding events, heavy rains and a hurricane, that occurred during this study limited rock pool collections in late June and early July, and again in early September. Although no conclusions could be based solely on the limited co-occurrence of these species during this study, these findings suggest that interspecific larval resource competition with *O. japonicus* may have had profound effects on populations of *O. atropalpus* in areas where these two species co-occur.

Differences in the overwintering strategies of these species have probably facilitated the decline of *O. atropalpus*, which is known to diapause in the egg stage rather than as larvae,

whereas *O. japonicus* may diapause in either stage (LaCasse and Yamaguti 1950, Kamimura 1976, Scott 2003, B. Harrison personal communication). Similar to that observed for *A. albopictus* and *O. japonicus* in artificial containers, differences in the cohort structures of these two species in rock pools may exacerbate larval competitive outcomes or promote hatching inhibition, intraguild predation, or cannibalism. The tendency of *O. atropalpus* to inhabit rock pools fully exposed to the sun, contrary to the preferences of *O. japonicus*, may allow this species to persist in this habitat, albeit probably in small numbers as highly flood prone rock pools tend to be in the more sunny locations (O'Meara et al. 1997). Survival of *O. atropalpus* in an environment frequently subjected to flooding may be facilitated by seasonal variation in ovipositional behavior, deposition of different types of eggs, or variable delays before hatching. It is possible that this finding is an artifact of sampling effort; therefore the rock pool microhabitat preferences of these species with respect to sun exposure should be researched further. Furthermore, as noted in Lounibos (2002), *O. atropalpus* has expanded its distribution by occupying artificial containers, primarily tires, and has itself been considered an invasive species both in the United States and abroad. Such tendencies may allow this species to persist through dispersal to new areas where *O. japonicus* does not occur, or may allow it to avoid competition to some extent in areas where the two species co-occur. However, it is interesting to note that *O. atropalpus* was not recovered from any tires during this study.

In conclusion, the invasion of *O. japonicus* seems to be associated with the possible displacement *O. atropalpus*, possibly through interspecific resource competition, in part of this species' rock pool habitat. In artificial containers, *O. japonicus* larvae are most likely to interact with *A. albopictus*, particularly late in the season, during the months of August and September, in small-sized shaded containers. The success of *O. japonicus* in artificial containers is most

likely attributed to the earlier seasonal appearance, older age, and capacity of this species to complete development in the presence of resident species. While surveillance cannot reveal any detrimental effects of *O. japonicus* on resident artificial container inhabiting mosquitoes, monitoring over subsequent years should continue to observe population trends. While this study focused predominantly on the potential interspecific interactions of *O. japonicus* with resident mosquito species in artificial containers and rock pools, other factors, particularly predators, may be influencing the structure of these communities and should be investigated. Selective preference of predators (Griswold and Lounibos 2005, 2006) and differential responses of prey species to predators (Holt and Lawton 1994) have been shown to influence interspecific interactions of container-inhabiting mosquitoes. Furthermore, the tolerance of these species to varying environmental conditions, intraguild predation, or differences in foraging behavior may be important in their interspecific interactions.

Table 2-1. Frequency of occurrence of *A. albopictus*, *O. hendersoni*, and *O. triseriatus* in ovitraps set at three sites and two heights in Fairfax, Virginia in 2006.

Site	Frequency (no. pos. traps <sup>a</sup> /total)		
	<i>A. albopictus</i>	<i>O. hendersoni</i>	<i>O. triseriatus</i>
Residential	0.1957a	0b	0b
Undisturbed forest	0.2128	0.1087	0.1304
Disturbed forest	0.3478a	0b	0.1739a
Height			
Ground	0.4265a	0.0588b	0.1471b
Above ground	0.0857	0.0571	0.0571

<sup>a</sup>Frequencies were calculated as the number of positive traps per total number of traps set at each site from May – September 2006. Lower case letters indicate significant differences among sites resulting from pairwise comparisons with *G*-tests (df = 1, *p* = 0.05), using the Bonferroni method to adjust for experimentwise error.

Table 2-2. Frequency of occurrence, by month, of *A. albopictus*, *O. hendersoni*, and *O. triseriatus* in ovitraps in Fairfax County, Virginia in 2006.

Species	Frequency (no. pos. traps <sup>a</sup> /total)				
	May	June	July	August	September
<i>A. albopictus</i>	0.1a	0.2222ab	0.4231b	0.3929b	0.1481a
<i>O. hendersoni</i> <sup>b</sup>	0	0.0370a	0.2692b	0	0
<i>O. triseriatus</i>	0a	0.1ab	0.3b	0.0333a	0.0333a

<sup>a</sup>Frequencies were determined as the number of positive traps per total number of traps set each month.

<sup>b</sup>Frequencies of collection of *O. hendersoni* were compared only for June and July; differences were significant at  $\alpha = 0.01$  with Cochran's *Q*-test,  $df = 1$ . Lower case letters indicate significant differences among sites resulting from pairwise comparisons with Cochran's *Q* test ( $df = 1, p = 0.05$ ), using the Bonferroni method to adjust for experimentwise error.

Table 2-3. Monthly abundance (mean number of eggs per trap) of *A. albopictus*, *O. hendersoni*, and *O. triseriatus* collected in ovitraps.

Species	Abundance (mean no. ( $\pm$ SE) /trap) <sup>a</sup>				
	May	June	July	August	September
<i>A. albopictus</i>	1.0 (0.93)a	1.19 (0.66)ab	11.92 (5.85)b	6.39 (2.73)b	0.67 (0.49)a
<i>O. hendersoni</i> <sup>b</sup>	0	0.15 (0.15)	4.08 (2.17)	0	0
<i>O. triseriatus</i>	0a	0.22 (0.13)ab	8.69 (4.24)b	0.79 (0.79)ab	0.15 (0.15)a
Total rainfall (cm)	5.61	35.61	9.04	2.62	16.03

<sup>a</sup>Abundance was determined as the mean number of eggs collected per trap, based on all ovitraps set each month.

<sup>b</sup>Mean abundances of *O. hendersoni* were compared only for June and July; differences were significant at  $\alpha = 0.05$  with Friedman's two-way analysis of variance,  $df = 1$ . Lower case letters indicate significant differences among sites resulting from pairwise comparisons with Friedman's two-way analysis of variance test ( $df = 1$ ,  $p = 0.05$ ), using the Bonferroni method to adjust for experimentwise error. All other pairwise comparisons were non-significant.

Table 2-4. Coefficients of association ( $C_8$ ) for the most abundant species in 191 artificial and natural container samples from Fairfax County, Virginia in 2006. Parentheses enclose  $C_8$  values that exclude rock pools.

Species	Coefficient of association ( $C_8$ )				
	<i>C. restuans</i>	<i>C. pipiens</i>	<i>O. triseriatus</i>	<i>O. japonicus</i>	<i>O. hendersoni</i>
<i>A. albopictus</i>	-0.056	-0.015	0.067***	-0.238*** (-0.207***)	0.037**
<i>O. hendersoni</i>	0	-0.272	0.232**	0 (0)	
<i>O. japonicus</i>	0.075 (0.105)	0.042 (0.133)	-0.048** (0)		
<i>O. triseriatus</i>	-0.122	-0.140			
<i>C. pipiens</i>	0.638***				

\*\*  $p < 0.01$ , \*\*\*  $< 0.001$  by  $\chi^2$ ; all other interspecific associations are non-significant.

Table 2-5. Intra- and interspecific mean crowding of the most abundant mosquito species collected from artificial and natural containers in Fairfax County, Virginia in 2006. Mean crowding is defined as the mean density of species y encountered by species x per liter volume. Values in bold indicate intraspecific mean crowding.

Species x	Mean crowding <sup>a</sup>					
	(density of species y encountered by species x per liter volume)					
	Species y					
	<i>A. albopictus</i>	<i>O. hendersoni</i>	<i>O. japonicus</i>	<i>O. triseriatus</i>	<i>C. pipiens</i>	<i>C. restuans</i>
<i>A. albopictus</i>	<b>395.80</b>	3.013	53.01	31.59	351.88	57.37
<i>O. hendersoni</i>	40.73	<b>32.49</b>	18.06	56.27	1.77	5.74
<i>O. japonicus</i>	139.3	9.03	<b>127.56</b>	12.85	51.56	83.06
<i>O. triseriatus</i>	136.7	11.90	19.21	<b>34.47</b>	25.93	39.26
<i>C. pipiens</i>	1098.43	2.48	37.56	12.96	<b>126.98</b>	52.33
<i>C. restuans</i>	20.16	2.51	57.41	4.33	34.05	<b>116.60</b>

<sup>a</sup>Interspecific mean crowding was determined from only those containers in which both species co-occurred.

Table 2-6. Species rank abundances compared for different container habitats sampled in Fairfax County, Virginia from May – September 2006. Kendall’s  $\tau$  was used as an index of similarity.

Container habitat	No. of samples	No. of species	$\tau$	$t_s^a$	$P$
Small artificial containers	97	7	0.714	8.101	< 0.001
Large artificial containers	33	9			
Tires	29	7	0.200	0.537	0.709
Rock pools	59	7			
All artificial containers	130	9	0.467	1.917	0.055
Rock pools	59	7			

<sup>a</sup>Significance of Kendall’s  $\tau$  was tested by calculating the test statistic,  $t_s$ , which makes use of a normal approximation to test the null hypothesis that the true value of  $\tau = 0$ :  $t_s = \tau / \text{sqrt} [(2(2n + 5)) / (9n(n - 1))]$ , where  $n$  is the number of data pairs.

Table 2-7. Number of larvae and average instar number for *A. albopictus* and *O. japonicus* collected in Fairfax County, Virginia by month, May – September 2006.

Month	<i>A. albopictus</i>		<i>O. japonicus</i>	
	No. larvae	Average instar number ( $\pm$ SE)	No. larvae	Average instar number ( $\pm$ SE)
May	5	3.0 (0.71)	16	3.13 (0.49)
June	204	2.67 (0.08)	98	4.18 (0.13)
July	574	2.94 (0.05)	1836	3.67 (0.04)
August	3427	2.69 (0.02)	998	4.19 (0.04)
September	227	3.64 (0.05)	2	4.0 (1.0)
Total	4437	2.77 (0.02)	2950	3.86 (0.03)

Table 2-8. Tests for significant heterogeneity of monthly instar distributions of *A. albopictus* and *O. japonicus* from June – August 2006, based on log-rank statistics.

Month	Paired comparisons with <i>z</i> -test							
	<i>A. albopictus</i>				<i>O. japonicus</i>			
	July		August		July		August	
	<i>z</i>	<i>P</i>	<i>z</i>	<i>P</i>	<i>z</i>	<i>P</i>	<i>z</i>	<i>P</i>
June	3.66	< 0.001	0.203	0.416	4.86	< 0.001	3.06	0.001
July			2.34	0.01			4.61	< 0.001
Test	$\chi^2$	df	<i>P</i>		$\chi^2$	df	<i>P</i>	
Log-rank	13.46	2	< 0.001		24.22	2	< 0.001	
Wilcoxon	16.46	2	< 0.001		45.94	2	< 0.001	

Table 2-9. Least square means ( $\pm$ SE) for intraspecific crowding (transformed by  $\log_{10}(x + 1)$ ) among larvae of *A. albopictus* and *O. japonicus* June – August 2006. Means followed by lower case letters that are not commonly shared are significantly different by pairwise comparisons ( $p < 0.05$ ) with the Bonferroni adjustment for experimentwise error.

Species	Least square means ( $\pm$ SE)		
	June	July	August
<i>A. albopictus</i>	0.6489 (0.1928)a	1.6176 (0.2164)b	1.8092 (0.1163)b
<i>O. japonicus</i>	1.2286 (0.3570)a	2.9186 (0.2558)b	2.3577 (0.2408)ab

Table 2-10. Frequencies of collection of *A. albopictus*, *O. japonicus*, and *O. triseriatus* adult females in CO<sub>2</sub>-baited CDC light traps and gravid traps, 2004 – 2006. Means followed by letters that are not commonly shared are significantly different by pairwise comparisons ( $p < 0.05$ ) with the Bonferroni adjustment for experimentwise error.

Species	Mean ( $\pm$ SE) frequency of collection (no. pos. traps/total <sup>a</sup> )					
	CO <sub>2</sub> -baited CDC light traps			Gravid traps		
	2004	2005	2006	2004	2005	2006
<i>A. albopictus</i>	0.423 (0.40)	0.433 (0.066)	0.327 (0.054)	0.064 (0.013)	0.046 (0.012)	0.061 (0.012)
<i>O. japonicus</i>	0.213 (0.20)a	0.137 (0.016)b	0.046 (0.008)c	0.454 (0.034)a	0.281 (0.367)b	0.186 (0.023)c
<i>O. triseriatus</i>	0.241 (0.30)a	0.176 (0.27)ab	0.135 (0.23)b	0.094 (0.015)a	0.046 (0.009)b	0.046 (0.12)b

<sup>a</sup>Mean frequency of collection was determined from 23 weekly frequencies (the number of positive traps per total traps set each week) for each species.

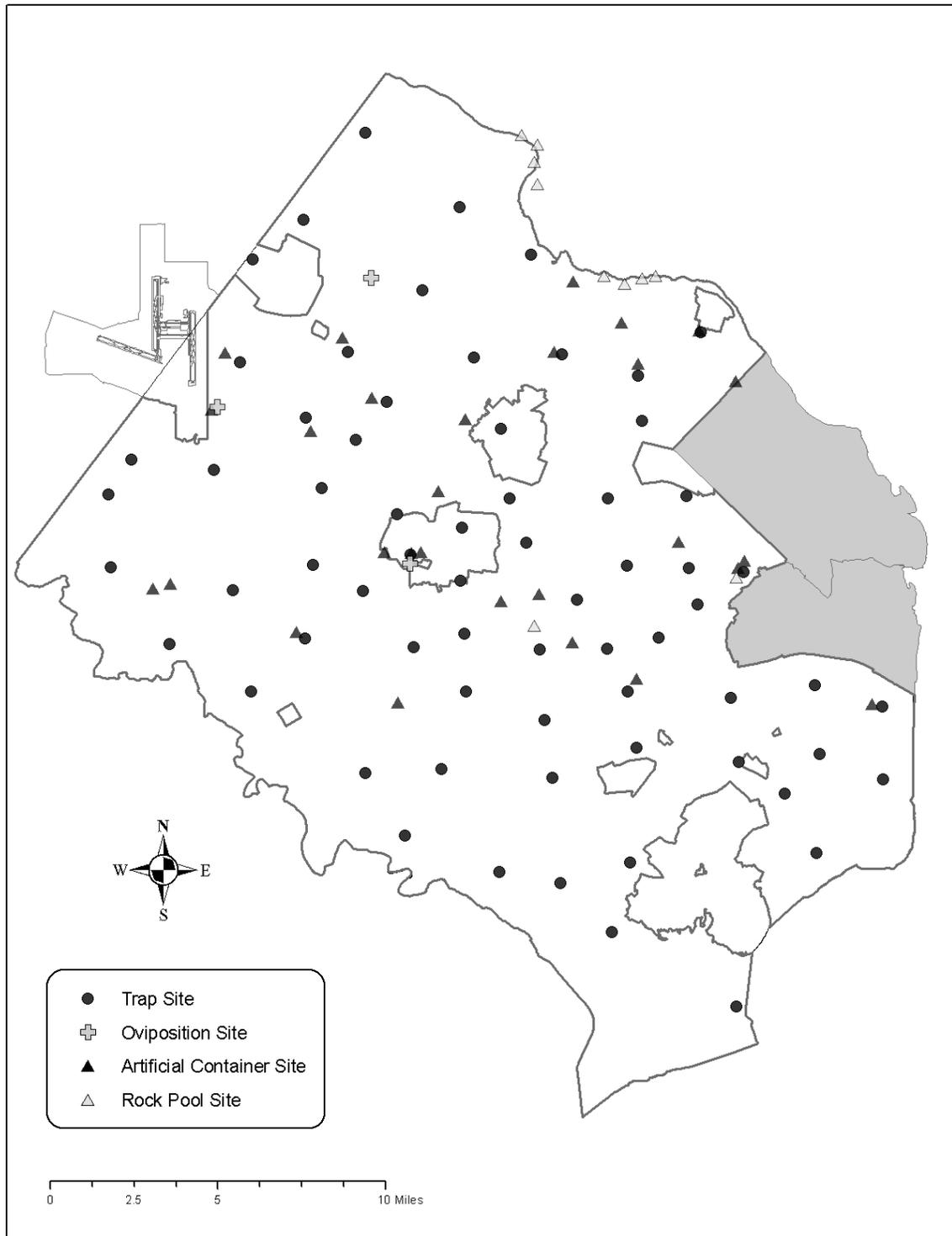


Figure 2-1. Map of Fairfax County, Virginia showing locations of study areas that were sampled or censused repeatedly. Trap sites include both a CO<sub>2</sub>-baited light trap and a gravid trap.

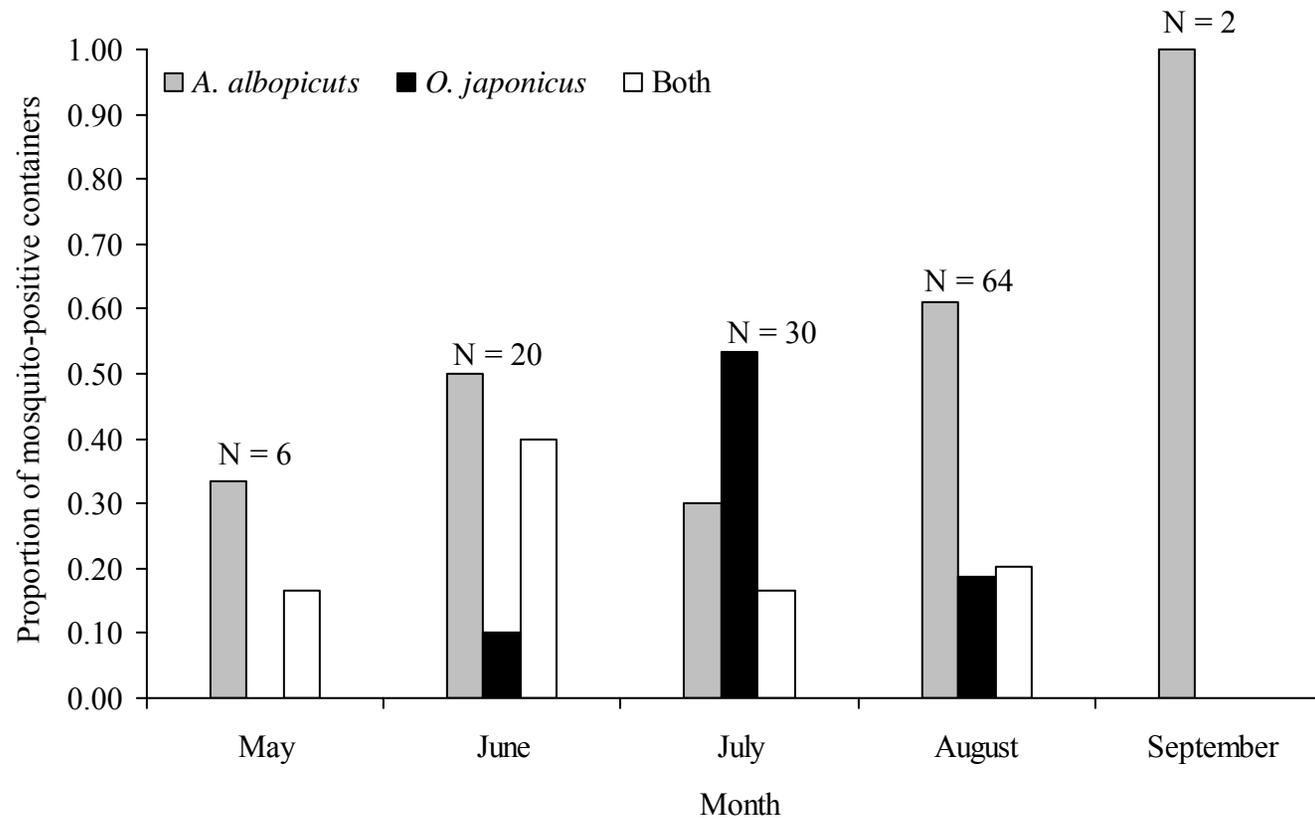


Figure 2-2. Proportion of mosquito-positive containers containing *A. albopictus*, *O. japonicus*, or both *A. albopictus* and *O. japonicus* May – September 2006. Total numbers of mosquito-positive containers sampled are indicated at the top of each histogram bar.

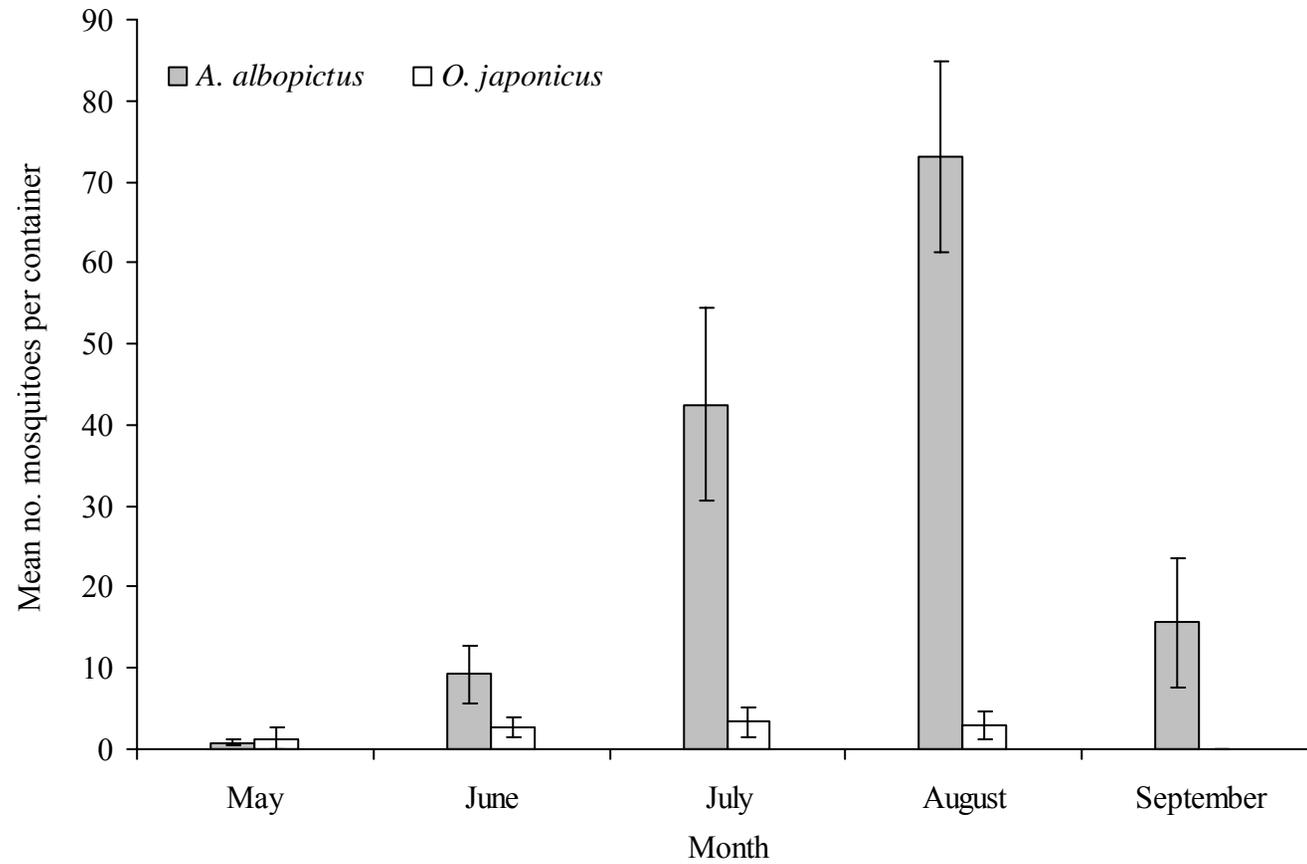


Figure 2-3. Monthly abundance (mean number of mosquitoes collected per container) of *A. albopictus* and *O. japonicus* ( $\pm$ SE) from 91 mosquito-positive artificial containers.

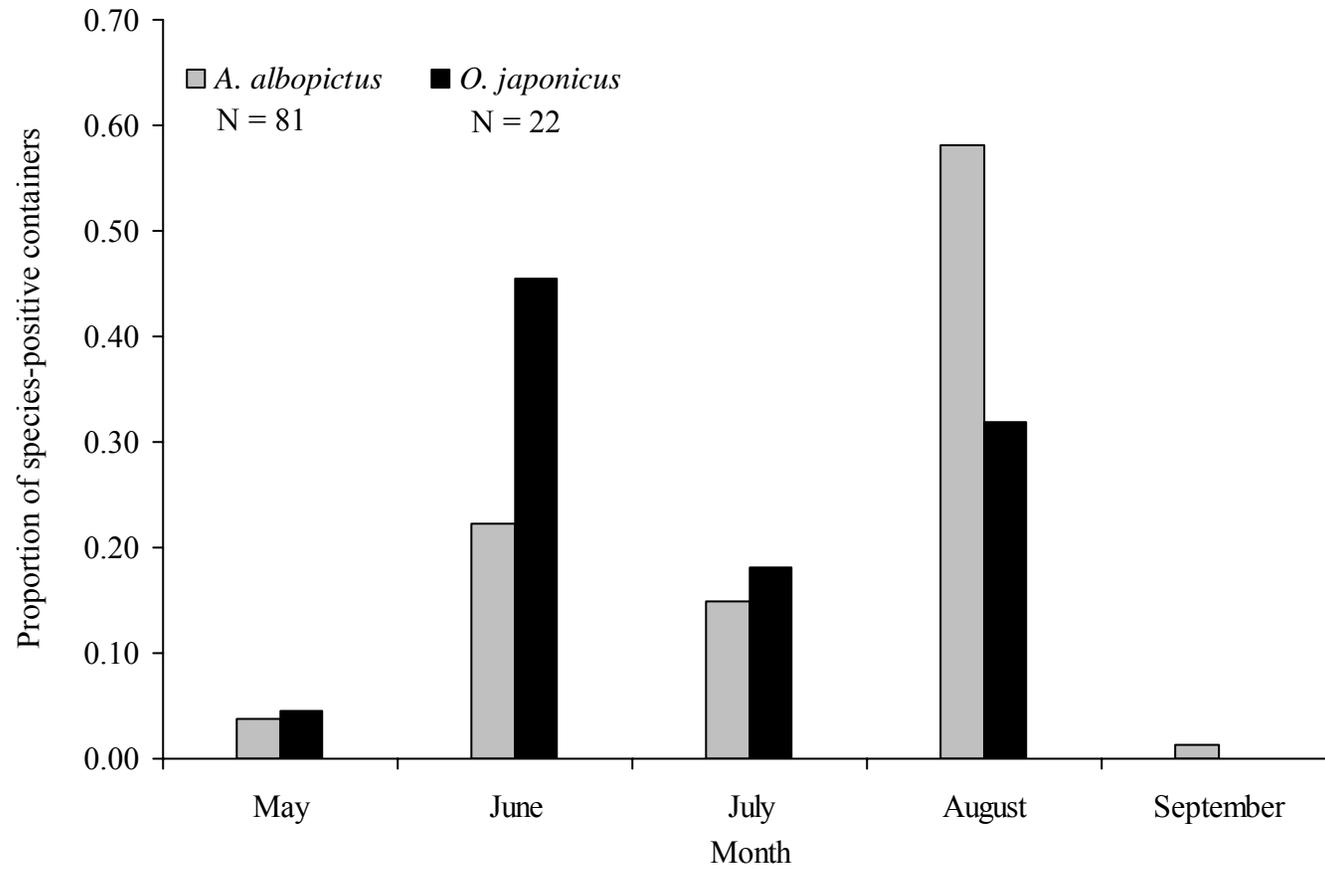


Figure 2-4. Seasonal occurrences (proportion of species-positive containers per month) of *A. albopictus* and *O. japonicus* collected from artificial containers in 2006. The total numbers of species-positive containers sampled from May – September are indicated in the figure legend.

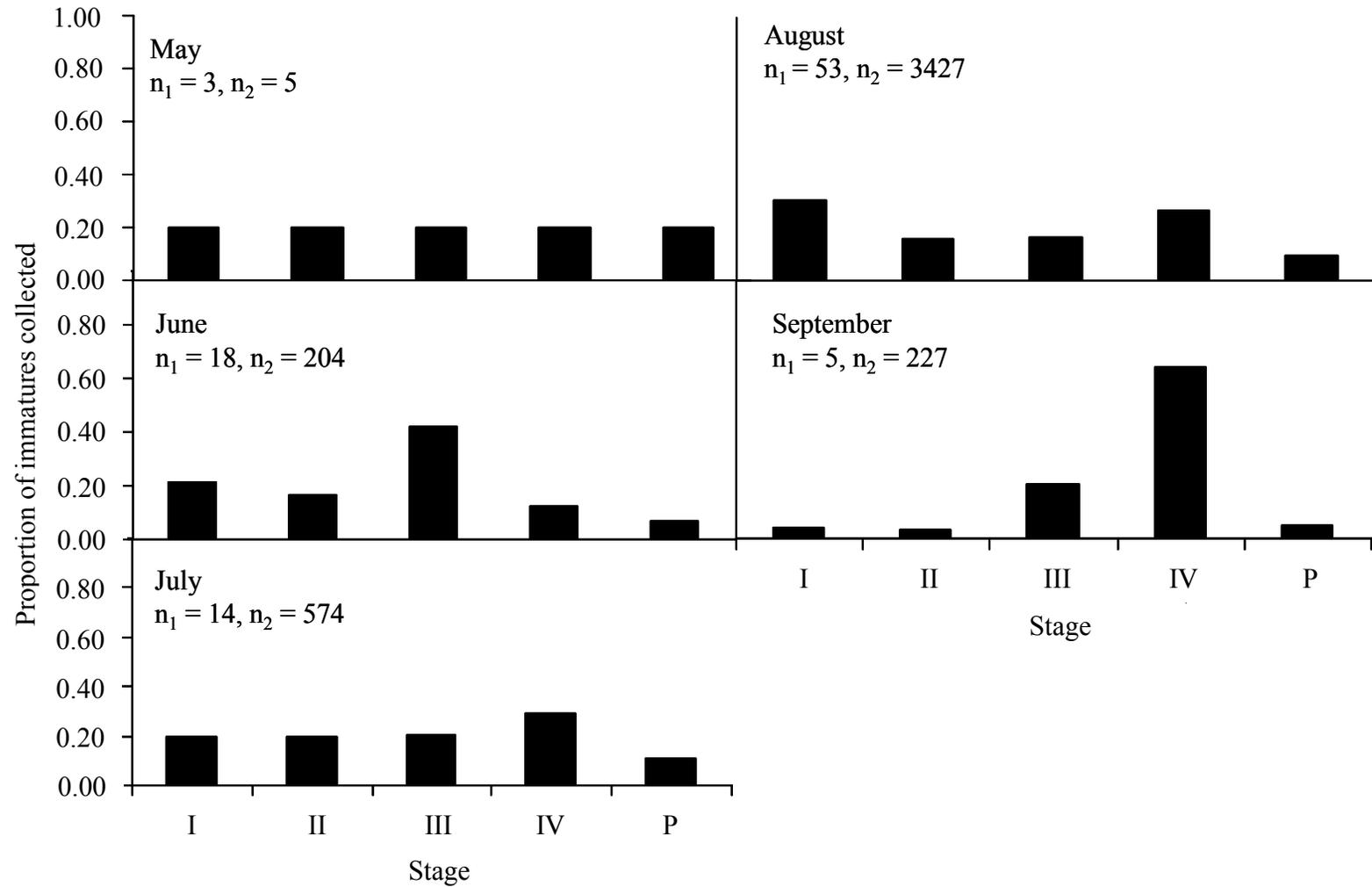


Figure 2-5. Monthly instar distributions of *A. albopictus* from May – September 2006 in Fairfax, Virginia. Total numbers of positive containers ( $n_1$ ) sampled and larvae collected ( $n_2$ ) are indicated for each month.

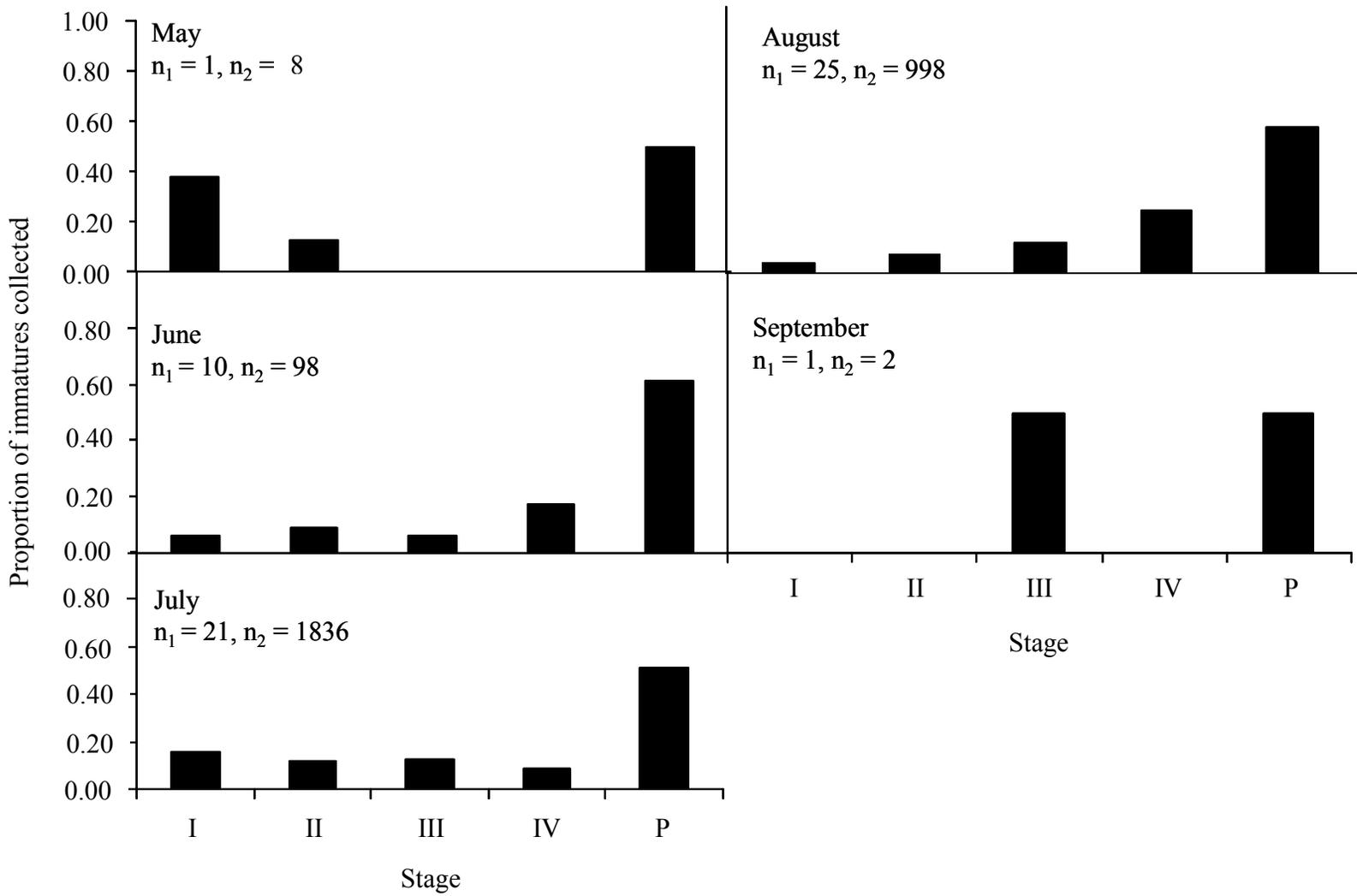


Figure 2-6. Monthly instar distributions of *O. japonicus* from May – September 2006 in Fairfax, Virginia. Total numbers of positive containers ( $n_1$ ) sampled and larvae collected ( $n_2$ ) are indicated for each month.

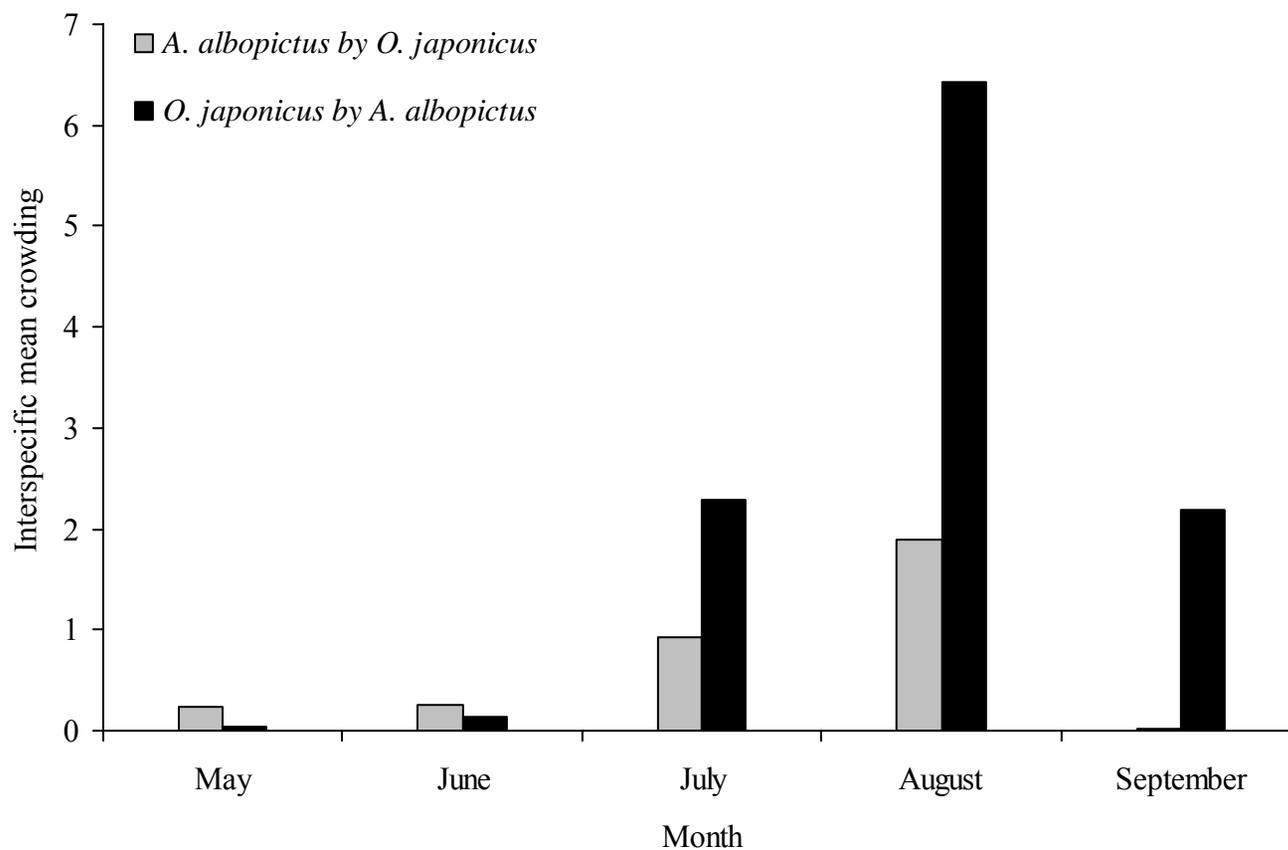


Figure 2-7. Interspecific mean crowding of *A. albopictus* by *O. japonicus* and *O. japonicus* by *A. albopictus*. Interspecific mean crowding was calculated by month as the density of species  $y$  encountered by species  $x$  per unit resource  $a$ , in this case, container volume. Interspecific mean crowding was quantified as  $\sum(x_i y_i / a_i) / \sum x_i$ .

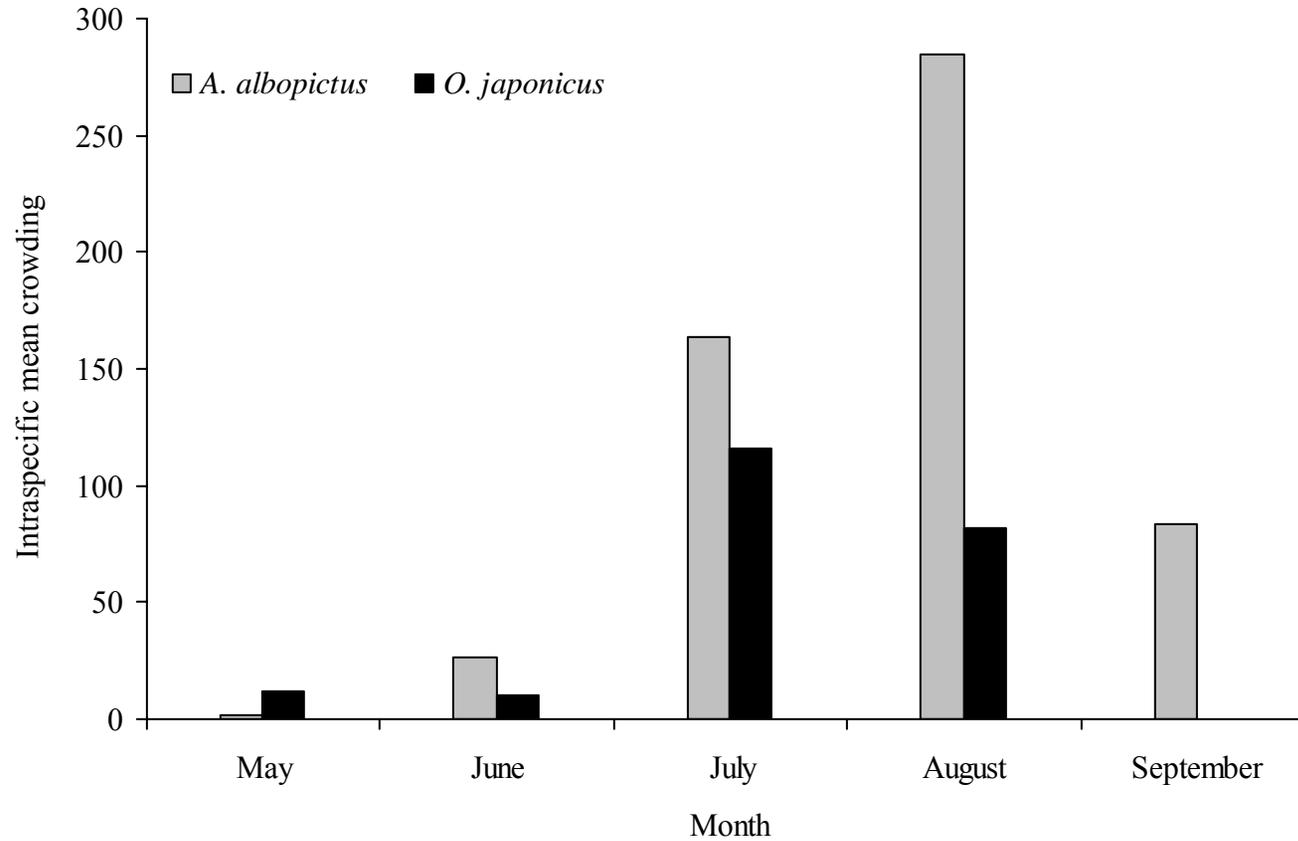


Figure 2-8. Intraspecific mean crowding (density of conspecifics encountered per unit resource,  $a$ ) of *A. albopictus* and *O. japonicus* by month. Intraspecific mean crowding was quantified as  $\sum[x_i(x_i - 1)/a_i]/\sum x_i$ , where  $a$  is container volume.

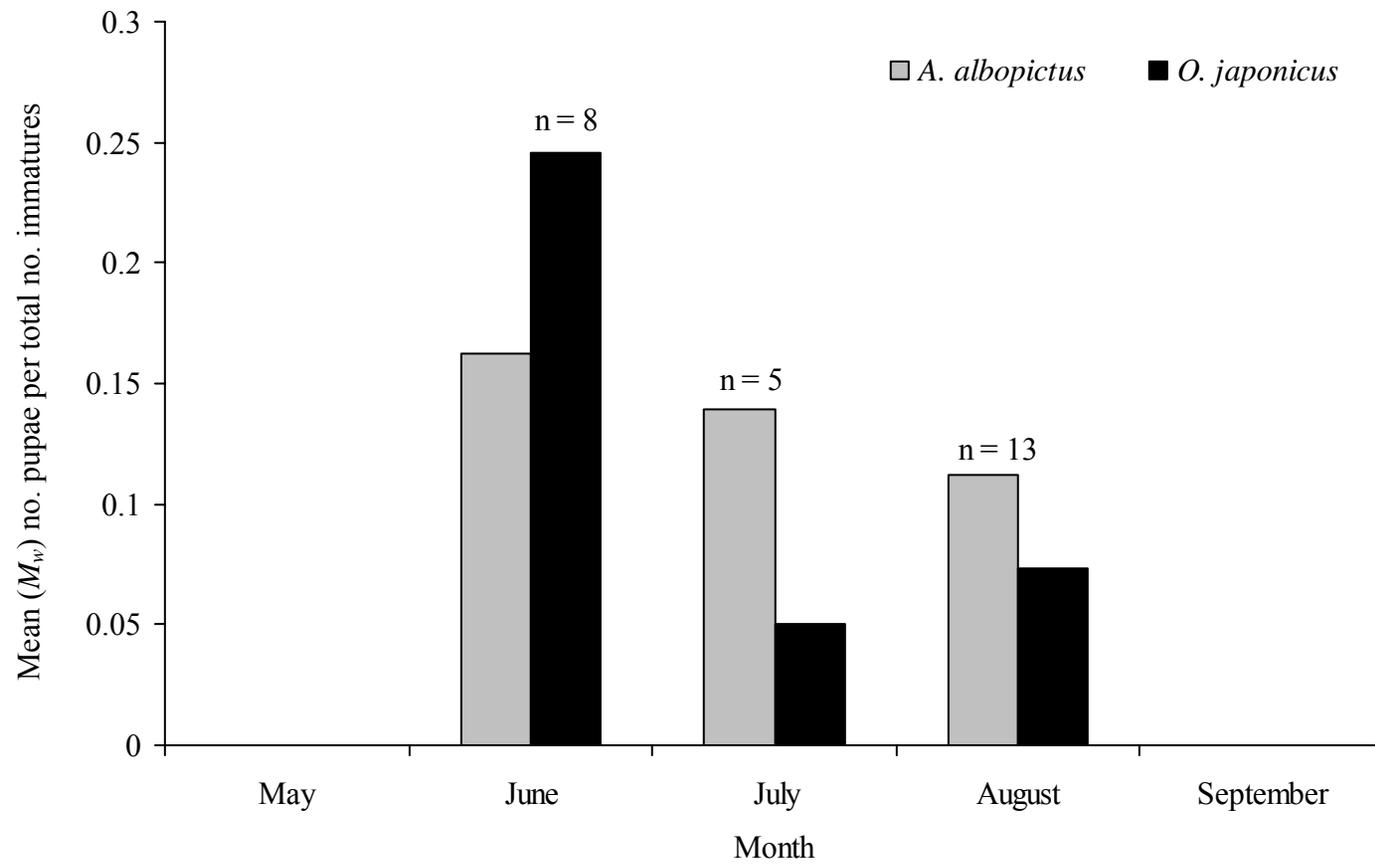


Figure 2-9. Metamorphic success of *A. albopictus* and *O. japonicus* collected from containers in which the two species co-occurred. Numbers of samples for each month are indicated above each histogram bar.

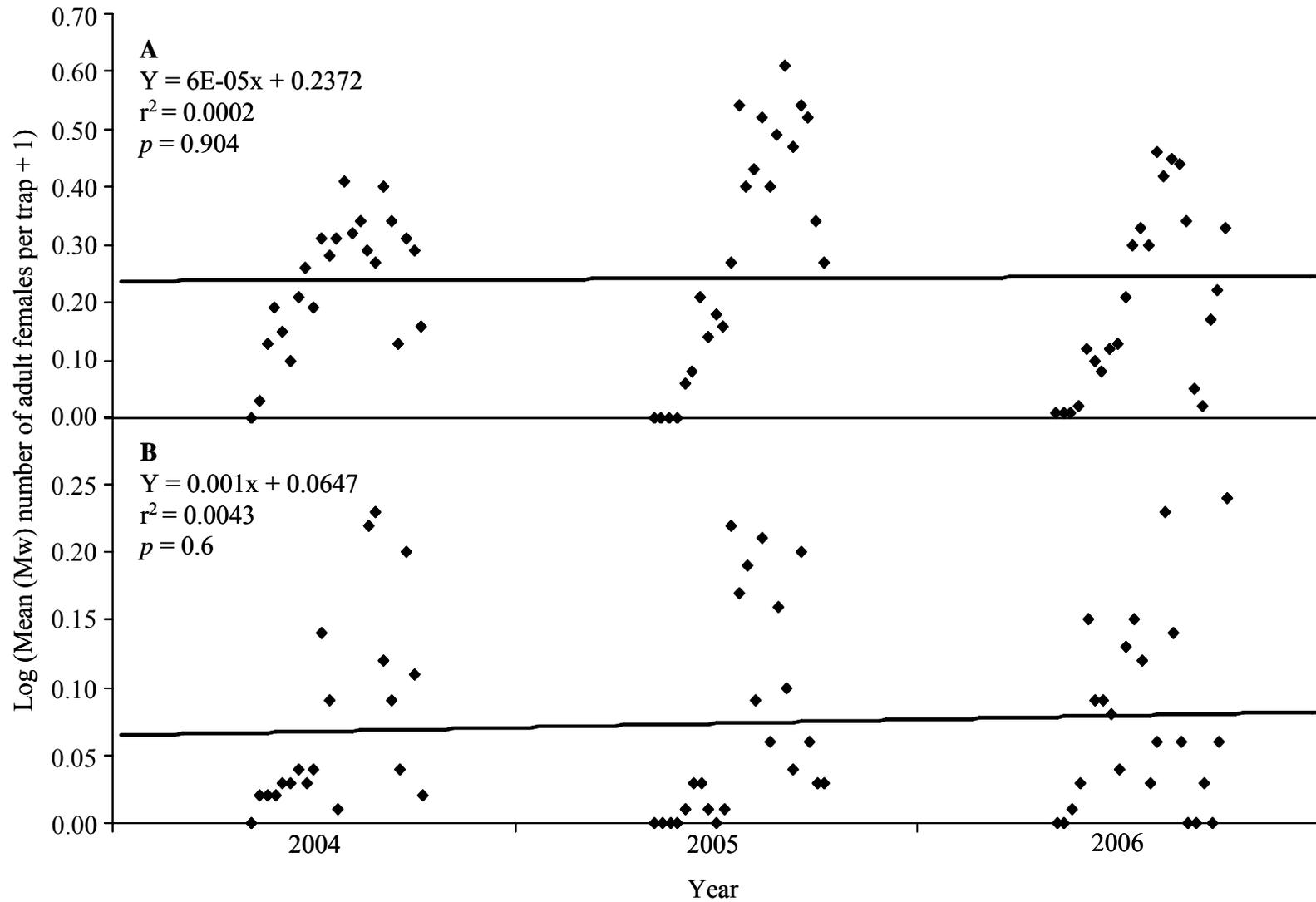


Figure 2-10. Mean weekly abundance of *A. albopictus* collected in (A) CO<sub>2</sub>-baited light traps and (B) gravid traps over time, from 2004 through 2006, in Fairfax County, Virginia. Williams means ( $W_M$ ) were transformed by  $\log_{10}(x + 1)$  to meet assumptions of normality and homogeneity of variance.

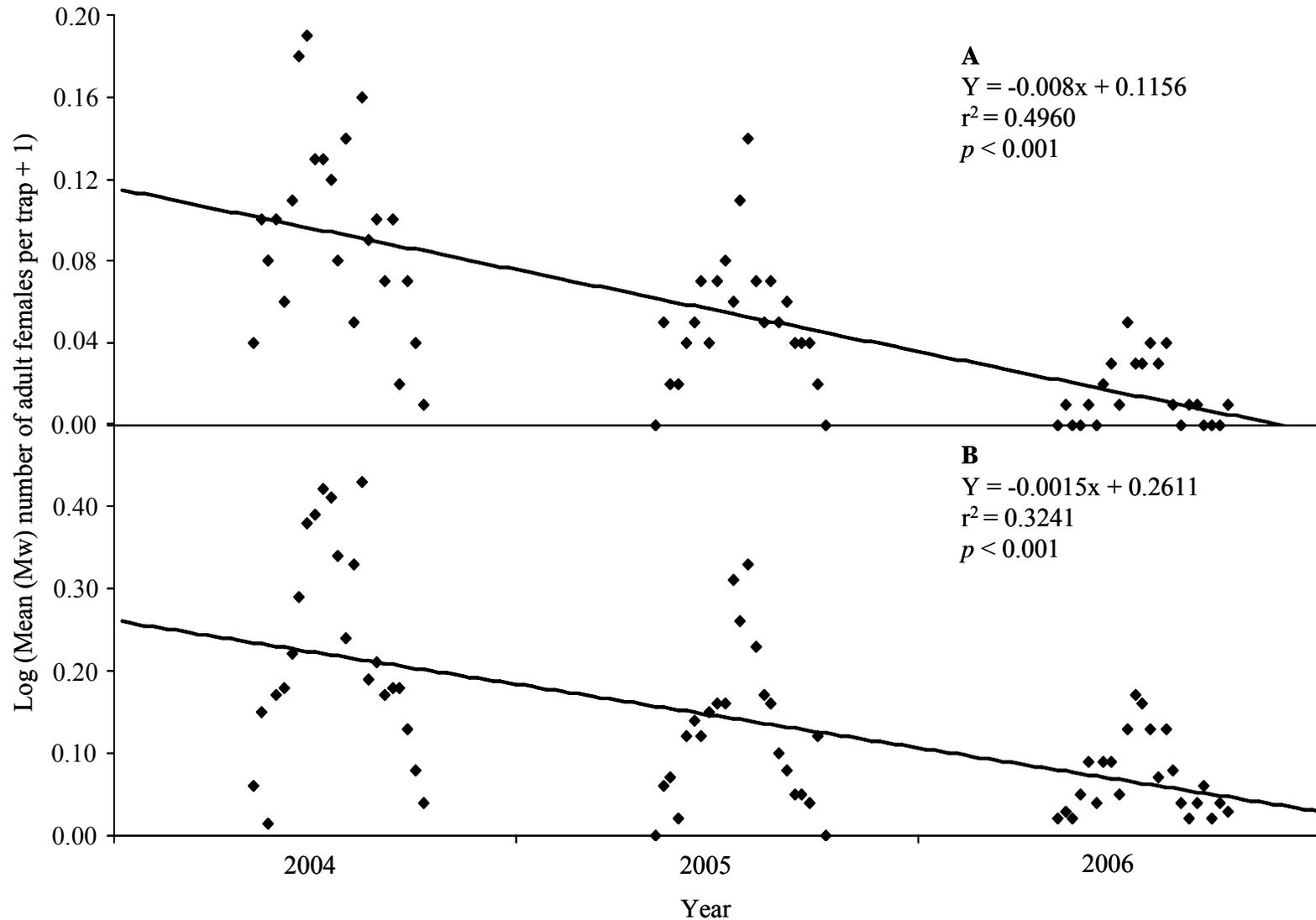


Figure 2-11. Mean weekly abundance of *O. japonicus* collected in (A) CO<sub>2</sub>-baited light traps and (B) gravid traps over time, from 2004 through 2006, in Fairfax County, Virginia. Williams means ( $W_M$ ) were transformed by  $\log_{10}(x + 1)$  to meet assumptions of normality and homogeneity of variance.

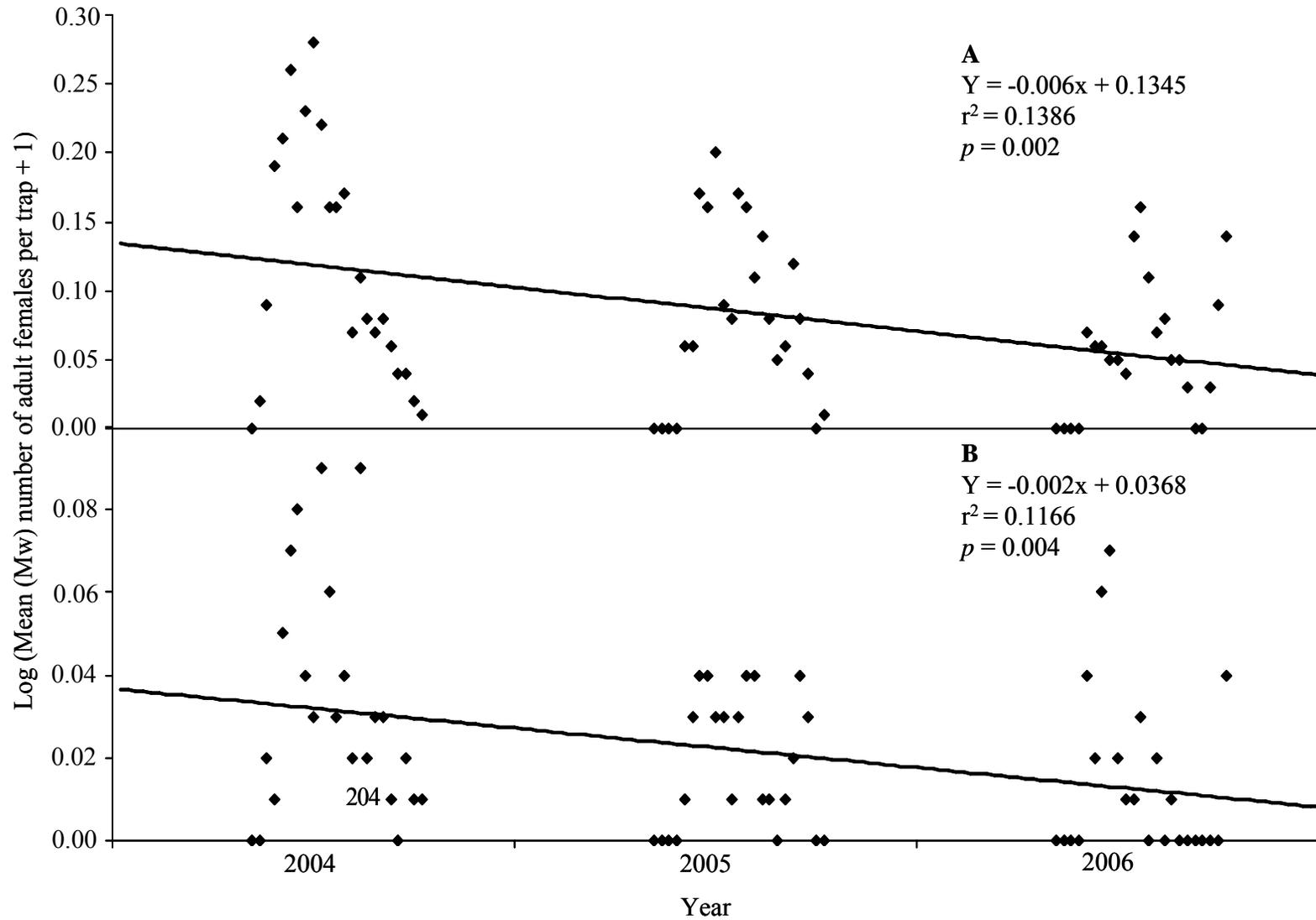


Figure 2-12. Mean weekly abundance of *O. triseriatus* collected in (A) CO<sub>2</sub>-baited light traps and (B) gravid traps over time, from 2004 through 2006, in Fairfax County, Virginia. Williams means ( $W_M$ ) were transformed by  $\log_{10}(x + 1)$  to meet assumptions of normality and homogeneity of variance.

## CHAPTER 3

### INTERSPECIFIC COMPETITION BETWEEN *AEDES ALBOPICTUS* AND *OCHERLOTATUS JAPONICUS*

#### **Introduction**

*Aedes albopictus* and *Ochlerotatus japonicus* are two of the most recently recognized exotic mosquito species to become established in the United States. *Aedes albopictus* was introduced into the United States from Japan by way of tire shipments (Hawley et al. 1987, Reiter and Sprenger 1987), which led to its establishment in Texas in 1985 (Sprenger and Wuithiranyagool 1986). State and local mosquito surveillance records indicate that it has since spread rapidly, becoming established across much of the eastern United States from southern Florida to New Jersey, Illinois, Indiana, and Ohio (Moore 1999). The westward spread of this invader has been much slower, presumably due to the drier summers in this region (Nawrocki and Hawley 1987). *Aedes albopictus* has been intercepted and destroyed on the west coast in California (Linthicum et al. 2003) and Washington (Craven et al. 1988). The invasion success and rapid spread of *A. albopictus* in the United States has been attributed to its generalized habitat and food requirements, ability to live in human-dominated habitats (Hawley 1988), desiccation resistant eggs (Focks et al. 1994, Juliano et al. 2002), and superior larval competitive ability (Juliano 1998, Juliano et al. 2004).

The introduction of *O. japonicus* into the United States was initially reported by Peyton et al. (1999) from light trap collections from New York and New Jersey in August and September 1998. However, an archival search by Andreadis et al. (2001) revealed that this species was actually first detected one month earlier in Connecticut. *Ochlerotatus japonicus* has since been detected along the East coast with reports as far south as Georgia (Reeves and Korecki 2004), north as Maine (Foss and Dearborn 2001), and west as Missouri (Gallitano et al. 2006), from

what appear to be multiple introductions from Japan (Fonseca et al. 2001). *Ochlerotatus japonicus* appears to have become established on the west coast in Washington (Roppo et al. 2004), and has recently been detected in Mississippi and Nevada (Moore 2005). Like *A. albopictus*, the used tire trade is the suspected mechanism of introduction of *O. japonicus* into the United States (Peyton et al. 1999, Lounibos 2002).

The current US distributions of these two species overlap considerably, although *O. japonicus* appears to be more cold tolerant (Tanaka et al. 1997) than *A. albopictus* as evidenced by the former species more northerly native range. It has been predicted that the range of *A. albopictus* may eventually expand northward as far as the  $-5^{\circ}\text{C}$  isotherm, as it does in Asia, however at such latitudes populations would likely not overwinter (Nawrocki and Hawley 1987). Furthermore, both species are container-inhabiting mosquitoes commonly found in water-filled artificial container habitats such as automobile tires, bird baths, and flower pot saucers; however rock pools are the preferred habitat of *O. japonicus* in its native range (Tanaka et al. 1979). The aquatic larvae of both species feed on microorganisms and particulate matter in the water column as well as on leaves and other organic detritus (Merritt et al. 1992).

Severe crowding and limiting resources are frequent in these habitats, thus it is likely that larval resource competition, inter- or intraspecific, may have important effects on the growth, survivorship, and reproductive success of these species (Juliano and Lounibos 2005); therefore larval conditions may have a significant impact on overall population growth. Those species that can maintain positive population growth under interspecific conditions of greater density or lower resource availability than a competitor are considered to have a competitive advantage. Such a competitive advantage is even greater if one species can maintain positive population growth under conditions that result in negative population growth for a competitor.

The role of interspecific competition in structuring communities of container-dwelling mosquitoes has been well documented, perhaps best so for *A. albopictus* and *A. aegypti* in the southeastern United States, where interspecific larval competition (Barrera 1996, Juliano 1998, Braks et al. 2004) was the probably cause of the decline in range and abundance of the latter species throughout most of this area (O'Meara et al. 1995, Juliano et al. 2004, Juliano and Lounibos 2005). *Aedes albopictus* has also been indicated experimentally to be a superior larval competitor to *O. triseriatus* (Livdahl and Willey 1991) and *Culex pipiens* (Costanzo et al. 2005). While the invasion of an introduced species may negatively impact native or other introduced species as a result of interspecific larval competition, the effects of other interactions such as predation, habitat alteration, or apparent competition mediated by shared enemies should also be considered when assessing interspecific interactions.

Understanding the invasion dynamics of *A. albopictus* and *O. japonicus* is important not only because of the ecological consequences resulting from their interactions with native container-inhabiting mosquitoes, but also because these species may be of epidemiological significance. The invasion and establishment of *A. albopictus* and *O. japonicus* in the United States is cause for concern because of potential involvement of these species in the transmission cycle of human arboviruses. In its native range *A. albopictus* is a known vector of dengue virus, which has been isolated from wild-caught individuals of this species in Mexico (Ibañez-Bernal et al. 1997). This species was also implicated as the vector in the 2001 outbreak of dengue in Hawaii (Effler et al. 2005). Wild-caught *A. albopictus* in the United States have also been recovered infected with eastern equine encephalitis (Mitchell et al. 1992) and LaCrosse encephalitis viruses (Gerhardt et al. 2001).

Although *O. japonicus* is not considered an important disease vector in its native range in Asia, it may transmit Japanese encephalitis virus (Takashima and Rosen 1989) and has also been indicated as a competent experimental vector of eastern equine encephalitis (Sardelis et al. 2002a), LaCrosse encephalitis virus (Sardelis et al. 2002b), and St. Louis encephalitis viruses (Sardelis et al. 2003). Both *A. albopictus* and *O. japonicus* have shown to be competent vectors of West Nile virus in the laboratory (Turell et al. 2001), and wild-caught adults of both species have been recovered infected with this virus (Holick et al. 2002, Scott 2003, Godsey et al. 2005). The demonstrated ability of these species to be infected by and transmit numerous arboviruses indicates that their introductions and competitive interactions in the United States may have important public health consequences (Lounibos 2002).

While considerable data exist regarding the interspecific interactions and competitive outcomes of *A. albopictus* with numerous other container-inhabiting mosquito species, there are no comparable reports on *O. japonicus*. As *A. albopictus* and *O. japonicus* are known to frequently co-occur in container habitats, an investigation of larval competitive interactions between these two species was proposed. An experiment designed similar to those of Juliano (1998) and Braks et al. (2004), with the exception that only a single resource level was implemented, was conducted to measure the performance of larvae of Virginia populations of these species competing for resources under field conditions in Fairfax, Virginia. Comparisons were made between species for intra- and interspecific effects of larval density on survivorship, development time, body size, and population growth.

### **Materials and Methods**

The experiment was conducted in a heavily forested streambed located directly behind the Fairfax County Department of Health in Fairfax, Virginia (latitude 38°50'57" N, longitude

77°19'W) from August to October 2006. Routine surveillance data collected by the Department of Health indicated that both *A. albopictus* and *O. japonicus* were commonly detected on this property as both larvae in artificial containers and as adults in CO<sub>2</sub>-baited CDC light traps and gravid traps. The *A. albopictus* and *O. japonicus* used in this experiment were the first generation progeny of gravid individuals collected from Fairfax, Virginia.

Inter- and intraspecific larval competition was investigated by monitoring the development of larvae at different densities in 400-ml black polypropylene cups (10.5 cm in height, 6.5 cm base diameter). Field surveys of container habitats in the area indicated that these species co-occur in containers of similar shape and size in nature. Three density combinations of *A. albopictus*:*O. japonicus* (10:0, 50:0, 0:10, 0:50, and 25:25) were evaluated using a completely randomized block design. One replicate of each combination was placed at each of five experimental sites, spaced approximately 30m apart, for a total of five replicates per treatment and 25 cups. Although both species have been found in containers with varying sun exposure (i.e., none, partial, and full exposure), all five sites used were completely shaded to maintain experimental consistency.

On 10 August, each cup was randomly labeled with a unique number and letter corresponding to one of the five treatments and sites, where they were secured to plastic stakes to prevent toppling. Food consisted of fallen pin oak leaves (*Quercus palustris*) that had been collected, washed, and dried at room temperature for one week prior to quartering, weighing, and sorting. To allow for the leaves to soak and be colonized by microorganisms, four days prior to the start of the experiment 1 g was added to each of the 25 cups containing 200 ml of distilled water. Each container was covered with fiberglass screen (0.5 mm) and secured with a rubber band to prevent entry of other macrofauna and detritus. In the laboratory, eggs of *A. albopictus*

and *O. japonicus* were synchronously hatched (Novak and Shroyer 1978), and 24 hours later larvae were counted into aliquots of 10, 25, and 50. Within one hour after counting, the larvae were distributed into appropriate cups.

Each container was monitored daily for the presence of pupae, which were collected and housed singly in sealed 50 ml vials containing water from their respective field cup. Each vial was labeled with the appropriate site and treatment identifier before being secured to a plastic stake at the field site with a rubber band. Upon emergence, adults were killed by freezing before scoring by container, species, sex, and day of emergence. The experiment ended on 11 October when the final adult emerged. Ambient temperature was monitored hourly for the duration of the experiment with three Onset HOBO data loggers located in the middle and at either end of the experimental area. The average ( $\pm$ SE) hourly ambient temperature recorded was  $18.77\pm 0.06^{\circ}\text{C}$ , with a range of  $7.76^{\circ}\text{C}$  to  $29.73^{\circ}\text{C}$  ( $n = 4197$ ). For two days of the experiment (31 August and 1 September), the area was subjected to intense wind and rain due to Hurricane Ernesto. To prevent damage to experimental apparatus and loss of data, the cups at each experimental site were successfully covered and secured with a tarpaulin during this time.

## **Data Analysis**

### **Population Growth Correlates**

To quantify the effects of inter- and intraspecific competition on *A. albopictus* and *O. japonicus*, the mean survivorship, median development time of males and females, and median body size at adulthood of females were analyzed by one-way ANOVA, followed by Tukey's honestly significantly different (HSD) post-hoc tests for pairwise comparisons of means in SPSS (SPSS 2002). Survivorship was calculated as the proportion of adults that emerged from the initial cohort of first instar larvae. Development time was calculated as the number of days from

hatching to adult emergence. Adult body size was estimated from the length of one wing, which was removed from each female and measured under a dissecting microscope with an ocular micrometer (Packer and Corbet 1989). Median rather than mean development time and wing length were calculated for each species and treatment because of the non-normal distributions of these variables within cohorts.

### Composite Index of Population Performance

Survivorship, female development time and wing length were used to calculate a composite index of mosquito population performance ( $\lambda'$ ), which is an analog of the finite rate of increase as defined by Juliano (1998):

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

where  $N_0$  is the initial number of females (assumed to be 50% of the cohort),  $A_x$  is the number of females eclosing on day  $x$ ,  $w_x$  is the mean wing length of females eclosing on day  $x$ , and  $f(w_x)$  is a function relating egg production to wing length.  $D$  is the time from adult eclosion to reproduction, taken as 14 days for *A. albopictus* (Livdahl and Willey 1991) and 12 days for *O. japonicus* (see below). Values of  $\lambda'$  greater than one indicate that the population is increasing, approximately equal to one that the population is stable, and less than one that the population is decreasing.

$D$  for *O. japonicus* was determined experimentally under controlled conditions of 26°C and 12h:12h light:dark in an insectary at the Florida Medical Entomology Laboratory in Vero Beach, Florida. *Ochlerotatus japonicus* eggs used for this experiment were obtained from the

colony maintained at the Headlee Research Laboratory Mosquito Research and Control Unit at Rutgers University in New Brunswick, New Jersey. This colony originated from larval collections from a horse farm in New Egypt, Ocean County, New Jersey in 2001 (L. McCuiston personal communication).

In the laboratory, eggs were hatched by flooding with water, and cohorts of larvae hatching within the same 24-hour period were grouped together and placed in plastic trays within separate 0.028 m<sup>3</sup> (30.5 cm x 30.5 cm x 30.5 cm) cages. Each cohort was fed 100 mg of an artificial diet consisting of one part Brewer's yeast and one part lactalbumen every other day. Beginning two days after emergence, adult females of each cohort were offered a bloodmeal from a restrained chicken placed within the cage daily for one hour. Cotton soaked in a 20% sucrose solution was provided as a source of carbohydrates for adults at all times. Upon visual inspection immediately following the bloodfeeding opportunity, those females that appeared to have fed to repletion were removed from the cage using a mouth aspirator and placed singly in 12-dram plastic vials containing a 2.54 cm by 7.62 cm strip of wet seed germination paper (Anchor Paper, St. Paul, MN) to serve as an oviposition substrate (Steinly et al. 1991). The germination paper was checked daily for the presence of eggs, and was replenished with water if necessary. The date of oviposition for each female was recorded, from which the average time from adult eclosion to oviposition for *O. japonicus* was calculated to be 12 days, with a range of 4 to 17 days (N = 144).

A regression relating female wing length to fecundity for *A. albopictus* was obtained from Lounibos et al. (2002):

$$f(w_X) = 78.02 w_X - 121.240 \quad (r^2 = 0.713, N = 91, p < 0.001)$$

where  $w_x$  is the wing length in millimeters on day  $x$ , while that for *O. japonicus* was obtained from Lounibos et al. (unpublished data), who used individuals originating from the same colony from Rutgers University mentioned previously, as follows:

$$f(w_x) = 53.078 w_x - 113.91 \quad (r^2 = 0.319, N = 79, p < 0.001),$$

where  $w_x$  is the wing length in millimeters on day  $x$ .

For analyses of  $\lambda'$  for *A. albopictus* and *O. japonicus* a one-way ANOVA was used with Tukey's honestly significantly different (HSD) tests performed post-hoc for pairwise comparisons of means (SPSS 2002).

## Results

### Survivorship to Adulthood

Mean survivorship to adulthood of *A. albopictus* was affected by treatment but that of *O. japonicus* was not (Table 3-1, Figure 3-1). Mean survivorship of *A. albopictus* varied significantly among treatments ( $F_{2,12} = 7.442, p = 0.008$ ), and was significantly higher in the 10:0 treatment than the 50:0 or 25:25 treatments, which were not different from one another. With respect to individual density treatments, mean survivorship of *A. albopictus* was consistently higher than that of *O. japonicus*.

### Developmental Time

Median time from hatch to adulthood of both sexes was significantly affected by density treatment for both males ( $F_{2,12} = 7.560, p = 0.008$ ) and females ( $F_{2,12} = 19.114, p < 0.001$ ) of *A. albopictus*. Comparisons of means for *A. albopictus* showed that development time was significantly shorter for both males and females in the 10:0 and 25:25 density treatments (Table 3-1). Median development time of both male ( $F_{2,12} = 7.09, p = 0.009$ ) and female ( $F_{2,12} = 10.194, p = 0.003$ ) *O. japonicus* was significantly affected by treatments; however significant differences in pairwise comparisons differed between the sexes in this species (Table 3-1). With respect to

individual density treatments, median development times of both male and female *A. albopictus* were consistently faster than those of *O. japonicus* (Figures 3-2, 3-3).

### **Female Wing Length**

Median wing lengths of both *A. albopictus* and *O. japonicus* females were significantly affected by density treatments ( $F_{2,12} = 4.837, p = 0.029$ ;  $F_{2,12} = 9.584, p = 0.003$ ). For both species, median wing length was significantly greater for females from 10:0 treatments; however for *A. albopictus* this difference was only significant in comparison to females from the 25:25 density treatment (Table 3-1, Figure 3-4).

### **Estimated Finite Rate of Increase ( $\lambda'$ )**

The mean estimated finite rate of increase of both species was significantly affected by density treatments (Table 3-1, Figure 3-5), although more pronounced for *A. albopictus* ( $F_{2,12} = 23.585, p < 0.001$ ) than *O. japonicus* ( $F_{2,12} = 16.366, p < 0.001$ ). Comparisons of means showed that  $\lambda'$  of *A. albopictus* was significantly higher for the 10:0 treatment than the 25:25 treatment, which was significantly higher than that of the 50:0 density treatment (Table 3-1). For *O. japonicus*,  $\lambda'$  of the low-density treatment was significantly higher than either of the high-density treatments. With respect to individual density treatments, mean estimated finite rates of increase of *A. albopictus* were consistently higher than those of *O. japonicus*.

## **Discussion**

It is common opinion that the invasion success and spread of non-native species is enhanced by superiority in interspecific competition, particularly when similar species and limited resources are encountered (Williamson 1996). It has been demonstrated that interspecific larval resource competition plays an important role in structuring the mosquito communities of artificial container habitats (Juliano and Lounibos 2005). Given the results of this experiment, *A. albopictus* does appear to be a superior competitor to *O. japonicus*; however the non-significant

impact of interspecific larval resource competition on population performance suggests that *O. japonicus* will be able to coexist with *A. albopictus* in artificial container habitats in nature.

Continued coexistence of *A. albopictus* and *O. japonicus* in artificial containers is supported by the relatively high survivorship of both species, which were not significantly different under interspecific (25:25 treatment) versus intraspecific conditions (50:0 treatment) of the same mosquito density. Furthermore, the estimated finite rate of increase,  $\lambda'$ , remained greater than one in all species/density treatments, indicating a population increase for both species under all experimental conditions (Table 3-1). However, the mean  $\lambda'$  for *A. albopictus* was actually significantly higher under interspecific conditions than intraspecific conditions of the same mosquito density (Figure 3-5). This appears to be due to the median development time of females from interspecific density treatments being significantly shorter than those from intraspecific treatments of the same mosquito density (Figure 3-2). These findings suggest that intraspecific competition may be more important for regulating *A. albopictus* population growth in container habitats than interspecific competition with *O. japonicus*.

While neither species appeared to be detrimentally affected under interspecific conditions with respect to population performance, *A. albopictus* may have a slight competitive advantage over *O. japonicus*. This is supported by the consistently higher survivorship, shorter development time, and higher finite rate of increase of *A. albopictus* compared to *O. japonicus* across density treatments. Under interspecific conditions, median development time of both male ( $F_{1,8} = 18.375, p = 0.003$ ) and female ( $F_{1,7} = 26.940, p < 0.001$ ) *A. albopictus* was significantly shorter than that of *O. japonicus*. Similarly, the mean estimated finite rate of increase was significantly greater for *A. albopictus* than *O. japonicus* ( $F_{1,8} = 11.016, p = 0.011$ ). However, there was no difference in survivorship between the two species under interspecific conditions

( $F_{1,8} = 3.240$ ,  $p = 0.110$ ). While the difference in development times may simply be the result of intrinsic metabolic differences between the species, when coupled with the higher mean estimated rate of finite increase it may suggest that perhaps *A. albopictus* is able to forage and acquire resources more efficiently or employs different feeding behaviors that are more effective in this type of larval habitat. Ho et al. (1973) suggested that perhaps the higher content of proteinases in the gut of *A. albopictus* facilitates a more efficient feeding style, which ultimately allows the species to develop faster than other container-inhabiting mosquitoes.

These results imply that while *A. albopictus* may have a slight competitive advantage over *O. japonicus*, the two will likely continue to coexist in containers in areas where their distributions overlap. However, these findings should be viewed in context with field observations of co-occurrences, as well as seasonal distributions, habitat preferences, and overwintering behaviors, as they may ultimately influence the community structure of the artificial containers in which these species coexist. In addition to these ecological consequences, these findings may potentially have epidemiological implications, particularly with respect to LaCrosse encephalitis virus, of which both *A. albopictus* and *O. japonicus* are suspected vectors. Their continued coexistence in containers in LaCrosse endemic regions may be important in epizootic, or potentially even epidemic, transmission of the disease, although this will require further investigation. Because larval competition has been linked to greater infection and dissemination rates of dengue and Sindbis viruses for *A. albopictus* (Alto et al. 2005), similar effects are possible with respect to arboviruses circulating in areas in which this species is sympatric with *O. japonicus*.

Although this experiment was conducted in the field under manipulated but ecologically realistic conditions, it is important to note that variations in resource level, type, or frequency

(Braks et al. 2004), temperature (Lounibos et al. 2002), sun exposure, container type (Livdahl and Willey 1991), larval density, and season (Teng and Apperson 2000) may influence larval competition differently among these mosquito species. Similarly, while interspecific larval competition is likely an important factor influencing the survivorship, growth, reproductive success, and population performance of mosquitoes in container environments with limited resources, other factors such as predation (Griswold and Lounibos 2005, 2006), intraguild predation (Edgerly et al. 1999) apparent competition mediated by shared enemies (Munstermann and Wesson 1990, Blackmore et al. 1995, Juliano 1998), habitat alteration (Bertness 1984), and differences in foraging behavior (Yee et al. 2004) may also be important and warrant further research with respect to interactions between these two species.

Table 3-1. Means ( $\pm$ SE) of population growth correlates for *A. albopictus* and *O. japonicus*. Means followed by letters that are not commonly shared are significantly different by pairwise comparisons ( $p < 0.05$ ).

Response	Density – species treatments					
	<i>Aedes albopictus</i>			<i>Ochlerotatus japonicus</i>		
	10:0	50:0	25:25	10:0	50:0	25:25
Mean survivorship	.94 (.04)a	.704 (.038)b	.728 (.061)b	.78 (.049)	.524 (.096)	.584 (.052)
Median female development time (d)	15.4 (.51)a	29.6 (2.91)b	17.5 (.71)a	20.8 (2.84)a	32.8 (2.85)b	35.4 (3.08)b
Median male development time (d)	12.8 (.56)a	15.2 (.58)b	12.7 (.37)a	14.8 (.52)a	20.3 (1.22)ab	25.9 (2.70)b
Median female wing length (mm)	2.71 (.092)a	2.51 (.053)ab	2.41 (.056)b	3.55 (.060)a	3.05 (.108)b	3.08 (.10)b
$\lambda'$	1.162 (.007)a	1.093 (.008)b	1.120 (.007)c	1.135 (.007)a	1.071 (.011)b	1.076 (.007)b

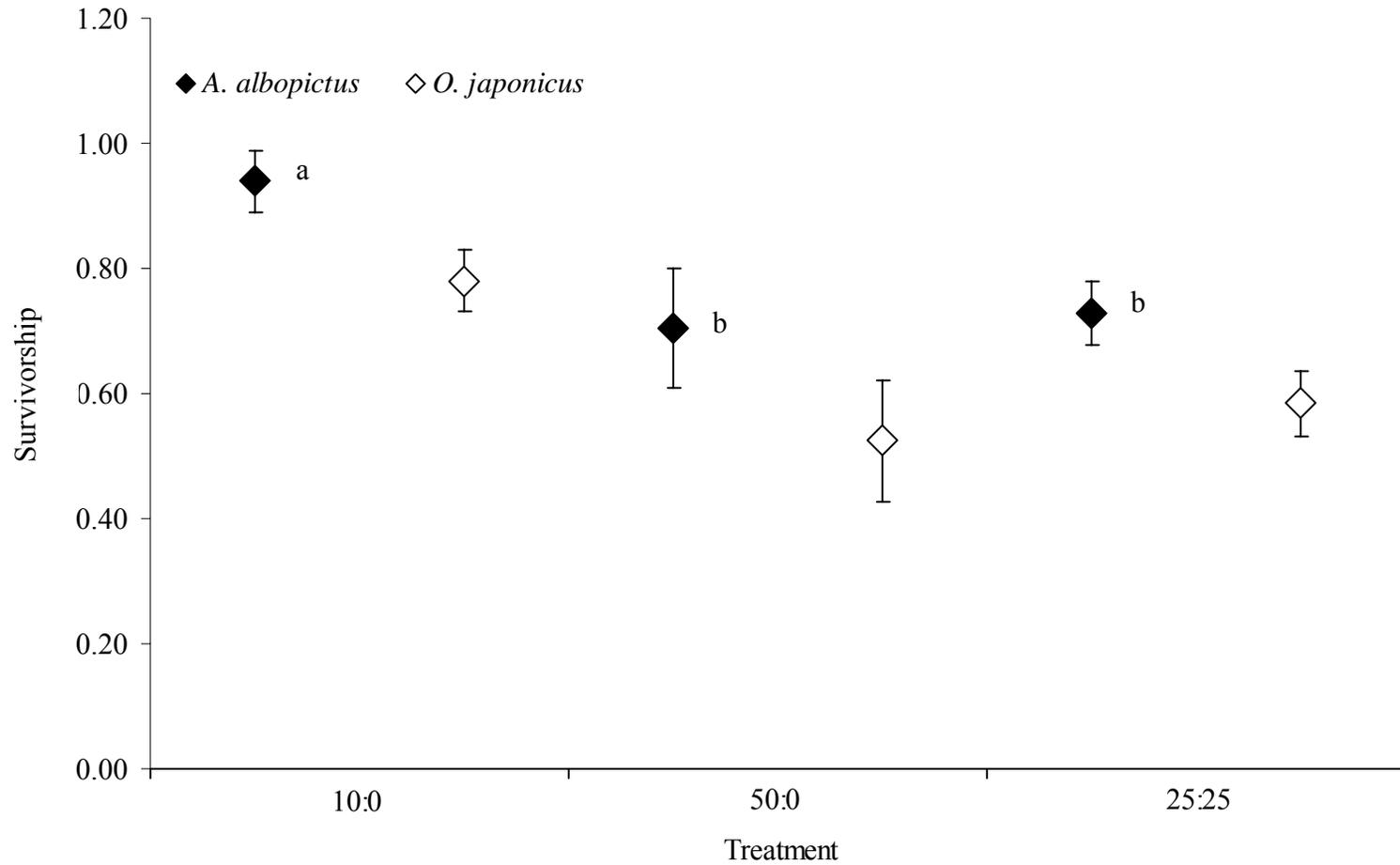


Figure 3-1. Mean survivorship (proportion of the original number of larvae surviving to adulthood) of *A. albopictus* and *O. japonicus* ( $\pm$ SE). Lower case letters indicate significant differences among competition treatments resulting from pairwise comparisons ( $p < 0.05$ ) for *A. albopictus*. Analysis of variance did not indicate a significant difference in survivorship among treatments for *O. japonicus*.

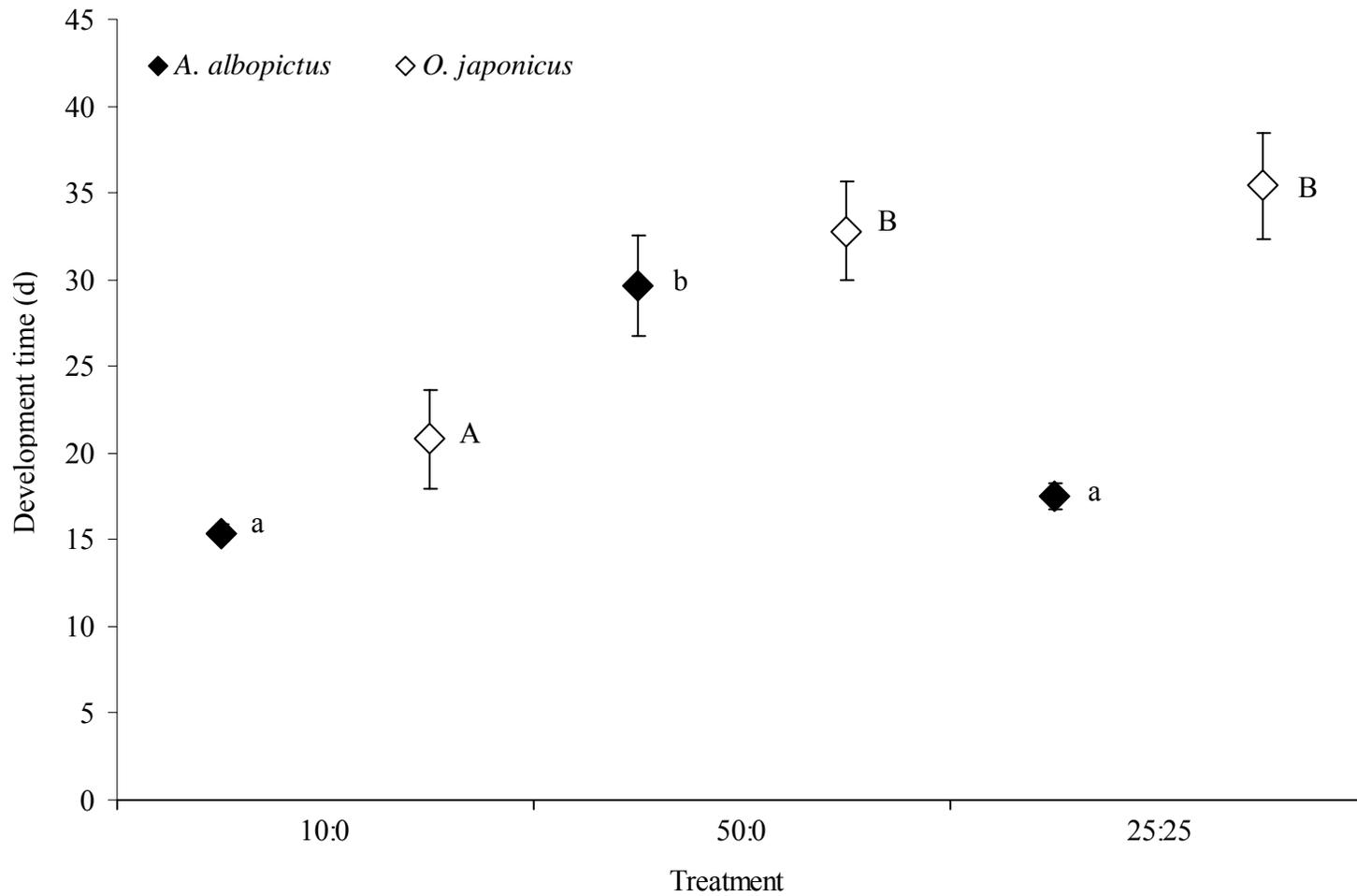


Figure 3-2. Means of median time to adulthood for female *A. albopictus* and *O. japonicus* ( $\pm$ SE). Lower case and upper case letters indicate significant differences among competition treatments resulting from pairwise comparisons ( $p < 0.05$ ) for *A. albopictus* and *O. japonicus* respectively.

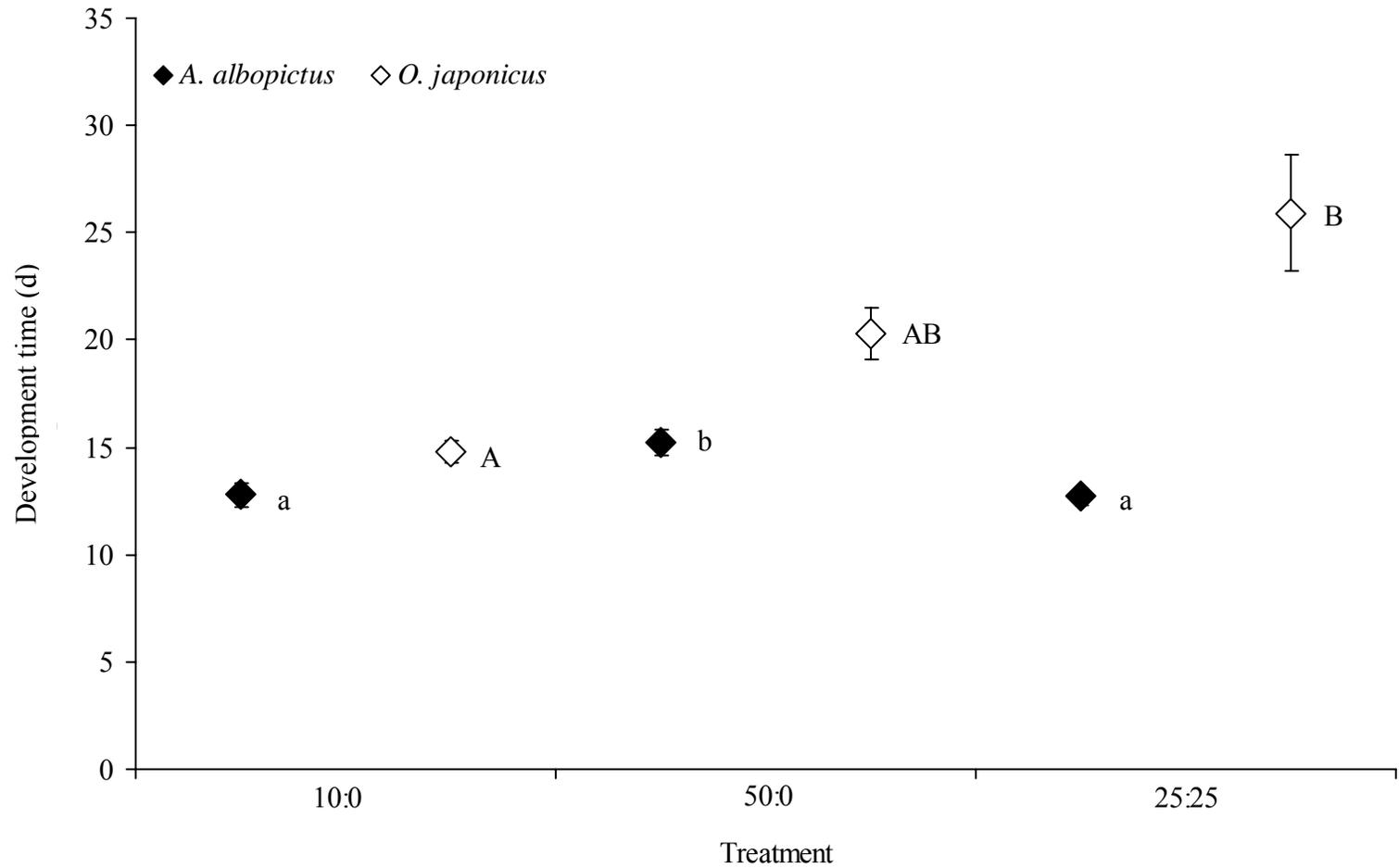


Figure 3-3. Means of median time to adulthood for male *A. albopictus* and *O. japonicus* ( $\pm$ SE). Lower case and upper case letters indicate significant differences among competition treatments resulting from pairwise comparisons ( $p < 0.05$ ) for *A. albopictus* and *O. japonicus* respectively.

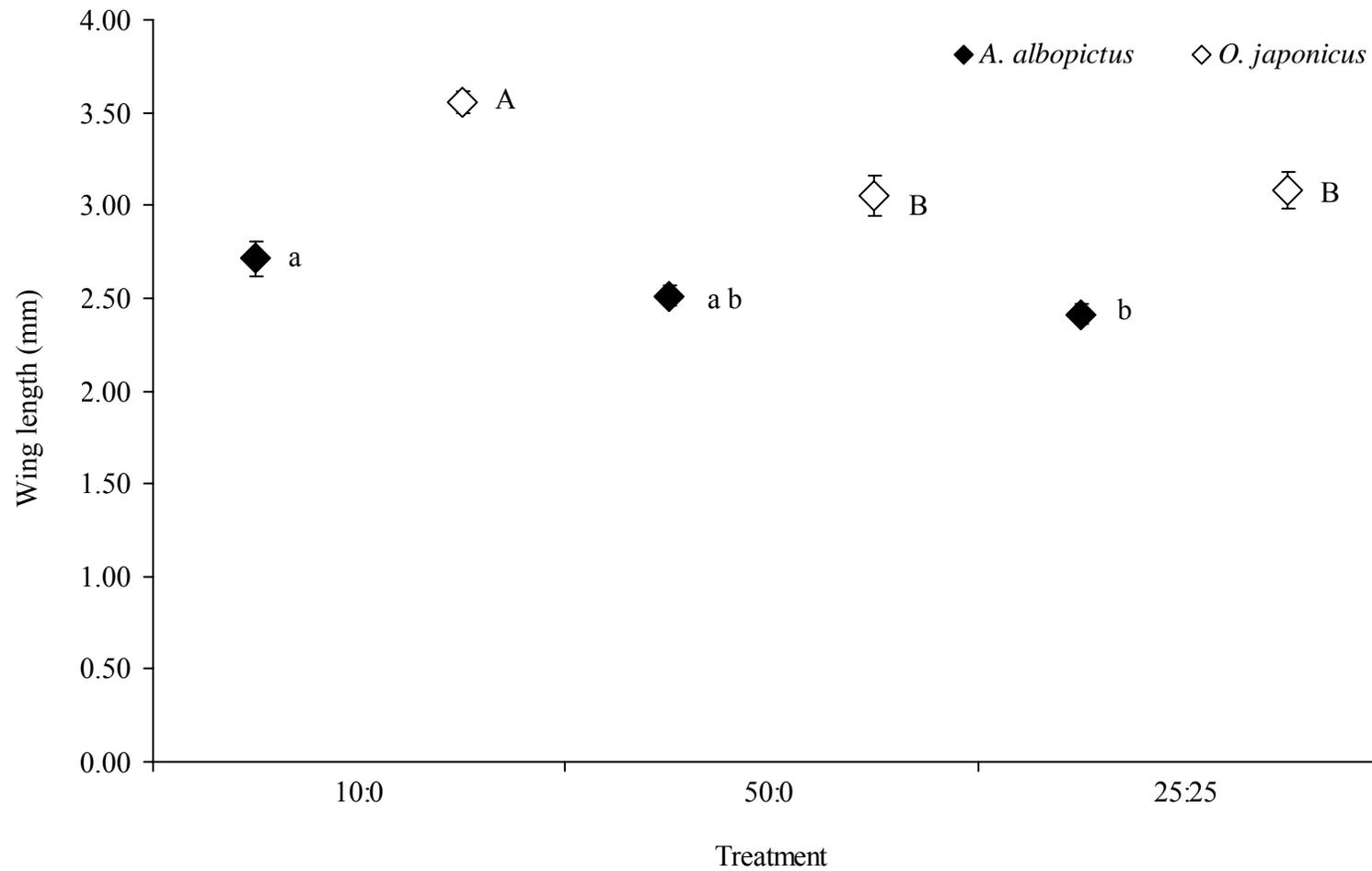


Figure 3-4. Means of median wing lengths of *A. albopictus* and *O. japonicus* adult females ( $\pm$ SE). Lower case and upper case letters indicate significant differences among competition treatments resulting from pairwise comparisons ( $p < 0.05$ ) for *A. albopictus* and *O. japonicus* respectively.

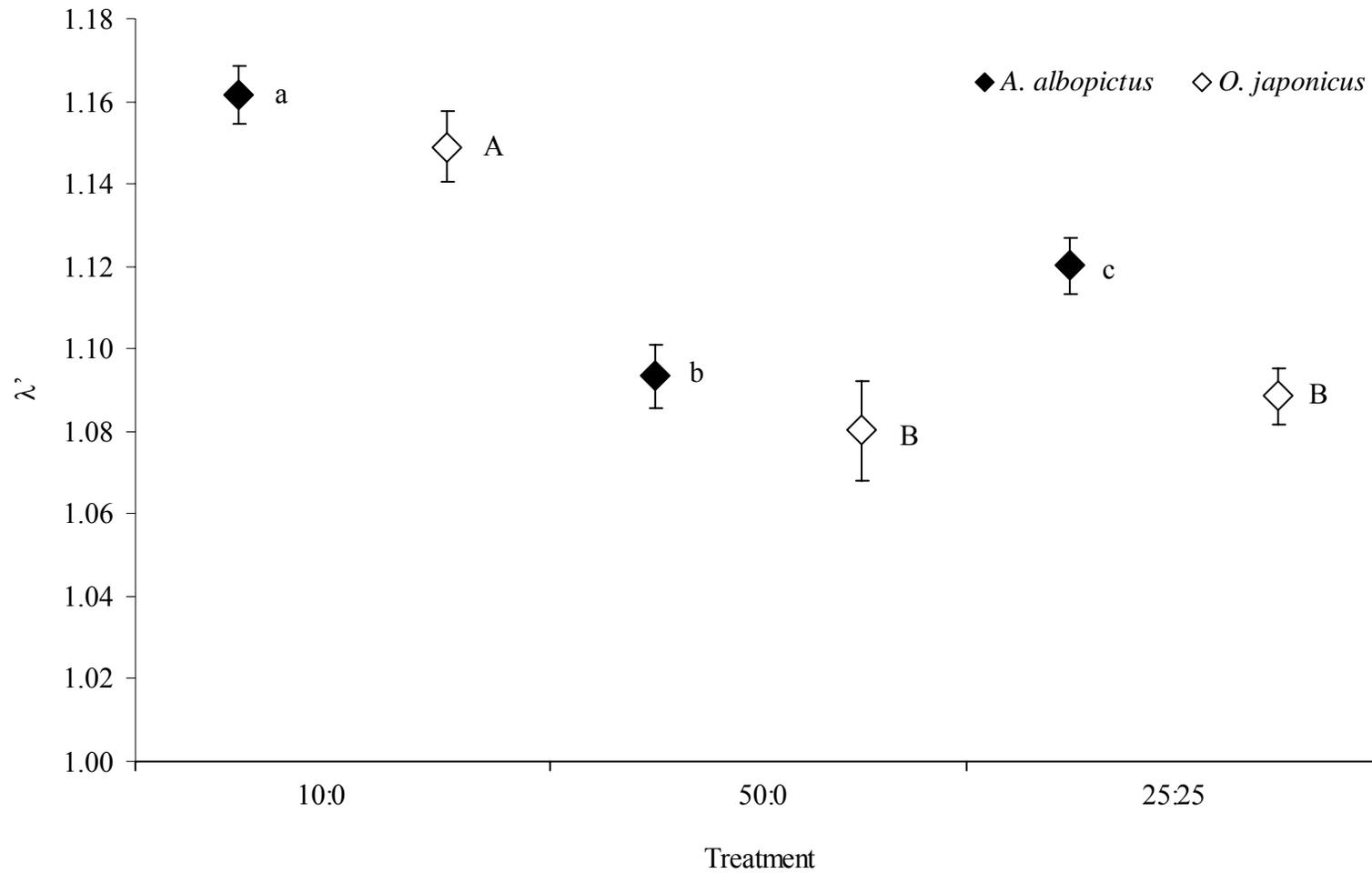


Figure 3-5. Mean estimates of population performance ( $\lambda'$ , an estimate of the finite rate of increase for the cohort) for female *A. albopictus* and *O. japonicus* adults ( $\pm$ SE). Lower case and upper case letters indicate significant differences among competition treatments resulting from pairwise comparisons ( $p < 0.05$ ) for *A. albopictus* and *O. japonicus* respectively.

## CHAPTER 4

### INTERSPECIFIC COMPETITION BETWEEN *OCHLEROTATUS ATROPALPUS* AND *OCHLEROTATUS JAPONICUS*

#### **Introduction**

Within its native range in Asia, *Ochlerotatus japonicus* larvae are found in a wide variety of natural and artificial containers; however rock pools are the preferred habitat (LaCasse and Yamaguti 1950, Tanaka et al. 1979). This species has colonized a similar ecological niche since its introduction to the United States in 1998 via used tire shipments (Peyton et al. 1999, Andreadis et al. 2001) from Japan (Fonseca et al. 2001). As an invasive species, the likelihood of *O. japonicus* to become established and propagate in this niche depends partly on resource availability and its ability to compete with ecologically similar resident species. Interspecific competition is instrumental in determining the outcome of an introduction regardless of whether it promotes or limits the spread of an invader, and may only be avoided if an invader is filling a vacant niche by exploiting a previously unoccupied habitat or unused resource (Williamson 1996).

The effects of interspecific competition due to severe crowding and limited resources among the larvae of container-inhabiting mosquitoes have been well documented (e.g. Livdahl and Willey 1991, Barrera 1996, Juliano 1998, Braks et al. 2004, Juliano et al. 2004, Costanzo et al. 2005), however the majority of these investigations centered around the invasion of *Aedes albopictus*. Such studies have demonstrated both the success (O'Meara et al. 1995, Juliano et al. 2004) and failure (O'Meara et al. 1989, Lounibos et al. 2003) of invasive species to spread. Interspecific competition may potentially impact the overall population performance of container-inhabiting species by affecting growth, survivorship, and reproductive success (Juliano

and Lounibos 2005). Under conditions of severe interspecific competition, such effects may lead to the decline or elimination of a resident species following the introduction of a competitively superior invader (e.g. Juliano 1998).

Due to the propensity of *O. japonicus* to inhabit rock pools, the indigenous mosquito most likely to be affected by the invasion of this species is the North American rock pool mosquito, *O. atropalpus*. The two species are highly sympatric, with distributions that overlap in parts of the eastern United States. Frequent and abundant collections of *O. japonicus* larvae co-occurring with *O. atropalpus* in rock pools provides a natural setting for interspecific larval resource competition (Andreadis et al. 2001), which some have speculated may limit its invasion success (Juliano and Lounibos 2005). However, recent field studies have indicated that competitive displacement of *O. atropalpus* by *O. japonicus* may be occurring in rock pools in New Jersey (Scott et al. 2001a), North Carolina (B. Harrison personal communication), and Virginia (see previous chapter). Because of the specialized primary larval habitat of *O. atropalpus* in rock pools, the distribution of this species tends to be sparsely and irregularly distributed despite its large geographical range. Such conditions may promote the localized decline or extinction of this species in areas where these two species co-occur, particularly if *O. japonicus* is a superior competitor. However, as noted in Lounibos (2002), *O. atropalpus* has expanded its distribution by occupying discarded tires and has itself been considered an invasive species.

The decline and potential competitive exclusion of *O. atropalpus* suggest that the competitive superiority of *O. japonicus* may be important in the invasion success of this species. While interspecific larval resource competition is a likely mechanism for such ecological processes, no formal research has been conducted to confirm such speculation. Thus an investigation of larval competitive interactions between these two species was proposed.

Comparisons were made in the laboratory between species for intra- and interspecific effects of larval density on survivorship, development time, body size, and population growth.

### **Materials and Methods**

The experiment was conducted in an insectary at the Florida Medical Entomology Laboratory in Vero Beach, Florida under controlled conditions of  $25.5 \pm 0.001^\circ\text{C}$ ,  $86.7 \pm 0.08\%$  RH, and a 12L:12D photoperiod, from October to November 2006. Ambient temperature was monitored hourly for the duration of the experiment with a single Onset HOBO data logger. The *O. japonicus* and *O. atropalpus* used in this experiment were harvested from eggs obtained from colonies maintained at the Headlee Research Laboratory Mosquito Research and Control Unit at Rutgers University in New Brunswick, New Jersey. The *O. japonicus* colony originated from larval collections from a horse farm in New Egypt, Ocean County, New Jersey in 2001, while the *O. atropalpus* colony originated from larvae collected in 1993 from Monmouth, Salem, Cumberland, and Burlington Counties, New Jersey (L. McCuiston personal communication).

To investigate inter- and intraspecific larval competition between these two species, the development of larvae in surrogate rock pools in different density – species combinations was monitored. Surrogate rock pools were constructed within plastic planters (91.4 x 18 x 12 cm) using a fast setting, high strength concrete mix consisting of coarse sand aggregate, and cement (Sakrete®). Approximately 15 kg of concrete mixed with 1.6 l water was poured into each of six planters, and within each, five individual indentations (7 x 11.5 cm) were made using glass canisters to create the rock pools. Field surveys in Fairfax County, Virginia indicated that these species occur in rock pools of similar shape and size in nature. Once dry, each planter was completely flooded with water for 24 hours to ensure structural reliability. A randomized complete block design was used for the experiment, with five density combinations of *O.*

*japonicus:O.atropalpus* (20:0, 60:0, 0:20, 0:60, and 30:30) as treatments and individual planters as blocks. Each planter contained one replicate of each density – species composition combination in a separate rock pool, for a total of six replicates per treatment and 30 individual rock pools. Two months prior and up to the start of the experiment, surrogate rock pools were flooded with water to allow for any potentially toxic chemicals to leech out of the cement in an effort to reduce or prevent larval mosquito mortality.

On 19 October, each planter was randomly labeled with a number, and each of its rock pools, was randomly labeled with a letter corresponding to one of the five treatments. Food consisted of fallen pin oak leaves (*Quercus palustris*) that had been collected in Fairfax, VA, washed, and dried at room temperature for one week prior to quartering, weighing, and sorting. Four days prior to the start of the experiment, 1.0 g of leaves was added with 300 ml of distilled water to each of the 30 rock pools. The appropriate water level was marked in each rock pool, and was checked every five days for evaporation and refilled with distilled water as necessary. Each planter was covered with fiberglass screen (0.5 mm) and secured with a large rubber band to prevent entry of other macrofauna. In the laboratory, eggs of *O. japonicus* and *O. atropalpus* were synchronously hatched (Novak and Shroyer 1978), and 24 hours later larvae were counted into aliquots of 20, 30, and 60. Within one hour after counting, the larvae were distributed into appropriate rock pools.

Each rock pool was monitored daily for the presence of pupae, which were collected and housed singly in sealed 10 dram (36.7 ml) vials containing water from their respective rock pool. Each vial was labeled with the appropriate planter and treatment identifier and placed in a rack until adult eclosion. Upon emergence, adults were killed by freezing before scoring by container,

species, sex, and day of emergence. The experiment ended on 26 November when the final adult emerged.

## **Data Analysis**

### **Population Growth Correlates**

To quantify the effects of inter- and intraspecific competition on *O. japonicus* and *O. atropalpus*, the mean survivorship, median development time of males and females, and median body size at adulthood of females were analyzed by one-way ANOVA was used with Tukey's honestly significantly different (HSD) tests performed post-hoc for pairwise comparisons of means in SPSS (SPSS 2002) with species-density treatments as independent variables.

Survivorship was calculated as the proportion of adults that emerged from the initial cohort of first instar larvae. Development time was calculated as the number of days from hatching to adult emergence. Adult body size was estimated from the length of one wing, which was removed from each female and measured under a dissecting microscope with an ocular micrometer (Packer and Corbet 1989). Median rather than mean development time and wing length were calculated for each species and treatment because of the non-normal distributions of these variables within cohorts.

### **Composite Index of Population Performance**

Survivorship, female development time and wing length were used to calculate a composite index of mosquito population performance ( $\lambda'$ ), which is an analog of the finite rate of increase as defined by Juliano (1998):

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

where  $N_0$  is the initial number of females (assumed to be 50% of the cohort),  $A_x$  is the number of females eclosing on day  $x$ ,  $w_x$  is the mean wing length of females eclosing on day  $x$ , and  $f(w_x)$  is a function relating egg production to wing length.  $D$  is the time from adult eclosion to reproduction, taken as 8 days for *O. atropalpus* and 12 days for *O. japonicus* (see below). Values of  $\lambda'$  greater than one indicate that the population is increasing, approximately equal to one that the population is stable, and less than one that the population is decreasing. If no individuals survive to reproduction,  $\lambda'$  equals zero (Léonard and Juliano 1995, Grill and Juliano 1996).

$D$  for both species was determined experimentally under controlled conditions of 26°C and 12L:12D photoperiod in an insectary at the Florida Medical Entomology Laboratory in Vero Beach, Florida. In the laboratory, eggs were hatched by flooding with water, and cohorts of approximately 100 larvae hatching within the same 24-hour period were grouped together and placed in plastic trays within separate 0.028 m<sup>3</sup> (30.5 cm x 30.5 cm x 30.5 cm) cages. Each cohort was fed 100 mg of an artificial diet consisting of one part Brewer's yeast and one part lactalbumen every other day. Beginning two days after emergence, adult females of each cohort of *O. japonicus* were offered a bloodmeal from a restrained chicken placed within the cage daily for one hour. As *O. atropalpus* females can mature their first egg batch without blood (O'Meara and Craig 1970, O'Meara and Krasnick 1970), cohorts of this species were not offered a bloodmeal. Cotton soaked in a 20% sucrose solution was provided as a source of carbohydrates for adults of both species at all times. Upon visual inspection immediately following the

bloodfeeding opportunity, those females that appeared to have fed to repletion were removed from the cage using a mouth aspirator and placed singly in 12-dram plastic vials containing a 2.54 cm by 7.62 cm strip of wet seed germination paper to serve as an oviposition substrate (Steinly et al. 1991). The germination paper was checked daily for the presence of eggs, and was replenished with water if necessary. The date of first oviposition for each female was recorded, from which the average time from adult eclosion to oviposition for *O. japonicus* was calculated to be 12 days, with a range of 4 to 17 days (N = 144), while that for *O. atropalpus* was calculated to be 8 days, with a range of 4 to 17 days (N = 153).

Regressions relating female wing length to fecundity were obtained from Lounibos et al. (unpublished data), who used individuals originating from the same colonies as these experiments, as follows:

*O. japonicus*:

$$f(w_x) = 53.078 w_x - 113.91 \quad (r^2 = 0.319, N = 79, p < 0.001)$$

*O. atropalpus*:

$$f(w_x) = 66.148 w_x - 150.28 \quad (r^2 = 0.526, N = 74, p < 0.001),$$

where  $w_x$  is the wing length in millimeters on day  $x$ .

For analyses of  $\lambda'$  for *O. japonicus*, no transformation yielded data that met assumptions of normality and homogeneous variance. Therefore, randomization ANOVA (Manly 1991, 1997) was used to analyze  $\lambda'$  as a randomized complete block design, with three different density – species composition combinations as treatments and six different planters as blocks. Following ANOVA, pairwise comparisons of all treatment means were conducted using randomization methods (Manly 1991, 1997) at an experimentwise  $\alpha = 0.05$ , using the sequential Bonferroni

method to correct for experimentwise error (Rice 1989). Because all values of  $\lambda'$  for *O. atropalpus* were zero, no comparisons could be performed.

## Results

### Survivorship to Adulthood

Mean survivorship to adulthood of *O. atropalpus* was affected by treatment but that of *O. japonicus* was not (Table 4-1, Figure 4-1). Mean survivorship of *O. atropalpus* varied significantly among treatments ( $F_{2,15} = 3.79, p = 0.047$ ), and was significantly higher in the 20:0 treatment than the 60:0 treatment. Mean survivorship of *O. atropalpus* in interspecific treatments was not significantly different from either intraspecific treatment. With respect to individual density – species composition combination treatments, mean survivorship of *O. atropalpus* was higher than that of *O. japonicus* in all instances, except the 60:0 treatment. There was no significant difference in survivorship between the two species under interspecific conditions ( $F_{1,10} = 1.456, p = 0.225$ ).

### Developmental Time

Median time from hatch to adulthood was not affected by density treatment for either sex of *O. japonicus* (Table 4-1, Figures 4-3, 4-4). Median development time of female ( $F_{2,15} = 8.184, p = 0.004$ ), but not male ( $F_{2,15} = 1.484, p = 0.263$ ), *O. atropalpus* was significantly affected by treatments, and was significantly shorter in the 20:0 treatment than the 60:0 or 30:30 treatments (Table 4-1, Figure 4-1), which were not different from one another. With respect to individual density treatments, median development times of female *O. japonicus* were shorter than those of *O. atropalpus* in all instances, except 20:0 treatments. Median development times of males of *O. atropalpus* were shorter than those of *O. japonicus* in all instances, except the interspecific treatment. There was no significant difference between male ( $F_{1,10} = 0.328, p = 0.581$ ), or

female ( $F_{1,10} = 0.995$ ,  $p = 0.342$ ) development time of *O. japonicus* and *O. atropalpus* under interspecific conditions.

### **Female Wing Length**

Median wing lengths of both *O. japonicus* and *O. atropalpus* females were significantly affected by density treatments ( $F_{2,14} = 18.522$ ,  $p < 0.001$ ;  $F_{2,15} = 39.474$ ,  $p < 0.001$ ). For both species, median wing length was significantly greater for females from the 20:0 treatment (Table 4-1, Figure 4-1).

### **Estimated Finite Rate of Increase ( $\lambda'$ )**

The mean estimated finite rate of increase of *O. japonicus* was significantly affected by density treatments ( $F_{2,14} = 3.87$ ,  $p = 0.058$ ), with  $\lambda'$  less than one for both the 60:0 and 30:30 treatments, indicating population decline. Pairwise comparisons of means showed that  $\lambda'$  of *O. japonicus* from the 20:0 treatment was significantly higher than that from the interspecific treatment; no other comparisons were significant (Table 4-1, Figure 4-1). For *O. atropalpus*,  $\lambda'$  was zero in all instances, indicating that no individuals were able to reproduce autogenously (Table 4-1, Figure 4-1).

## **Discussion**

This experiment indirectly supported predictions that interspecific competition between larvae of *O. japonicus* and *O. atropalpus* contributed to the decline and potential displacement of the latter species in some rock pool communities. However, results indicate that interspecific larval competition was not detectably different from intraspecific competition for either *O. japonicus* or *O. atropalpus*, as there was no significant difference in the mean survivorship, median development time, or median wing length for either species under inter- or intraspecific conditions of the same mosquito density. The mean estimated finite rate of increase,  $\lambda'$ , was zero

in all instances for *O. atropalpus*, indicating that no individuals were able to reproduce autogenously, while that of *O. japonicus* did not significantly change between intra- and interspecific treatments of the same mosquito densities. This suggests better overall population performance for this species than *O. atropalpus* across all experimental treatments. The  $\lambda'$  values for *O. atropalpus* were a direct result of the zero fecundity values resulting from the small size of emergent *O. atropalpus* females from all density – species combination treatments. Considering the autogenous reproduction of *O. atropalpus* (O'Meara and Craig 1970, O'Meara and Krasnick 1970), in which the fecundity of this species depends on nutrient reserves obtained in the larval stage, this result is not surprising. Fecundity of *O. japonicus* also appears to be affected, as the median wing lengths of females from this experiment, which ranged from approximately 2.0 – 2.5 mm, were noticeably smaller than those from the previous field competition experiment with *A. albopictus*, where they were approximately 1 mm longer (Figures 3-4, 4-4).

An attempt was made to simulate the ecological conditions experienced by these species in nature; however the somewhat equivocal results as evidenced by the declining or zero estimated finite rates of increase for both species suggest that perhaps these experimental conditions were excessively stressful. It has been demonstrated that the type and quantity of detritus in a container can influence microorganism populations and communities (Walker et al. 1991, Kaufman et al. 2001), and therefore can influence mosquito population performance (Lounibos et al. 1993, Walker et al. 1997). Furthermore, interspecific differences in feeding behavior are common among container-inhabiting mosquitoes (Yee et al. 2004); larvae may browse on hard surfaces or filter fluid to gather microorganisms (Merritt et al. 1992), and the efficiency of these feeding modes may depend on habitat structure and complexity. The use of increased or multiple levels of food, a different food source, or pulse delivery of food in this experiment may have

provided experimental conditions more comparable to those in nature in which interspecific competition might be expressed. Furthermore, it is possible that the cement from which the rock pools were constructed imposed some toxic effect on the mosquito larvae, although it is important to note that there was no observable difference in the survivorship of *O. japonicus* in this experiment of the previous field competition experiment of this species with *A. albopictus* (Figures 3-1, 4-1).

Although it is evident that experimental conditions were stressful for both species, and thus interspecific interactions between these two species as they occur in nature may have been obscured, it is possible that *O. japonicus* may have a competitive advantage resulting from more efficient feeding behaviors or better resistance to starvation. Although the latter was not directly demonstrated in this experiment in terms of differences in survivorship, it is interesting to note that male *O. atropalpus* on average developed faster than that of *O. japonicus*, but the reverse applied to females, indicating the need for females of the former species to lengthen larval development to accumulate nutrient reserves for egg production. This suggests that the autogenous reproduction of *O. atropalpus* (O'Meara and Craig 1970, O'Meara and Krasnick 1970) may render this species more sensitive to competitive stress. Condition-specific competition, wherein competitive superiority varies with the abiotic environment, is known to occur among various life stages of container-inhabiting mosquitoes (Barrera 1996, Daugherty et al. 2000, Costanzo et al. 2005), and may be an important factor in understanding the competitive outcomes among *O. atropalpus* and *O. japonicus*.

These results emphasize the importance of experimental conditions and suggest that multiple factors other than interspecific larval resource competition may be important in determining the current abundance and distribution of these species. It has been demonstrated

that larvae of *O. japonicus* can survive at low temperatures for extended time periods (Scott 2003), an ability that appears to allow this species to hatch and/or begin development before other mosquitoes in the early spring, and apparently overwinter in the larval as well as the egg stage in temperate areas, even where the surface of larval habitats freeze completely (Kamimura 1976, Scott 2003, B. Harrison personal communication). *Ochlerotatus japonicus* has been found as larvae in every month of the year within its natural range (Nakata 1962) and in North Carolina (B. Harrison personal communication), and in all months except February in New Jersey (Scott 2003). This cold tolerance of *O. japonicus* may confer an ecological advantage in obtaining resources over *O. atropalpus*, which diapauses in the egg stage (Hedeen 1953) and emerges later in the season. Furthermore, it may facilitate asymmetric intraguild predation of newly hatched *O. atropalpus* larvae by fourth instar *O. japonicus*; however the importance of this ecological process in structuring container-inhabiting mosquito communities has yet to be demonstrated in nature (Edgerly et al. 1999).

Predation is a prominent feature of rock pools in North America with predacious diving beetles of the family Dytiscidae playing a significant role in regulating the numbers of *O. atropalpus* (Shaw and Maisey 1961, James 1964a,b). Larvae and adult *Laccophilus maculosus* are the most efficient predators of this rock pool mosquito because of their habit of crawling about on the bottom and sides of the pool where they came in contact with the bottom-feeding larvae of *O. atropalpus* (James 1964b). *Hydra oligactis* will capture *O. atropalpus*, killing but not ingesting young larvae and paralyzing later stages, thereby limiting the abundance of this species (James 1964b). If *O. atropalpus* and *O. japonicus* tend to occupy different spaces within a rock pool (i.e., at the bottom, surface, or in the water column), selective predation (Griswold

and Lounibos 2005, 2006) by these diving beetles may be important in facilitating the invasion of the latter species in these habitats and should be investigated further.

The tendency of *O. atropalpus* larvae to frequently congregate under leaves and other organic debris at the bottom of their rock pools (Hedeon 1953) has been shown to result from a negative phototropic reaction (Beach and Craig 1979). Because fully exposed rock pools tend to be more flood prone, *O. atropalpus* may have adapted this behavior in response to larval mortality associated with such environmental conditions. While this reaction may promote a preference of this species for areas of lesser concentrations of light, under enough selective pressure, this protective mechanism could potentially allow *O. atropalpus* to inhabit rock pools fully exposed to the sun, therefore partitioning, at least to some extent, rock pool habitats with *O. japonicus*, which is less frequently found under such conditions (B. Byrd personal communication, previous chapter).

These experimental findings appear ambiguous with respect to the nature of interspecific larval resource competition between *O. atropalpus* and *O. japonicus* because of the stressful experimental conditions; however, a slight competitive advantage for *O. japonicus* seems likely. Numerous mechanisms for the perceived reduction in numbers of *O. atropalpus* in rock pool communities have been proposed here, however it is important to note that variations in temperature (Lounibos et al. 2002), habitat (Bertness, 1984, Livdahl and Willey 1991), larval density, season (Teng and Apperson 2000), and oviposition attraction and repellency (Maire 1985, Zahiri et al. 1997) may also influence larval competition differently among mosquito species and warrant further research with respect to interactions between these two species. Finally, field observations of co-occurrences of these species, seasonal distributions, habitat preferences, and overwintering behaviors should be made on a large geographical scale, as they

may ultimately influence the community structure of the rock pools in which these species coexist.

Table 4-1. Means ( $\pm$ SE) of population growth correlates for *O. japonicus* and *O. atropalpus*. Means followed by letters that are not commonly shared are significantly different by pairwise comparisons ( $p < 0.05$ ).

Response	Density – species treatments					
	<i>Ochlerotatus japonicus</i>			<i>Ochlerotatus atropalpus</i>		
	20:0	60:0	30:30	20:0	60:0	30:30
Mean survivorship	.53 (0.09)	.575 (0.107)	.317 (0.057)	.575 (0.107)a	.26 (0.049)b	.432 (0.076)ab
Median female development time (d)	13.5 (0.55)	18.25 (1.22)	18.6 (1.22)	12.8 (0.95)a	21.1 (2.35)b	21.7 (1.60)b
Median male development time (d)	12.4 (0.75)	13.5 (0.56)	13.9 (0.64)	11.7 (1.26)	13.3 (0.73)	15.3 (2.18)
Median female wing length (mm)	2.48 (0.018)a	2.02 (0.064)b	2.05 (0.069)b	2.02 (0.022)a	1.65 (0.051)b	1.60 (0.031)b
$\lambda$ ' <sup>a</sup>	1.082 (0.007)a	0.490 (0.22)ab	0.349 (0.22)b	0	0	0

<sup>a</sup>Multiple comparisons of mean  $\lambda$ ' among treatments for *O. japonicus* were conducted using randomization methods at an experimentwise  $\alpha = 0.05$ , using the sequential Bonferroni method.

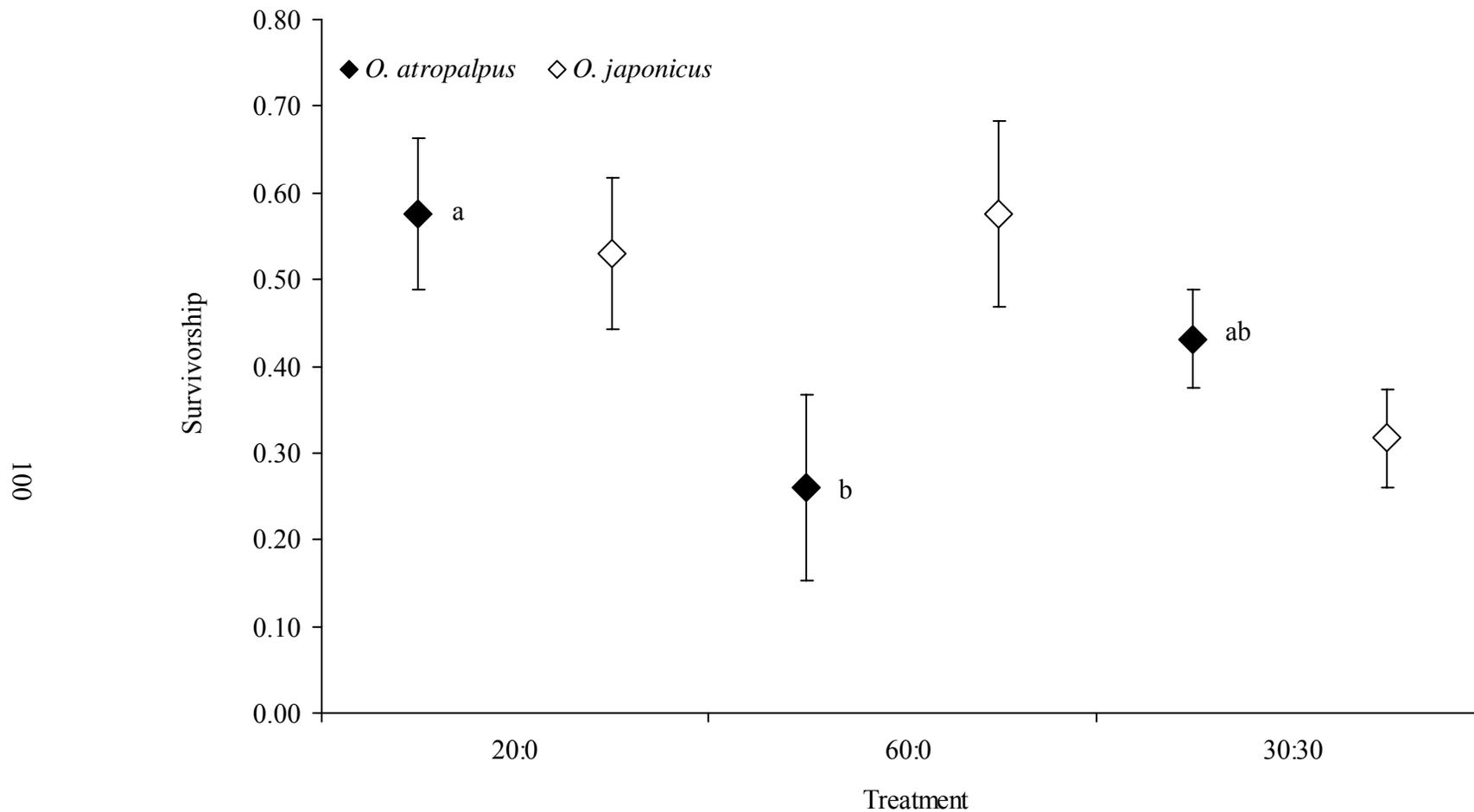


Figure 4-1. Mean survivorship (proportion of the original number of larvae surviving to adulthood) of *O. japonicus* and *O. atropalpus* ( $\pm$ SE). Lower case letters indicate significant differences among competition treatments resulting from pairwise comparisons ( $p < 0.05$ ) for *O. atropalpus*. Analysis of variance did not indicate significant differences in survivorship among treatments for *O. japonicus*.

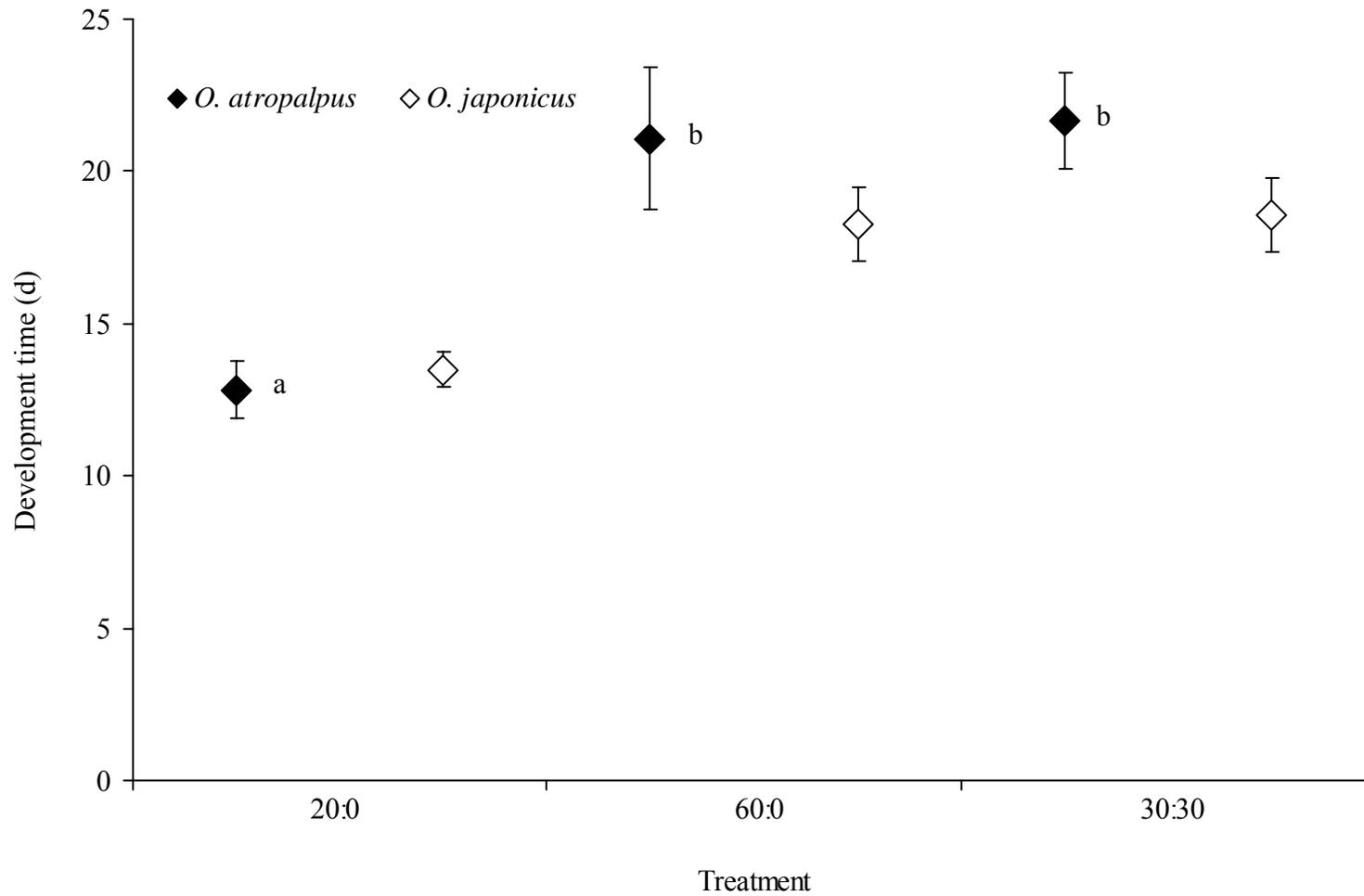


Figure 4-2. Means of median time to adulthood for female *O. japonicus* and *O. atropalpus* ( $\pm$ SE). Lower case letters indicate significant differences among competition treatments resulting from pairwise comparisons ( $p < 0.05$ ) for *O. atropalpus*. Analysis of variance did not indicate a significant variation in survivorship among treatments for *O. japonicus*.

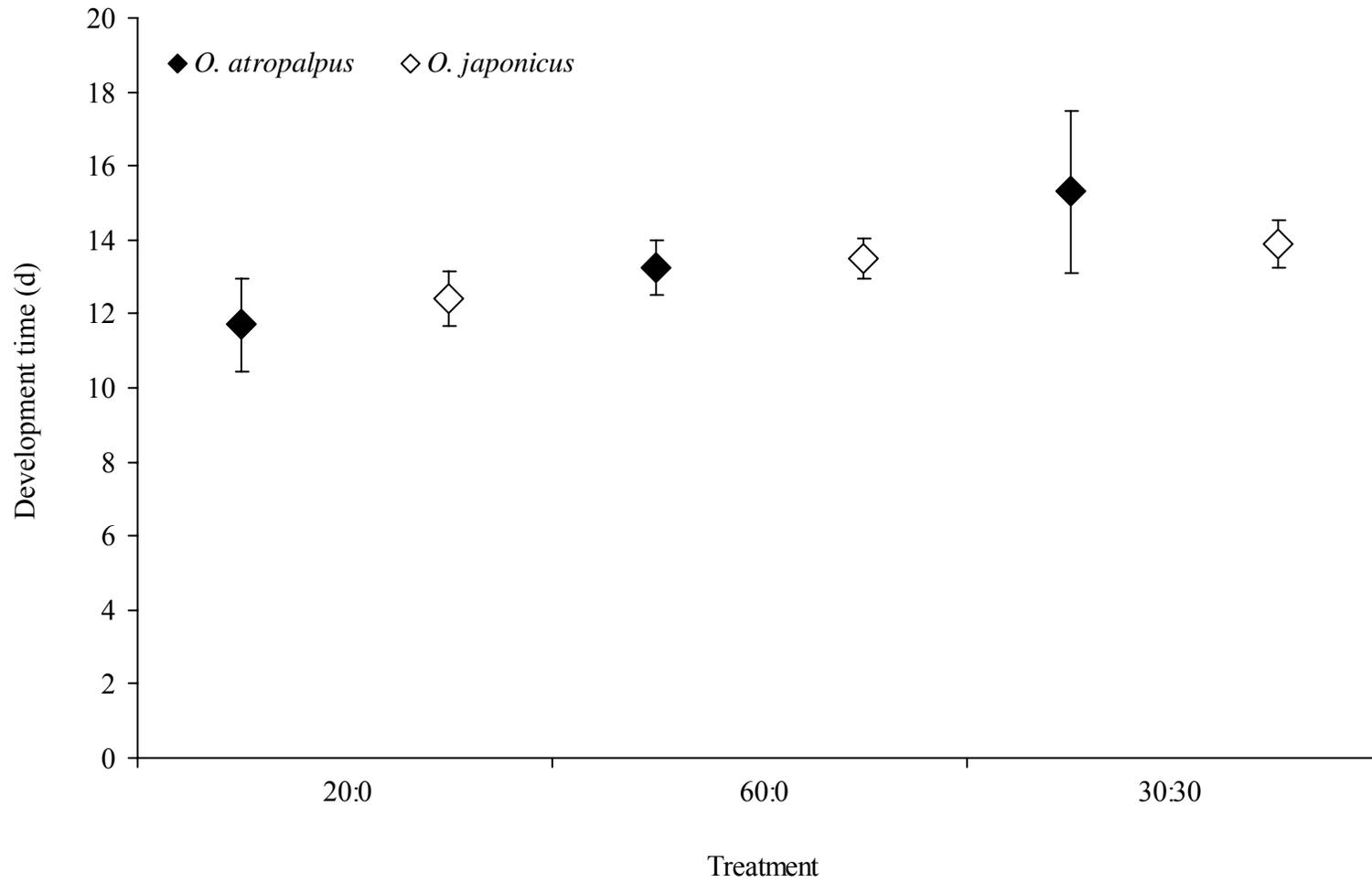


Figure 4-3. Means of median time to adulthood for male *O. japonicus* and *O. atropalpus* ( $\pm$ SE). Analysis of variance did not indicate significant variations in development time among treatments for males of either species.

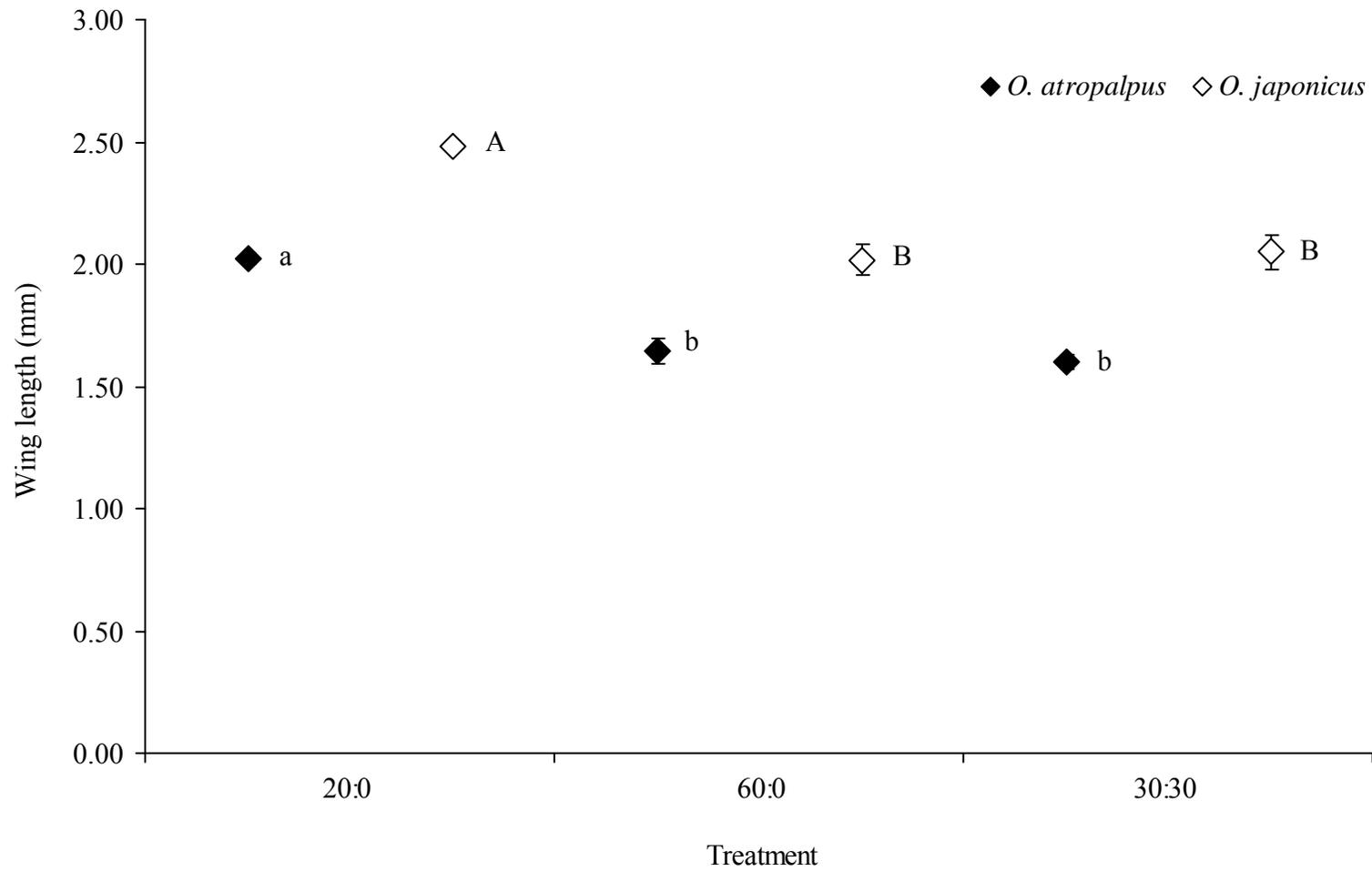


Figure 4-4. Means of median wing lengths of *O. japonicus* and *O. atropalpus* adult females ( $\pm$ SE). Lower case and upper case letters indicate significant differences among competition treatments resulting from pairwise comparisons ( $p < 0.05$ ) for *O. atropalpus* and *O. japonicus*, respectively.

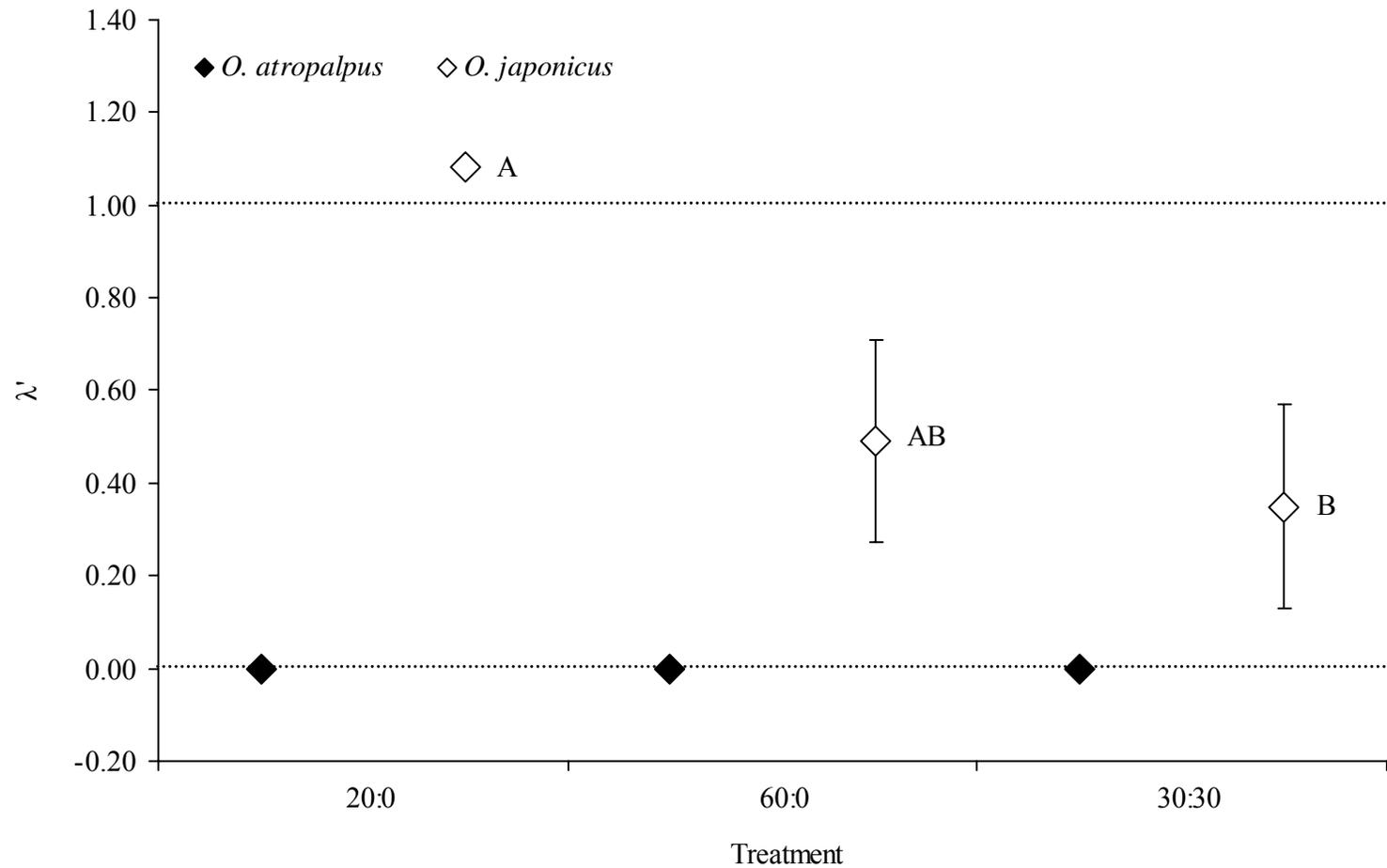


Figure 4-5. Mean estimates of population performance ( $\lambda'$ , an estimate of the finite rate of increase for the cohort) for female *O. japonicus* and *O. atropalpus* adults ( $\pm$ SE). Upper case letters indicate significant differences among competition treatments resulting from pairwise comparisons ( $p < 0.05$ ) for *O. japonicus*. Analysis of variance did not indicate a significant difference in survivorship among treatments for *O. atropalpus*. The line at  $\lambda' = 1$  is where the population is being replaced, neither increasing nor decreasing, and the line at  $\lambda' = 0$  is where no individuals survive to reproductive age.

## CHAPTER 5

### CONCLUSIONS

The establishment of an invasive species in a new region depends on its ability to compete with resident species that occupy a similar ecological niche (Williamson 1996). In mosquito container communities, interactions among invasive and resident species may determine invasion success (O'Meara et al. 1995, Juliano et al. 2004) or serve as a barrier to invasion (Rosen et al. 1976, O'Meara et al. 1989). Invasion success and spread may be associated with forms of competition that result in either coexistence with resident species or their decline and elimination (Juliano 1998). Both types of competitive outcomes were observed during the establishment of *O. japonicus* in Fairfax County, Virginia.

The propensity of *O. japonicus* immatures to inhabit rock pools was associated with, as predicted, what appears to be the localized decline and possible displacement of the native rock pool mosquito, *O. atropalpus*. Investigations on the causes of this decline were impeded by both infrequent collections of *O. atropalpus* from rock pools and stressful conditions used in laboratory experiments to determine the effects of inter- and intraspecific larval competition on the population performance of *O. atropalpus* and *O. japonicus*. Although no strong conclusions could be made, results suggest that interspecific competition between the two species is probable, and likely to favor the success of *O. japonicus* over *O. atropalpus* in the resource-limited conditions expected in rock pool habitats. The autogenous reproduction of *O. atropalpus*, which renders this species more vulnerable to conditions of limited resources because nutrient reserves for egg production must be acquired during development (Telang and Wells 2004), may disadvantage this species in larval competition.

While rock pools were clearly the preferred habitat of *O. japonicus*, this species was frequently collected from various types of artificial containers as well. Unlike the competitive

exclusion of *O. atropalpus* that appears to have resulted from the invasion of *O. japonicus* in rock pools, resident mosquito species, namely *A. albopictus* and *O. triseriatus*, appear to coexist with *O. japonicus* in artificial containers in Fairfax County, Virginia. While *O. triseriatus* may be a superior larval competitor, the infrequent collection of this species from artificial containers suggests that its coexistence with *O. japonicus* in such habitats may be facilitated by species-specific differences in habitat preference, with a suspected preference of the former species for treeholes.

Results of experiments on interactions of *O. japonicus* with *A. albopictus* in artificial containers support the coexistence of these two species in this habitat. Although inter- and intraspecific effects of larval density on the population performance of these two species in a field experiment indicated *A. albopictus* to be the superior competitor, the lack of a significant difference between intra- and interspecific larval resource competition on the population performance of *O. japonicus* suggests that the two species should be able to coexist in artificial container habitats in nature. The superior population performance of *A. albopictus* in the presence of *O. japonicus* compared to the same larval density of conspecifics suggests that intraspecific competition may be more important than interspecific competition for regulating population growth of this species in container habitats in Virginia. This is supported by high larval densities and intraspecific mean crowding of *A. albopictus* observed in the field, which was much higher than the interspecific mean crowding of this species by *O. japonicus*, or by any other co-occurring species in field samples. Because of their potentials as vectors of pathogens, the coexistence of *A. albopictus* and *O. japonicus* may have practical consequences, thus their involvement in arboviral transmission cycles, such as that of LaCrosse encephalitis virus, should be scrutinized further in areas of co-occurrence.

Species-specific differences in seasonality appear to have influenced the interactions of *O. japonicus* with other species in container communities in Fairfax County, Virginia that resulted in the decline of *O. atropalpus* and coexistence of this species with *A. albopictus*. The ability of *O. japonicus* to overwinter in the larval stage allows this species to resume development earlier in the spring, while other container-inhabiting aedine species remain in the egg stage. This difference in life history strategies gives *O. japonicus* a developmental head start over *A. albopictus*, and the consequent co-occurrence of older *O. japonicus* with younger *A. albopictus* larvae may have favored *O. japonicus* during competition between these species, and allowed it to persist later into the season despite the high abundance of *A. albopictus* during this time (Figure 2-3). On the other hand, the co-occurrence of older *O. japonicus* larvae with early hatchlings of *O. atropalpus* has likely exacerbated the asymmetry of interspecific larval resource competition between these two species, which may have contributed to the local decreases in numbers and distribution of the native rock pool mosquito.

The impact of interspecific larval resource competition on *O. japonicus* and *O. atropalpus* in rock pools warrants further investigation, but will require more ecologically realistic experimental conditions than those used here. Future studies should also focus on the role of cohort structure in density-dependent interspecific competition between *O. japonicus* and resident container-inhabiting mosquitoes, as such research would likely provide more insight into the interspecific interactions among these species as they occur in nature. The roles of other ecological processes in structuring mosquito container communities should also be investigated, particularly the prey preferences of predators and the responses of prey species to these predators. Furthermore, the tolerance of these species to varying environmental conditions, particularly temperature, and differences in foraging behaviors should be explored. Finally, to

fully appreciate the ecological processes operating in mosquito container communities, and to observe any significant changes that may occur therein following the invasion of *O. japonicus*, continued monitoring of all life stages of these species over several years would be necessary.

APPENDIX A

DESCRIPTIVE STATISTICS AND INFORMATION: CHAPTER 2

Table A-1. Descriptive statistics and information for mosquito species collected in a survey of natural and artificial container habitats in Fairfax County, Virginia in 2006.

Species	Container types <sup>a</sup>	No. pos. containers <sup>b</sup> (%)	No. of larvae (%)	Mean. no. larvae/container <sup>c</sup>	Mean ( $\pm$ SE) water vol. (l) of pos. containers	Range of water vol. (l) of pos. containers
<i>A. albopictus</i>	B, D, F, P, R, R, RH, V, O	91 (47.6)	4437 (51.0)	48.76 (6.92)	1.37 (0.32)	0.01 – 21.0
<i>A. punctipennis</i>	P, R, T, O	7 (3.7)	17 (0.2)	2.43 (0.84)	5.35 (3.8)	0.06 – 28.0
<i>C. pipiens</i>	B, D, F, P, R, T, V, O	51 (26.7)	1073 (12.3)	21.04 (5.47)	2.20 (0.68)	0.02 – 28.0
<i>C. restuans</i>	F, P, R, T, O	36 (18.8)	1198 (14.3)	33.28 (10.01)	1.99 (0.79)	0.06 – 28.0
<i>O. atropalpus</i>	R	4 (2.0)	74 (0.9)	18.5 (15.26)	2.04 (1.04)	0.45 – 5.0
<i>O. hendersoni</i>	T, TH, V, O	10 (5.24)	50 (0.6)	5.0 (1.6)	0.98 (0.29)	0.15 -2.5
<i>O. japonicus</i>	B, D, F, P, R, T, TH, O	58 (30.4)	1553 (17.9)	26.78 (5.13)	1.93 (0.58)	0.015 – 28.0
<i>O. triseriatus</i>	P, R, T, TH, V, O	17 (8.9)	203 (2.3)	11.94 (4.93)	0.87 (0.23)	0.03 – 2.5
<i>O. signifera</i>	F	1 (0.5)	28 (0.3)	-	0.15	-
<i>T. rutilus</i>	F, T, TH, V, O	5 (2.6)	60 (0.7)	12.0 (10.0)	0.63 (0.47)	0.08 – 2.5

<sup>a</sup>Container types include birdbaths (B), 55-gallon drums (D), flower pot saucers (F), plastic such as tarps or garbage bags (P), rock pools (R), tires (T), treeholes (TH), cemetery vases (V), or other miscellaneous artificial containers (O).

<sup>b</sup>Number of and percent positive containers was determined from a total of 191 containers sampled.

<sup>c</sup>Calculations of mean number of larvae per container were based only on positive containers.

APPENDIX B

HABITAT COMPARISONS: RANK ORDERS OF MOSQUITO SPECIES

Table B-1. Rank orders of immature mosquito abundances used for habitat comparisons of rock pools, tires, small and large artificial containers. Kendall's coefficient of rank correlation,  $\tau$ , was used for comparisons.

	Rock pools		Tires		Small artificial <sup>a</sup>		Large artificial <sup>b</sup>		All artificial	
	No. collected	Rank	No. collected	Rank	No. collected	Rank	No. collected	Rank	No. collected	Rank
<i>A. albopictus</i>	242	2	1190	1	2620	1	1553	1	4174	1
<i>A. punctipennis</i>	14	6	-	-	-	-	3	9	3	9
<i>C. pipiens</i>	169	4	216	3	214	3	590	2	904	3
<i>C. restuans</i>	184	3	488	2	455	2	559	3	1014	2
<i>O. atropalpus</i>	74	5	-	-	-	-	-	-	-	-
<i>O. hendersoni</i>	-	-	22	6	27	6	14	8	41	7
<i>O. japonicus</i>	1296	1	66	5	167	5	81	5	248	4
<i>O. triseriatus</i>	4	7	140	4	58	4	125	4	183	5
<i>O. signifera</i>	-	-	-	-	-	-	28	7	28	8
<i>T. rutilus</i>	-	-	3	7	3	7	3	9	3	9

<sup>a</sup>Small artificial containers include those with less than or equal to 1 l of fluid volume.

<sup>b</sup>Large artificial containers include those with more than 1 l of fluid volume; this included 12 of 29 tires sampled.

## LIST OF REFERENCES

- Aliabadi, B.K. and S.A. Juliano. 2002. Escape form gregarine parasites affects the competitive impact of an invasive mosquito. *Biol. Invasions* 4: 283-297.
- Alto, B.W., L.P. Lounibos, S. Higgs and S.A. Juliano. 2005. Larval competition differentially affects arbovirus infections in *Aedes* mosquitoes. *Ecology* 86: 3279-3288.
- Andreadis, T.G., J.F. Anderson, L.E. Munstermann, R.J. Wolfe and D.A. Florin. 2001. Discovery, distribution, and abundance of the newly introduced mosquito *Ochlerotatus japonicus* (Diptera: Culicidae) in Connecticut, USA. *J. Med. Entomol.* 38: 774-779.
- Apperson, C.H. B.A. Harrison, T.R. Unnasch, H.K. Hassan, W.S. Irby, H.M. Savage, S.E. Aspen, D.W. Watson, L.M. Rueda, B.R. Engber and R.S. Nasci. 2002. Host-feeding habits of *Culex* and other mosquitoes (Diptera: Culicidae) in the borough of Queens in New York City, with characters and techniques for identification of *Culex* mosquitoes. *J. Med. Entomol.* 39: 777-785.
- Barrera, R. 1996. Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. *Ecol. Entomol.* 21: 117-127.
- Beach, R.F. and G.B. Craig. 1979. Photoinhibition of diapause in field populations of *Aedes atropalpus*. *Environ. Entomol.* 8: 392-396.
- Bédhomme, S., and P. Agnew, C. Sidbore and Y. Michalakis. 2005. Pollution by conspecifics as a component of intraspecific competition among *Aedes aegypti* larvae. *Ecol. Entomol.* 30: 1-7.
- Beier, J.C., W.J. Berry and G.B. Craig. 1982. Horizontal distribution of adult *Aedes triseriatus* (Diptera: Culicidae) in relation to habitat structure, oviposition, and other mosquito species. *J. Med. Entomol.* 19: 239-247.
- Bertness, M.D. 1984. Habitat and community modification by an introduced herbivorous snail. *Ecology* 65: 370-381.
- Blackmore, M.S., G.A. Scoles and G.B. Craig. 1995. Parasitism of *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae) by *Ascogregarina* spp. (Apiomplexa: Lecudinidae) in Florida. *J. Med. Entomol.* 32: 847-852.
- Braks, M.A.H., N.A. Honório, L.P. Lounibos, R. Lourenço-de-Oliveira and S.A. Juliano. 2004. Interspecific competition between two invasive species of container mosquitoes in Brazil. *Ann. Entomol. Soc. Am.* 97: 130-139.
- Brooks, J.L. and S.I. Dodson. 1965. Predation, body size, and composition of the plankton. *Science* 150: 28-35.

- Caldwell, N.D., R.R. Gerhardt and C.J. Jones. 2005. First collection of *Ochlerotatus japonicus japonicus* in the state of Tennessee. *J. Am. Mosq. Control Assoc.* 21: 322-324.
- Chagin, K.P. and P.I. Kondratiev. 1943. Vectors of autumnal (Japanese) encephalitis in Soviet Far East and their Control. *Med. Parasit. And Parasitic Dis.* 12: 34-44.
- Christophers, S.R. 1960. *Aedes aegypti, the yellow fever mosquito*. Cambridge University Press, Cambridge., UK. 739 pp.
- Clark, G.G. and G.B. Craig. 1985. Oviposition behavior of *Aedes triseriatus* and *Aedes hendersoni* on the Delmarva Peninsula. *J. Am. Mosq. Control Assoc.* 1: 526-528.
- Coquillett, D.W. 1902. Three new species of *Culex*. *Can. Entomol.* 34: 292-293.
- Costanzo, K.S., K. Mormann and S.A. Juliano. 2005. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *J. Med. Entomol.* 42: 559-572.
- Craven, R.B., D.A. Eliason, D.B. Francy, P. Reiter, E.G. Campos, W.L. Jakob, G.C. Smith, C.J. Bozzik, G.G. Moore, G.O. Maupin and T.P. Monath. 1988. Importation of *Aedes albopictus* and other exotic mosquito species into the United States in used tires from Asia. *J. Am. Mosq. Control Assoc.* 4: 138-142.
- Daehler, C.C. 2001. Two ways to be an invader, but one is more suitable for ecology. *Bull. Ecol. Soc. Am.* 81: 101-2.
- Darsie, R.F. and R.A. Ward. 2005. *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico*. Univ. Press of Florida, Gainesville, FL. USA. 383 pp.
- Daugherty, M.P., B.W. Alto and S.A. Juliano. 2000. Invertebrate carcasses as a resource for competing *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* 37: 364-372.
- Edgerly, J.S. and T.P. Livdahl. 1992. Density-dependent interactions within a complex life cycle: the roles of cohort structure and mode of recruitment. *J. Anim. Ecol.* 61: 139-150.
- Edgerly, J.S., M.S. Willey and T. Livdahl. 1993. The community ecology of *Aedes* hatching: implications for a mosquito invasion. *Ecol. Entomol.* 18: 123-128.
- Edgerly, J.S., M.S. Willey and T. Livdahl. 1999. Intraguild predation among treehole mosquitoes *Aedes albopictus*, *Ae. aegypti*, and *Ae. triseriatus* (Diptera: Culicidae), in laboratory microcosms. *J. Med. Entomol.* 36: 394-399.
- Effler, P.W., L. Pang, P. Kitsutani, V. Vorndam, M. Nakata, T. Ayers, J. Elm, T. Tom, P. Reiter, J.G. Rigau-Perez, J.M. Hayes, K. Mills, M. Napier, G..G. Clark and D.J. Gubler for the

- Hawaii Dengue Outbreak Investigation Team. Dengue Fever, Hawaii, 2001-2002. 2005 *Emerg. Infect. Dis.* 11: 742-749.
- Focks, D.A., S.B. Linda, G.B. Craig, W.A. Hawley and C.B. Pumpuni. 1994. *Aedes albopictus* (Diptera: Culicidae) – a statistical model of the role of temperature, photoperiod, and geography in the induction of egg diapause. *J. Med. Entomol.* 31: 278-286.
- Fonseca, D.M. S. Campbell, W.J. Crans, M. Mogi, I. Miyagi, T. Toma, M. Bullians, T.G. Andreadis, R.L. Berry, B. Pagac, M.R. Sardelis and R.C. Wilkerson. 2001. *Aedes (Finlaya) japonicus* (Diptera: Culicidae), a newly recognized mosquito in the United States: analyses of genetic variation in the United States and putative source populations. *J. Med. Entomol.* 38: 135-146.
- Foss, A.F. and R.G. Dearborn. 2001. *Preliminary faunistic survey of mosquito species (Diptera: Culicidae) with a focus on population densities and potential breeding sites in greater Portland, Maine*. Technical Report No. 42. Maine Department of Conservation, Maine Forest Service, Forest Health and Monitoring Division, Augusta, Maine. 36 pp.
- Fox, G.A. 1993. Failure-time analysis: emergence, flowering, survivorship, and other waiting times. In: S.M. Scheiner and J. Gurevitch (eds.), *Design and Analysis of Ecological Experiments*. pp. 253-289. Chapman and Hall, NY.
- Gallitano, S., L. Blaustein and J. Vonesh. 2006. First occurrence of *Ochlerotatus japonicus* in Missouri. *J. Vector Ecol.* 30: 347-348.
- Gerhardt, R.R., K.L. Gottfried, C.S. Apperson, B.S. Davis, P.C. Erwin, A.B. Smith, N.A. Panella, E.E. Powell and R.S. Nasci. 2001. First isolation of LaCrosse virus from naturally infected *Aedes albopictus*. *Emerg. Infect. Dis.* 7: 807-811.
- Ghent, A.W. 1963. Kendall's "tau" coefficient as an index of similarity in comparisons of plant or animal communities. *Can. Entomol.* 95: 568-575.
- Godsey, M.S., M.S. Blackmore, N.A. Panella, K. Burkhalter, K. Gottfried, L.A. Halsey, R. Rutledge, S.A. Langevin, R. Gates, K.M. Lamonte, A.M. Lambert, R.S. Lanciotti, C.G.M. Blackmore, T. Loyless, L. Stark, R. Oliveri, L. Conti and N. Komar. 2005. West Nile virus epizootiology in the southeastern United States, 2001. *Vector-Borne Zoonot.* 5: 82-89.
- Graham, A.C. and J. Turmel. 2001. Distribution records of Vermont's first introduced mosquito species, *Ochlerotatus japonicus* (Diptera: Culicidae). In: *Proceedings of the 47<sup>th</sup> Annual Meeting of the Northeastern Mosquito Control Association*. December 3-5, 2001, Salem, MA.
- Grill, C.P. and S.A. Juliano. 1996. Predicting species interactions based on behaviour: predation and competition in container-dwelling mosquitoes. *J. Anim. Ecol.* 65: 63-76.

- Griswold, M.W. and L.P. Lounibos. 2005. Does differential predation permit invasive and native mosquito larvae to coexist in Florida? *Ecol. Entomol.* 30: 122-127.
- Griswold, M.W. and L.P. Lounibos. 2006. Predator identity and additive effects in a treehole community. *Ecology* 87: 987-995.
- Gubler, D.J. 1997. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: D.J. Gubler and G. Kuno (eds.), *Dengue and Dengue Hemorrhagic Fever*. pp 1-22. CABI International, NY.
- Haddow, A.J. 1960. Studies on the biting habits and medical importance of East African mosquitoes in the genus *Aedes*. I. Subgenera *Aedimorphus*, *Banksinella* and *Dunnius*. *B. Entomol Res.* 50: 759-779.
- Harrison, B.A., P.B. Whitt, S.E. Cope, G.R. Payne, S.E. Rankin, L.J. Bohn, F.M. Stell and C.J. Neely. 2002. Mosquitoes (Diptera: Culicidae) collected near the Great Dismal Swamp: new state records, notes on certain species, and a revised checklist for Virginia. *Proc. Entomol. Soc. Wash.* 104: 655-662.
- Hawley, W.A. 1988. The biology of *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* 4 (Suppl.): 1-40.
- Hawley, W.A., C.B. Pumpuni, R.H. Brady and G.B. Craig. 1987. *Aedes albopictus* in North America: probable introduction in used tires from Northern Asia. *Science* 236: 1114-1116.
- Heardon, M., C. Skelly and P. Weinstein. 1999. Improving the surveillance of mosquitoes with disease-vector potential in New Zealand. *NA Public Health Rep.* 6: 25-28.
- Hedeen, R.A. 1953. The biology of the mosquito *Aedes atropalpus* Coq. *J. Kansas Entomol. Soc.* 26: 1-10.
- Ho, B.C., K.L. Chan and Y.C. Chan. 1973. The biology and bionomics of *Aedes albopictus* (Skuse). In: Y.C. Chan, K.L. Chan, and B.C. Ho (eds.), *Vector Control in Southeast Asia*. pp. 125-143. Proceedings, 1<sup>st</sup> Southeast Asian Ministers for Education Organization Workshop, Singapore.
- Ho, B.C., A. Ewert and L. Chew. 1989. Interspecific competition among *Aedes aegypti*, *Ae. albopictus*, and *Ae. triseriatus* (Diptera: Culicidae): larval development in mixed cultures. *J. Med. Entomol.* 26: 615-623.
- Holick, J., A. Kyle, W. Ferraro, R.R. Delaney and M. Iwaseczko. 2002. Discovery of *Aedes albopictus* infected with West Nile virus in southeastern Pennsylvania. *J. Am. Mosq. Control Assoc.* 18: 131.
- Holt, R.D. and J.H. Lawton. 1994. The ecological consequences of shared natural enemies. *Ann. Rev. Ecol. Syst.* 25: 495-520.

- Hurlbert, S.H. 1969. A coefficient of interspecific association. *Ecology* 50: 1-9.
- Ibañez-Bernal, S., B. Briseño, J.-P. Mutebi, E. Argot, G. Rodriguez, C. Martinez-Campos, R. Paz, P. de la Fuente-San Roman, R. Tapia-Conyer and A. Flisser. First record in America of *Aedes albopictus* naturally infected with dengue virus during the 1995 outbreak at Reynosa, Mexico. *Med. Vet. Entomol.* 11: 305-309.
- James, H.G. 1964a. Insect and other fauna associated with the rock pool mosquito *Aedes atropalpus* (Coq.). *Mosq. News* 24: 325-329.
- James, H.G. 1964b. Predators of *Aedes atropalpus* (Coq.) (Diptera: Culicidae) and of other mosquitoes breeding in rock pools in Ontario. *Can. J. Zoolog.* 43: 155-159.
- Juliano, S.A. 1998. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? *Ecology* 79: 255-268.
- Juliano, S.A. and L.P. Lounibos. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecol. Lett.* 8: 558-574.
- Juliano, S.A., G.F. O'Meara, J.R. Morrill and M.M. Cutwa. 2002. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. *Oecologia* 130: 458-469.
- Juliano, S.A., L.P. Lounibos and G.F. O'Meara. 2004. A field test for competitive effects of *Aedes albopictus* on *Aedes aegypti* in south Florida: differences between sites of coexistence and exclusion? *Oecologia* 139: 583-593.
- Kamimura, K. 1976. On the Japanese species of the family Culicidae. In: M. Sasa (ed.), *Science of Mosquitoes* (Japanese). pp 150-188. Hokuryukan, Tokyo, Japan.
- Kaufman, M.G., S.N. Bland, M.E. Worthen, E.D. Walker and M.J. Klug. 2001. Bacterial productivity and fungal biomass responses to feeding by larval *Aedes triseriatus* (Say) (Diptera: Culicidae). *J. Med. Entomol.* 38: 711-718.
- Kesavaraju, B. and S.A. Juliano. 2004. Differential behavioral responses to water-borne cues to predation in two container dwelling mosquitoes. *Ann. Entomol. Soc. Am.* 97: 194-201.
- Kitching, R.L. 2000. *Food Webs and Container Habitats: the Natural History and Ecology of Phytotelmata*, Cambridge University Press, NY. 431 pp.
- Kitron, U.D., D.W. Webb and R.J. Novak. 1989. Oviposition behavior of *Aedes triseriatus* (Diptera: Culicidae): prevalence, intensity, and aggregation of eggs in oviposition traps. *J. Med. Entomol.* 26: 462-467.

- Knight, K.L. 1968. Contributions to the mosquito fauna of Southeast Asia. – IV, Species of the subgroup *Chrysolineatus* of Group D, Genus *Aedes*, Subgenus *Finlaya* Theobald. Contrib. Amer. Entomol. Inst. 2(5): 1-45.
- Koenekoop, R.K. and T.P. Livdahl. 1986. Cannibalism among *Aedes triseriatus* larvae. Ecol. Entomol. 11: 111-114.
- Koenraadt, D.J.M and W. Takken. 2003. Cannibalism and predation among larvae of the *Anopheles gambiae* complex. Med. Vet. Entomol. 17: 61-66.
- Koenraadt, C.J.M., S. Majambere, L. Hemerik and W. Takken. 2004. The effects of food and space on the occurrence of cannibalism and predation among larvae of *Anopheles gambiae* s.l. Entomol. Exp. Appl. 112: 125-134.
- Kramer, L.D. and K.A. Bernard. 2001. West Nile virus in the western hemisphere. Curr. Opin. Infect. Dis. 14: 519-525.
- LaCasse, W.J. and S. Yamaguti. 1950. *Mosquito Fauna of Japan and Korea. Parts I and II. Mosquito Survey data on Japan and their application in the control of mosquito-borne diseases*. Off. Surg., HQ 1 Corps APO 301 (Japan).
- Laird, M., L. Calder, R.C. Thornton, R. Syme, P.W. Holder and M. Mogi. 1994. Japanese *Aedes albopictus* among four mosquito species reaching New Zealand in used tires. J. Am. Mosq. Control Assoc. 10: 14-23.
- Linthicum, K.J., V.L. Kramer, M.B. Madon, K. Fujioka and the Surveillance-Control Team. 2003. Introduction and potential establishment of *Aedes albopictus* in California in 2001. J. Am. Mosq. Control Assoc. 19: 301-308.
- Livdahl, T.P. 1982. Competition within and between hatching cohorts of a treehole mosquito. Ecology 63: 125-136.
- Livdahl, T.P. and M.S. Willey. 1991. Prospects for an invasion: competition between *Aedes albopictus* and native *Aedes triseriatus*. Science 253: 189-191.
- Lloyd, M. 1967. Mean Crowding. J. Anim. Ecol. 36: 1-20.
- Lounibos, L.P. 1981. Habitat segregation among African treehole mosquitoes. Ecol. Entomol. 6: 129-154.
- Lounibos, L.P. 2002. Invasions by insect vectors of human disease. Ann. Rev. Entomol. 47: 233-266.
- Lounibos, L.P., N. Nishimura and R.L. Escher. 1993. Fitness of a tree hole mosquito: influences of food type and predation. Oikos 66: 114-118.

- Lounibos, L.P., G.F. O'Meara, R.L. Escher, N. Nishimura, M. Cutwa, T. Nelson, R.E. Campos and S.A. Juliano. 2001. Testing predictions of displacement of native *Aedes* by the invasive Asian tiger mosquito *Aedes albopictus* in Florida, USA. *Biol. Invasions* 3: 151-166.
- Lounibos, L.P., G.F. O'Meara, N. Nishimura, and R.L. Escher. 2003. Interactions with native mosquito larvae regulate the production of *Aedes albopictus* from bromeliads in Florida. *Ecol. Entomol.* 28: 551-558.
- Lounibos, L.P., S. Suárez, Z. Menéndez, N. Nishimura, R.L. Escher, S.M. O'Connell and J.R. Rey. 2002. Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*? *J. Vector Ecol.* 27: 86-95.
- Maire, A. and R. Langis. 1985. Oviposition responses of *Aedes (Ochlerotatus) communis* (Diptera: Culicidae) to larval holding water. *J. Med. Entomol.* 22: 111-112.
- Manly, B.F.J. 1991. *Randomization and Monte Carlo Methods in Biology*. Chapman and Hall, London. 292 pp.
- Manly, B.F.J. 1997. RT: a program for randomization testing. Version 2.1. West Incorporated, Cheyenne, WY.
- Merritt, R.W., R.H. Dadd and E.D. Walker. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Ann. Rev. Entomol.* 37: 349-376.
- Mitchell, C.J., M.L. Niebylski, N. Karabatsos, D. Martin, J.-P. Mutebi, G.B. Craig and M.J. Mahler. 1992. Isolation of eastern equine encephalitis from *Aedes albopictus* in Florida. *Science* 257: 526-527.
- Miyagi, I. 1971. Notes on the *Aedes (Finlaya) chrysolineatus* subgroup in Japan and Korea (Diptera: Culicidae). *Trop Med.* 13: 141-151.
- Moore, C.G. 1999. *Aedes albopictus* in the United States: current status and prospects for further spread. *J. Am. Mosq. Control Assoc.* 15: 221-227.
- Moore, C.G. 2005. Exotic and Invasive Vectors of Public Health Importance in the United States. Database. Arthropod-borne and Infectious Diseases Laboratory and Dept. of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO.
- Munstermann, L.E. and D.M. Wesson. 1990. First record of *Ascogregarina taiwanensis* (Apiomplexa: Lecudinidae) in North American *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* 6: 235-243.
- Nakata, G. 1962. Taxonomical and ecological studies on Japanese mosquitoes. *Sanitary Injurious Insects* 6: 43-173.

- Nasci, R.S., S.G. Hare and F.S. Willis. 1989. Interspecific mating between Louisiana strains of *Aedes albopictus* and *Aedes aegypti* in the field and laboratory. *J. Am. Mosq. Control Assoc.* 5: 416-421.
- Nawrocki, S.J. and W.A. Hawley. 1987. Estimation of the northern limits of distribution of *Aedes albopictus* in North America. *J. Am. Mosq. Control Assoc.* 3: 314-317.
- Novak, R.J. and D.A. Shroyer. 1978. Eggs of *Aedes triseriatus* and *A. hendersoni*: a method to stimulate optimal hatch. *Mosq. News* 38: 515-521.
- Oliver, J., R.G. Means and J.J. Howard. 2003. Geographical distribution of *Ochlerotatus japonicus* in New York State. *J. Am. Mosq. Control Assoc.* 19: 121-124.
- O'Meara, G.F. and G.B. Craig. 1970. Geographical variation in *Aedes atropalpus* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* 63: 1392-1400.
- O'Meara, G.F. and G.J. Krasnick. 1970. Dietary and genetic control of the expression of autogenous reproduction in *Aedes atropalpus* (Coq.) (Diptera: Culicidae). *J. Med. Entomol.* 7: 328-334.
- O'Meara, G.F., L.F. Evans, Jr. and M.L. Womack. 1997. Colonization of rock holes by *Aedes albopictus* in the southeastern United States. *J. Am. Mosq. Control Assoc.* 13: 270-274.
- O'Meara, G.F., V.L. Larson, D.H. Mook and M.D. Latham. 1989. *Aedes bahamensis*: its invasion of south Florida and association with *Aedes aegypti*. *J. Am. Mosq. Control Assoc.* 5: 1-5.
- O'Meara, G.F., L.F. Evans, A.D. Gettman and J.P. Cuda. 1995. Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera: Culicidae) in Florida. *J. Med. Entomol.* 32: 554-562.
- Packer, M.J. and P.S. Corbet. 1989. Size variation and reproductive success of female *Aedes punctor* (Diptera: Culicidae). *Ecol. Entomol.* 1: 297-309.
- Peyton, E.L., S.R. Campbell, T.M. Candeletti, M. Romanowski and W.J. Crans. 1999. *Aedes (Finlaya) japonicus japonicus* (Theobald), a new introduction into the United States. *J. Am. Mosq. Control Assoc.* 15: 238-241.
- Pielou, E.C. 1977. *Mathematical Ecology*. John Wiley and Sons, New York, NY. 385 pp.
- Qualls, W.A. and G.R. Mullen. 2006. Larval survey of tire-breeding mosquitoes in Alabama. *J. Am. Mosq. Control Assoc.* 22: 601-608.
- Rathcke, B.J. 1976. Competition and coexistence within a guild of herbivorous insects. *Ecology* 57: 76-87.

- Reeves, W.K. and J.A. Korecki. 2004. *Ochlerotatus japonicus japonicus* (Theobald) (Diptera: Culicidae), a new invasive mosquito for Georgia and South Carolina. *Proc. Entomol. Soc. Wash.* 106: 233-234.
- Reinert, J.F. 2000 new classification for the composite genus *Aedes* (Diptera: Culicidae: Aedini), elevation of subgenus *Ochlerotatus* to generic rank, reclassification of the other subgenera, and notes on certain subgenera and species. *J. Am. Mosq. Control Assoc.* 16: 175-188.
- Reiter, P. 1983. A portable, battery-powered trap for collection of gravid *Culex* mosquitoes. *Mosq. News* 43: 496-498.
- Reiter, P. and D. Sprenger. 1987. The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitoes. *J. Am. Mosq. Control Assoc.* 3: 494-501.
- Rhymer, J. and D. Simberloff. 1996. Extinction by hybridization and introgression. *Ann. Rev. Ecol. Syst.* 27: 83-109.
- Ribiero, J.M.C. and A. Spielman. 1986. The satyr effect: a model predicting paraptry and species extinction. *Am. Nat.* 128: 513-528.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223-225.
- Richardson, D.M., W.J. Bond, W.R.J. Dean, S.I. Higgins, G.F. Midgely, S.J. Milton, L.W. Powrie, M.C. Rutherford, M.J. Samways and R.E. Schulze. 2000. Invasive alien species and global change: a South African perspective. In: H.A. Mooney and R.J. Hobbs (eds.), *Invasive species in a changing world*. pp. 303-349. Island Press, Washington DC.
- Roppo, M.R., J.L. Lilya, F.A. Maloney and W.J. Sames. 2004. First occurrence of *Ochlerotatus japonicus* in the state of Washington. *J. Am. Mosq. Control Assoc.* 20: 83-84.
- Rosen, L., L.E. Rozeboom, W.C. Reeves, J. Saugrain and D.J. Gubler. 1976. A field trial of competitive displacement of *Aedes polynesiensis* by *Aedes albopictus* on a Pacific atoll. *Am. J. Trop. Med. Hyg.* 25: 906-913.
- Ross, R. 1911. *The Prevention of Malaria*. John Murray, London. 651 pp.
- Ruesink, J.L., I.M. Parker, M.J. Groom and P.M. Kareiva. 1995. Reducing the risks of nonindigenous species introductions. *BioScience* 45: 465-477.
- Sakai, A.K., F.W. Allendorf, J.S. Holt, D.M. Lodge and J. Molofsky. 2001. The population biology of invasive species. *Ann. Rev. Ecol. Syst.* 32: 305-332.
- Sardelis, M.R. and M.J. Turell. 2001. *Ochlerotatus j. japonicus* in Frederick County, Maryland: discovery, distribution, and vector competence for West Nile virus. *J. Am. Mosq. Control Assoc.* 17: 137-141.

- Sardelis, M.R., J.D. Dohm, B. Pagac, R.G. Andre and M.J. Turell. 2002a. Experimental transmission of eastern equine encephalitis virus by *Ochlerotatus j. japonicus* (Diptera: Culicidae). *J. Med. Entomol.* 39: 480-484.
- Sardelis, M.R., M.J. Turell and R.G. Andre. 2002b. Laboratory transmission of LaCrosse virus by *Ochlerotatus j. japonicus* (Diptera: Culicidae). *J. Med. Entomol.* 39: 635-639.
- Sardelis, M.R. M.J. Turell and R.G. Andre. 2003. Experimental transmission of St. Louis encephalitis virus by *Ochlerotatus j. japonicus*. *J. Am. Mosq. Control Assoc.* 19: 159-162.
- SAS Institute Inc. 1989. *SAS/STAT User's Guide*. SAS Institute, Cary, NC.
- Sasa, M. and K. Kamimura. 1971. Index and consideration on taxonomy of the Japanese Mosquitoes. In: M Sasa (ed.), *Progress in Medical Zoology I* (Japanese). p. 1-47 Gakutsusho Shuppankai, Tokyo.
- Sasa, M., Y. Nakahara, N. Ushiroku, H. Hashimoto, A. Uno, T. Ogino, T. Miyachi, F. Yokomizo, S. Koyama, A. Akagi, K. Yamaguchi, C. Saito and H. Kumazawa. 1947. Studies on mosquitoes (7). Species of lowlands and mountains, observations in the Okayama District. *Med. Biol. (Japanese)* 11: 152-154.
- Savignac, R., C. Back and J. Bourassa. 2002. Biological Notes on *Ochlerotatus japonicus* and other mosquito species new to Quebec. Abstract. In: *The Abstract Book of A Joint Meeting: 68<sup>th</sup> Annual Meeting of the American Mosquito Control Association and the West Central Mosquito & Vector Control Association*. pp 21-22. February 16-21, 2002, Denver, CO. American Mosquito Control Association, Eatontown, NJ.
- Schaffner, F., S. Chouin and J. Guilloteau. 2003. First record of *Ochlerotatus (Finlaya) japonicus japonicus* (Theobald, 1901) in metropolitan France. *J. Am. Mosq. Control Assoc.* 19: 1-5.
- Scott, J.J. 2003. The ecology of the exotic mosquito *Ochlerotatus (Finlaya) japonicus japonicus* (Theobald 1901) (Diptera: Culicidae) and an examination of its role in the West Nile virus cycle in New Jersey. Ph.D. Dissertation. Rutgers, The State University of New Jersey, New Brunswick, NJ.
- Scott, J.J. and W.J. Crans. 2003. Expanded Polystyrene (EPS) floats for *Ochlerotatus japonicus* (Theobald) surveillance. *J. Am. Mosq. Control Assoc.* 19: 376-381.
- Scott, J.J., F.L. Carle and W.J. Crans. 2001a. *Ochlerotatus japonicus* collected from natural rockpools in New Jersey. *J. Am. Mosq. Control Assoc.* 17: 91-92.
- Scott, J.J., S.C. Crans and W.J. Crans. 2001b. Use of an infusion-baited gravid trap to collect adult *Ochlerotatus japonicus*. *J. Am. Mosq. Control Assoc.* 17: 142-143.

- Shaw, F.R. and S.A. Maisey. 1961. The biology and distribution of the rockpool mosquito, *Aedes atropalpus* (Coq.). Mosq. News 24: 12-16.
- Scholl, P.J. and G.R. DeFoliart. 1977. *Aedes triseriatus* and *Aedes hendersoni*: vertical and temporal distribution measured by oviposition. Environ. Entomol. 6: 355-38.
- Simberloff, D. 1996. Impacts of introduced species in the United States. Consequences: Nat. Implic. Environ. Change 2: 13-22.
- Sinsko, M.U. and P.R. Grimstad. 1977. Habitat separation by differential vertical oviposition of two treehole *Aedes* in Indiana. Environ. Entomol. 6: 485-487.
- Slaff, M. and C.S. Apperson. 1989. A key to the mosquitoes of North Carolina and the Mid-Atlantic states. N. C. State Univ. Agric. Ext. Serv. Publ. AG. 412: 1-38.
- Sokal, R.R. and F.J. Rohlf. 1981 *Biometry*, 2<sup>nd</sup> ed. W.H. Freeman and Company, San Francisco, CA. 859 pp.
- Soper, F.L. and D.B. Wilson. 1943. *Anopheles gambiae* in Brazil 1930 – 1940. The Rockefeller Foundation, NY. 262 pp.
- Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas, USA. J. Am. Mosq. Control Assoc. 2: 217-219.
- SPSS or Macintosh, Rel. 11.0 2002. SPSS Inc., Chicago, IL.
- Steinly, B.A., R.J. Novak and D.W. Webb. 1991. A new method for monitoring mosquito oviposition in artificial and natural containers. J. Am. Mosq. Control Assoc. 7: 649-650.
- Sucharit, S., K. Surathin and S.R. Shrestha. 1989. Vectors of Japanese encephalitis virus (JEV): species complexes of the vectors. Southeast Asian J. Trop. Med. Public Health 20: 611-621.
- Sucharit, S., W. Tumrasvin, S. Vutikes and S. Viraboonchai. 1978. Interaction between larvae of *Aedes aegypti* and *Aedes albopictus* in mixed experimental populations. Southeast Asian J. Trop. Med. Publ. Health 9: 93-97.
- Sunahara, T. and M. Mogi. 2002. Priority effects of bamboo-stump mosquito larvae: influences of water exchange and leaf litter input. Ecol. Entomol. 27: 346-354.
- Tabachnick, W.J. 1991. Evolutionary genetics and arthropod-borne disease. The Yellow fever mosquito. Am. Entomol. 37: 14-24.
- Takashima, I. and N. Hashimoto. 1985. Getah virus in several species of mosquitoes. Trans. R. Soc. Trop. Med. Hyg. 79: 546-550.
- Takashima, I. and L. Rosen. 1989. Horizontal and vertical transmission of Japanese encephalitis virus by *Aedes japonicus* (Diptera: Culicidae). J. Med. Entomol. 26: 454-458.

- Tanaka, K., K. Mizusawa and E.S. Saugstad. 1979. A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). *Contrib. Am. Entomol. Inst.* 16(ii-vii): 1-987.
- Telang, A. and M.A. Wells. 2004. The effect of larval and adult nutrition on successful autogenous egg production by a mosquito. *J. Insect Phys.* 50: 677-685.
- Teng, H.J. and C.S. Apperson. 2000. Development and survival of immature *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) in the laboratory: effects of density, food, and competition on response to temperatures. *J. Med. Entomol.* 37: 40-52.
- Theobald, F.V. 1901. *A Monograph of the Culicidae of the World. Vol. I.* British Museum of Natural History, London.
- Thielman, A. and F.F. Hunter. 2006. Establishment of *Ochlerotatus japonicus* (Diptera: Culicidae) in Ontario, Canada. *J. Med. Entomol.* 43: 138-142.
- Trpis, M. 1970. A new bleaching and decalcifying method for general use in zoology. *Can. J. Zool.* 48: 892-893.
- Tsai, T.W. and J.C. Lien. 1950. A new species of *Aedes* (*Finlaya*) found in Taiwan. *J. Med. Assn. Formosa* 49: 177-183.
- Turell, M.J., M.L. O'Guinn, D.J. Dohm and J.W. Jones. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *J. Med. Entomol.* 38: 130-134.
- Walker, E.D., D.L. Lawson, R.W. Merritt, W.T. Morgan and M.J. Klug. 1991. Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. *Ecology* 72: 1529-1546.
- Walker, E.D., M.G. Kaufman, M.P. Ayres, M.H. Riedel and R.W. Merritt. 1997. Effects of variation in quality of leaf detritus on growth of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae). *Can. J. Zool.* 75: 706-718.
- Werner, B.G. 2001. Arbovirus Surveillance and Testing in Massachusetts, 2001. In: *Proceedings of the 47<sup>th</sup> Annual Meeting of the Northeastern Mosquito Control Association*. December 3-5, 2001, Salem, MA.
- White, D.J., L.D. Kramer, P.B. Backenson, G. Lukacik, G. Johnson, J. Oliver, J.J. Howard, R.G. Means, M. Eidson, I. Gotham, V. Kulasekerea, S. Campbell, the Arbovirus Research Laboratory and the Statewide West Nile Virus Response Teams. 2001. Mosquito surveillance and polymerase chain reaction detection of West Nile virus, New York State. *Emerg. Infect. Dis.* 7: 643-649.

- Widdel, A.K., L.J. McCuiston, W.J. Crans, L.D. Kramer and D.M. Fonseca. 2005. Finding needles in the haystack: single copy microsatellite loci for *Aedes japonicus* (Diptera: Culicidae). *Am. J. Trop. Med. Hyg.* 73: 744-748.
- Williams, C.B. 1937. The use of logarithms in the interpretation of certain entomological problems. *Ann. Appl. Biol.* 24: 404-414.
- Williamson, M. 1996. *Biological Invasions*. Chapman and Hall, New York, NY. 256 pp.
- Yee, D.A., B. Kesavaraju and S.A. Juliano. 2004. Interspecific differences in feeding behavior and survival under food-limited conditions for larval *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* 97: 720-728.
- Young, C.L.E., J.A. Beery, R.E. Sheffer and K.M. Rand. 2004. First record of *Ochlerotatus japonicus* (Diptera: Culicidae) in St. Joseph County, Indiana. *Great Lakes Entomologist* 37: 196-197.
- Zahiri, N., M.E. Rau and D.J. Lewis. 1997. Oviposition responses of *Aedes aegypti* and *Ae. atropalpus* (Diptera: Culicidae) females to waters from conspecific and heterospecific normal larvae and from larvae infected with *Plagiorchis elegans* (Trematoda: Plagiorchiidae). *J. Med. Entomol.* 34: 565-568.

## BIOGRAPHICAL SKETCH

Jennifer Star Armistead was born in Cocoa Beach, Florida, the second of three children. As the child of a military officer, she spent her childhood in various parts of the United States until her father retired and settled in Arlington, Virginia. Shortly after, Jennifer began her undergraduate degree at George Mason University where she studied biology. During this time, she also developed a passion for entomology, which led her to study the population ecology of butterflies on Andros Island, the Bahamas for her senior research project. Shortly after graduation, Jennifer was married to her now-husband of nearly five years, Paul Johnson. She works as a biologist for the Disease Carrying Insects Program of the Fairfax County Department of Health in Virginia, where she was involved in the surveillance and management of mosquitoes, ticks, and the diseases these arthropods vector. It was from her experiences in this position that she decided to pursue a master of science degree in entomology at the University of Florida. As a distance education student, Jennifer was able to continue her employment while taking Web-based courses and conducting her thesis research in Virginia. Her graduate studies have emphasized medical entomology, vector ecology, and invasion biology. Upon her graduation, Jennifer will matriculate at the Bloomberg School of Public Health at the Johns Hopkins University in Baltimore, Maryland to complete her graduate studies in the field of public health with a focus on vector-borne diseases.