LARVAL MOSQUITOES IN AGRICULTURAL WETLANDS OF THE GULF COASTAL PLAIN OF GEORGIA, U.S.A.: INVESTIGATING THE EFFECTS OF SURROUNDING LAND USE, NUTRIENT ENRICHMENT, AND FOOD RESOURCES

by

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(Under the Direction of Alan Covich and Stephen Golladay)

ABSTRACT

The expansion of mosquito-borne disease outbreaks has increased the need to determine how altered wetland conditions influence mosquito ecology. This research investigated the influence of selected landscape and environmental habitat variables, nutrient enrichment, and food resources on immature mosquito abundance, diversity, and species composition in agricultural wetlands of the Gulf Coastal Plain of Georgia, U.S.A. Results indicated that *Anopheles quadrimaculatus*, *Culex erraticus*, and *Psorophora columbiae* were indicator species of agricultural wetlands (LDI > 2.0), whereas *Anopheles crucians* and *Culex territans* were indicators of forested reference wetlands (LDI < 2.0). Nutrient enrichment influenced immature mosquito abundance, assemblage composition, and supported mosquitoes of medical concern. Wetland surveys and experimental results provide information on the value of using mosquitoes as bioindicators of wetland condition. Larval mosquitoes and potential food resources in agricultural wetlands had greater $\delta^{15}N$ stable isotope values compared with cypress-gum swamps.

INDEX WORDS: larval mosquito assemblages, agricultural wetlands, surrounding land use, nutrient enrichment, larval mosquito diet, stable isotope, bioindicators

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TABLE OF CONTENTS

CHAPTER 1

LITERATURE REVIEW AND SUMMARY OF OBJECTIVES

INTRODUCTION

In addition to their nuisance feeding behavior, adult mosquitoes in Georgia and other parts of the southeastern U.S.A. serve as vectors of important human and livestock pathogens, including West Nile virus (WNV), Eastern Equine encephalitis virus (EEEV), St. Louis encephalitis virus (SLEV), and LaCrosse encephalitis virus (LACV) (Lance-Parker, Rebmann et al. 2002; Buckner, Blackmore et al. 2011). Human land use has been linked to changes in mosquito community composition (Leisnham, Lester et al. 2004; Muturi, Shililu et al. 2006; Reiter and Lapointe 2007; Johnson, Gómez et al. 2008) and concurrent changes in disease transmission patterns (Patz, Graczyk et al. 2000; Norris 2004; DeGroote, Sugumaran et al. 2008). For example, human WNV incidence was strongly associated with rural agricultural and row crop settings in Iowa (DeGroote, Sugumaran et al. 2008). The suitability of a mosquito breeding site depends on complex interactions of abiotic factors such as precipitation, temperature, humidity, and soil moisture (Buckner, Blackmore et al. 2011), in addition to biotic factors such as competition (Reiskind and Wilson 2008), predation (Chase and Shulman 2009), and food availability (Blaustein and Kotler 1993; Walker, Merritt et al. 1997; Palik, Batzer et al. 2006; Yee and Juliano 2006). Altering landscapes through agricultural development can change the physical, chemical, and biological characteristics of larval mosquito habitats, in turn, affecting oviposition and larval survival (Norris 2004; Leisnham, Slaney et al. 2005; Reiter and Lapointe 2007).

MOSQUITOES IN GEORGIA: HISTORICAL SURVEILLANCE AND SPECIES OF CONCERN

Historically, mosquito surveillance in Georgia was conducted as a result of the prevalence of endemic malaria in southern Georgia (Womack 1997). Researchers at the Emory University Field Station in Baker County, Georgia contributed to the scientific literature on the *Anopheline* vectors of malaria and recorded distribution and ecological information on mosquito species in the state (Love and Goodwin 1959; Womack 1997). The introduction of the Asian tiger mosquito, *Aedes albopictus,* to Georgia in 1986 led to intensive surveys from 1991-1994 (Womack 1997; Smith and Floore 2001). *Aedes albopictus* is a species of concern because it is a major viral vector in southeast Asia and a potential disease vector of Chikungunya fever (Tsetsarkin, Vanlandingham et al. 2007).

In addition to the Asian tiger mosquito, two other introduced mosquito species have been reported in Georgia. *Ochlerotatus japonicus,* also endemic to Asia, has been collected in several counties since 2002 (Gray, Harrison et al. 2005). The occurrence of *Ochlerotatus japonicus* in Georgia is a concern because laboratory studies suggest it as a potential vector for WNV (Gray, Harrison et al. 2005). *Culex coronator*, which has a wide geographic distribution, including the American tropics and the U.S.A. (Arizona, New Mexico, and Texas), has recently expanded its range to the Gulf Coastal Plain of Georgia (Kelly, Mead et al. 2008). WNV and SLEV have been detected in this species (Mackay, Roy et al. 2008), but the ability of this species to transmit arboviruses in areas of recent range expansion remains undocumented (Kelly, Mead et al. 2008). Previous studies have linked *Culex coronator* with agricultural wetlands in southwestern Georgia (Buckner, Blackmore et al. 2011).

Several mosquito-borne viruses known to infect humans circulate in Georgia, including WNV, EEEV, SLEV, and LACV. Each of these viruses has a different transmission cycle, different vectors, and different environmental associations. The primary vector of WNV is *Culex quinquefasciatus*, which occurs throughout the state and is commonly associated with water rich in organic matter such as sewage treatment ponds and polluted ditches (Chaves, Keogh et al. 2009). EEEV is transferred within bird populations by *Culiseta melanura*, but humans and horses are infected by bridge vectors such as *Aedes*, *Ochlerotatus*, *Coquillettidia*, and *Culex* spp. (Harvala, Bremner et al. 2009). The primary mosquito vectors of SLEV are *Culex tarsalis*, *Culex quinquefasciatus*, and *Culex nigripalpus*. *Ochlerotatus triseriatus* is the primary vector of LACV virus and the major virus reservoirs are chipmunks and squirrels (Leisnham and Juliano 2012).

LAND USE AND HABITAT FACTORS ASSOCIATED WITH LARVAL MOSQUITO DISTRIBUTION AND ABUNDANCE

The Gulf Coastal Plain was historically dominated by longleaf pine forest, which was sustained by recurrent fires ignited by lightning and Native Americans (Kirkman, Mitchell et al. 2007). Since European settlement, the Gulf Coastal Plain landscape has been continually altered through fire suppression, timber harvest, and agricultural land conversion. Alteration of wetlands for agricultural use has been the primary driver of land cover change and wetland loss in the U.S.A. and around the world (Turner, Gardner et al. 2001). Isolated wetlands are generally small in size (Martin 2010) and experience periodic dry downs, which make them particularly vulnerable to human disturbance. Wetland loss because of agricultural drainage has reduced a variety of ecosystem services; including habitat provision for wetland-dependent species, hydrologic and nutrient retention, and flood storage and water quality functions (Tiner 2003; Blann, Anderson et al. 2009).

Agriculture is an important part of Georgia's economy and is the dominant economic activity in rural areas of southwestern Georgia. Since the 1970s, mechanized agricultural practices such as tilling, leveling of agricultural fields, and in particular, center-pivot irrigation, have shaped the landscape and hydrology of the region. This intensive land use and associated runoff alters the water chemistry of adjacent isolated wetlands, resulting in elevated pH levels, higher nitrate (NO_3-N), phosphate (PO_4-P), ammonium nitrogen (NH_4-N), and suspended sediments compared with reference wetlands (Battle, Golladay et al. 2001; Atkinson, Golladay et al. 2011). Immature mosquito (larvae and pupae) abundance is known to be greater in constructed water treatment wetlands enriched with NH_4-N (Sanford, Chan et al. 2005), and positively associated with dissolved nitrates and phosphates in natural wetlands (Mercer, Sheeley et al. 2005) Nutrient-rich conditions may favor mosquito species that serve as disease vectors (Chaves, Keogh et al. 2009).

Oviposition is an important stage in the mosquito life cycle, and females of different species exploit diverse habitats that increase the growth and survival of their offspring. Identifying individual factors influencing oviposition preference is difficult because of natural variability; but mesocosm studies have proved useful. For example, research examining nutrient addition and shade treatments found that mosquito productivity was highest in treatments receiving full sunlight and having medium detrital loads (5 g sheep manure/L of water) (Leisnham, Lester et al. 2004). Several studies indicated that mosquitoes preferentially select oviposition habitats that ensure a reliable food resource. For example, *Anopheles pseudopunctipennis* oviposited exclusively in containers treated with filamentous algae, and algae represented 47% of the gut contents of the larval mosquitoes (Bond, Arredondo-Jiménez et al. 2005). In Israel, *Culiseta longiareolata* oviposition rates were four times greater in artificial pools treated with extra food (ground fish and mouse chow), compared with unsupplemented pools (Blaustein and Kotler 1993). Results from constructed water treatment wetlands in California showed that adult mosquito production was nine times greater in wetlands enriched with NH_4-N, compared with unenriched wetlands (Sanford, Chan et al. 2005). Another mesocosm experiment demonstrated that female *Culex restuans*, a vector of WNV among bird populations, preferred nutrient-enriched containers (Reiskind and Wilson 2004). Similarly, the production of *Culex tarsalis*, another important vector of WNV, increased in California rice fields that were nutrient enriched from the incorporation of straw (Lawler and Dritz 2005).

Understanding the landscape distribution and ecological requirements of larval mosquito populations is important for managing potential breeding habitats and also for predicting vector-borne disease prevalence (Reiter and Lapointe 2007; DeGroote, Sugumaran et al. 2008). Several studies have examined the relationships between agricultural landscape level factors and the abundance and distribution of mosquito assemblages. For example, agriculture (e.g., cattle ranching, farming, and horticulture) and forest fragmentation increased the probability of capturing adult *Culex quinquefasciatus* in a mixed residential-agricultural landscape in Hawai'i (Reiter and Lapointe 2007). Similarly, pasture and urban lands in temperate, lowland areas had higher larval mosquito densities compared with native forests (Leisnham, Slaney et al. 2005). In Mwea, Kenya, adult mosquitoes that vector important arboviruses were more abundant, but species diversity was lower in rice agroecosystems compared with non-irrigated agroecosystems (Muturi, Shililu et al. 2006). In the Gulf Coastal Plain of Georgia, *Coquillettidia perturbans* and *Psorophora ferox* were associated with natural land cover such as wetlands and forested land, whereas *Aedes albopictus*, *Culex coronator*, *Culex quinquefasciatus*, and *Culex salinarius* were associated with sites that had the greatest human activity (Buckner, Blackmore et al. 2011).

Physical habitat features are also predictive of wetland mosquito species assemblages. For example, larval habitats dominated by filamentous algae were positively associated with *Anopheles* spp. in northern Belize (Rejmankova, Roberts et al. 1993). In California wetlands dominated by *Myrophyllum aquaticum*, oviposition rates of *Anopheles* females increased as stem density approached 2000 stems m^{-2} (Orr and Resh 1992). Similarly, the number of emerged *Culex erythrothorax* was positively correlated with the density of emergent vegetation and nearly absent from open water California wetland areas (Workman and Walton 2000).

The key landscape and environmental habitat variables that determine larval mosquito assemblages in isolated wetlands of the Gulf Coastal Plain remain largely undocumented. Previous studies have examined immature mosquitoes in rice and cattle agroecosystems (McLaughlin, Vidrine et al. 1987; Lawler and Dritz 2005; Leisnham, Slaney et al. 2005; Muturi, Shililu et al. 2006) and adult mosquitoes in row crop agricultural areas (Buckner, Blackmore et al. 2011). This is the first regional study to investigate the relationships between immature mosquitoes and landscape and environmental habitat characteristics in row crop agricultural wetlands during wet and dry hydrologic regimes.

LARVAL MOSQUITO DIETS IN ISOLATED WETLANDS

Invertebrate communities and food webs vary between forested and marsh wetland habitats (Battle and Golladay 2001; Taylor and Batzer 2010), and may be influenced by differences in the availability and quality of food resources (e.g., plant species composition, detritus, and algae). In the Gulf Coastal Plain, forested cypress-gum swamps typically have longer hydroperiods and large detrital inputs that reduce dissolved oxygen concentrations and lower aquatic invertebrate diversity (Battle and Golladay 2001). Grass-sedge marshes dominated by herbaceous vegetation have greater diversity and density of aquatic invertebrates, which is partially attributed to the availability of both macrophyte detritus and periphyton food resources (Battle and Golladay 2001). In contrast, agricultural wetlands in the region have fewer invertebrate species (Battle, Golladay et al. 2001). Previous studies indicated that wetland habitat type was important in determining larval mosquito diets. For example, *Anopheles quadrimaculatus* larvae consumed more water mites and rotifers in open water habitats compared with vegetated zones (Wallace and Merritt 2004). Different sources and types of detritus can influence oviposition preferences as well as larval mosquito survival and growth (Palik, Batzer et al. 2006; Yee and Juliano 2006; Reiskind, Greene et al. 2009; Ponnusamy, Xu et al. 2010; Winters and Yee 2012).

Understanding the relationship between larval mosquitoes and their food source and quality is essential to understanding the role and species composition of mosquitoes in wetland food webs. Larval mosquitoes are filter-feeders, collector-gatherers, and grazers with diets consisting of organic detritus and microorganisms, such as bacteria and algae. A variety of detrital food resources are suspended in the water column or associated with benthic substrates (Merritt, Craig et al. 1996). Larval *Anopheles*, *Culex*, and *Culiseta*, are considered filter-feeders, extracting suspended fine particulate organic matter (FPOM; size range 0.45 µm to 1 mm) in the water column (*Culex*) or at the air-water interface (*Anopheles*); whereas *Aedes* and *Psorophora* are considered collector-gatherers, feeding in both the water column and on sediments (Merritt, Dadd et al. 1992; Winters and Yee 2012). Knowledge of larval mosquito feeding behavior and diets have important implications for larval abundance, survival, and subsequent disease transmission (Yee, Kesavaraju et al. 2004; Yee and Juliano 2006; Winters and Yee 2012). For example, laboratory studies indicated that decreased food availability led to lower survival and reduced adult biomass of *Culex quinquefasciatus* and *Culex tarsalis* (Peck and Walton 2005). Similarly, containers with some animal detritus versus leaf-only containers produced larger *Aedes albopictus* and *Culex restuans* adults that had higher survival rates (Winters and Yee 2012). The body size of a female mosquito is important, as it can influence fecundity (Ameneshewa and Service 1996; Blackmore and Lord 2000) and vector potential (Hawley 1985; Paulson and Hawley 1991; Nasci and Mitchell 1994; Alto, Reiskind et al. 2008).

Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) is a powerful tool that has been used to identify food resources contributing to higher trophic levels in wetlands (Blaustein, Kiflawi et al. 2004; Opsahl, Golladay et al. 2010; Taylor and Batzer 2010). Primary food resources have consistent differences in the ratio of $\delta^{13}C$ / $\delta^{12}C$, derived from unique isotopic signatures in C_3 and C_4 plants, so that herbivore food preference is reflected in a consumer's tissue (Batzer and Wissinger 1996). Consumers with a diverse food base can be matched to their food supply using a mixing model (e.g., U.S. Environmental Protection Agency's IsoSource), which determines the proportion of diet derived from distinct food sources (Taylor and Batzer 2010). Mixing models indicated that invertebrates in forested wetlands primarily consume detritus from trees, epiphyton, detrital FPOM, sediment, and macrophyte detritus; whereas, algae, sediment, and macrophyte detritus were the primary food resources for invertebrates in marshes (Taylor and Batzer 2010). In Gulf Coastal Plain marshes, C and N isotope analyses suggested that filter-feeders, such as fairy shrimp and ostracods, used phytoplankton as important food resources (Opsahl, Golladay et al. 2010). Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) has also been used to show that plant detritus contributed up to 80% of mosquito pupal biomass in tree-hole ecosystems, while no more than 30% of tissue was animal detritus (Kaufman, Pelz-Stelinski et al. 2010). Similarly, container experiments indicated that *Aedes albopictus* had higher $\delta^{15}N$ values compared with *Culex restuans*, which is likely the result of higher feeding rates and the direct consumption of detritus-derived microorganisms by browsing feeding behavior (Winters and Yee 2012). Knowledge of larval mosquito feeding behavior and diets have important implications for the success of mosquito biological control agents, such as *Bascillus thuringiensis* (Bt), that must be ingested (Merritt, Craig et al. 1996).

RESEARCH OBJECTIVES

The overall objective of this research was to determine how agricultural land use influences immature mosquito assemblages in wetlands in the Gulf Coastal Plain of Georgia, U.S.A. Specifically, the first and second objectives were to investigate the relationships between immature mosquitoes and landscape and environmental habitat characteristics in wetlands associated with row crops during wet and dry hydrologic regimes. The third objective was to examine the relationship between nutrient enrichment and immature mosquito assemblages using field-based mesocosms adjacent to reference and agricultural wetlands. The fourth objective was to investigate the contributions of potential food resources assimilated by larval mosquitoes in reference and agricultural wetlands (Figure 1.1). Most mosquito surveillance focuses on urban areas and few observational data of mosquito assemblages are available from rural areas of the southeastern U.S.A. (Buckner et al. 2011). The expansion of mosquito-borne disease, and outbreak of WNV during the summer of 2012 (CDC 2012) has increased the need to determine how altered wetland conditions influence mosquito ecology.

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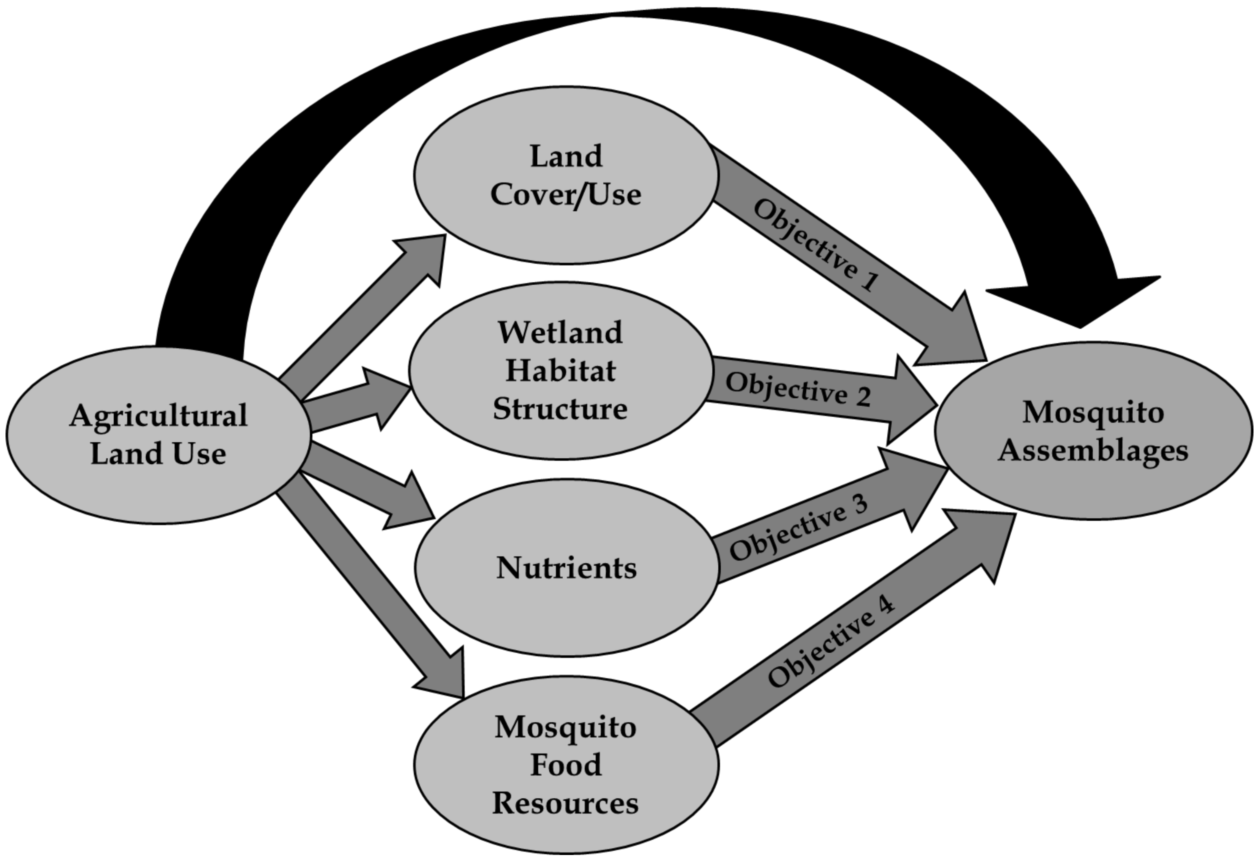
Figure 1.1: Conceptual model of research objectives



CHAPTER 2

IMMATURE MOSQUITOES IN AGRICULTURAL WETLANDS: EFFECTS OF LANDSCAPE AND ENVIRONMENTAL HABITAT CHARACTERISTICS DURING WET AND DRY HYDROLOGIC REGIMES

ABSTRACT

This study is the first to report on the relationships between immature mosquitoes and landscape and environmental habitat characteristics in wetlands associated with row crop agriculture during wet and dry hydrologic regimes. Indicator Species Analysis (ISA) was used to test for associations among mosquito species and groups of wetland sites with similar Landscape Development Intensity (LDI) values. Results indicated that *Anopheles quadrimaculatus*, *Culex erraticus*, and *Psorophora columbiae* were associated with agricultural wetlands (LDI > 2.0); whereas *Anopheles crucians* and *Culex territans* were associated with forested reference wetlands (LDI < 2.0) in both wet and dry years. The species fidelity to wetland type, regardless of the hydrologic regime, demonstrates these species are robust indicators of wetland condition. Data on immature mosquito assemblages were compared to selected landscape and environmental habitat variables using Akaike's Information Criterion (AIC_c) model selection. LDI indices, dissolved oxygen concentration, the proportion of emergent vegetation, and the proportion of bare ground in wetlands were important factors associated with the selected mosquito species. These results indicate that LDI indices are useful in predicting the distributions of disease vector or other nuisance mosquito species across broad geographic areas. Additionally, these results suggest mosquitoes are valuable bioindicators of wetland condition that reflect land use and hydrologic variability.

INTRODUCTION

In addition to their nuisance feeding behavior, adult mosquitoes in Georgia and other parts of the southeastern U.S.A. serve as vectors of important human and livestock pathogens, including West Nile virus (WNV), Eastern Equine encephalitis virus (EEEV), St. Louis encephalitis virus (SLEV), and LaCrosse encephalitis virus (LACV) (Lance-Parker, Rebmann et al. 2002; Buckner, Blackmore et al. 2011). Human land use has been linked to changes in mosquito community composition (Leisnham, Lester et al. 2004; Muturi, Shililu et al. 2006; Reiter and Lapointe 2007; Johnson, Gómez et al. 2008) and concurrent changes in disease transmission patterns (Patz, Graczyk et al. 2000; Norris 2004; DeGroote, Sugumaran et al. 2008). For example, human WNV incidence was strongly associated with rural agricultural and row crop settings in Iowa (DeGroote, Sugumaran et al. 2008). The suitability of a mosquito breeding site depends on complex interactions of abiotic factors such as precipitation, temperature, humidity, and soil moisture (Buckner, Blackmore et al. 2011), in addition to biotic factors such as competition (Reiskind and Wilson 2008), predation (Chase and Shulman 2009), and food availability (Blaustein and Kotler 1993; Walker, Merritt et al. 1997; Palik, Batzer et al. 2006; Yee and Juliano 2006). Altering landscapes through agricultural development can change the physical, chemical, and biological characteristics of larval mosquito habitats, in turn, affecting oviposition and larval survival (Norris 2004; Leisnham, Slaney et al. 2005; Reiter and Lapointe 2007).

Agriculture is an important part of Georgia's economy and is the dominant economic activity in rural areas of southwestern Georgia. Since the 1970s, mechanized agricultural practices such as tilling, leveling of agricultural fields, and in particular, center-pivot irrigation, have shaped the landscape and hydrology of the region. This intensive land use and associated runoff alters the water chemistry of adjacent isolated wetlands, resulting in elevated pH levels, higher nitrate (NO_3-N), phosphate (PO_4-P), ammonium nitrogen (NH_4-N), and suspended sediments compared with reference wetlands (Battle, Golladay et al. 2001; Atkinson, Golladay et al. 2011). Immature mosquito (larvae and pupae) abundance is known to be greater in constructed water treatment wetlands enriched with NH_4-N (Sanford, Chan et al. 2005), and positively associated with dissolved nitrates and phosphates in natural wetlands (Mercer, Sheeley et al. 2005) Nutrient-rich conditions may favor mosquito species that serve as disease vectors (Chaves, Keogh et al. 2009).

Because mosquitoes depend on aquatic habitats to complete their life cycle, differences in annual rainfall, runoff from cultivated fields, and wetland hydroperiod are likely to influence mosquito abundance and community composition. Isolated reference wetlands in the southeastern U.S.A. have hydroperiods that are driven by precipitation, evapotranspiration, basin morphology and topographic position (Kirkman, Goebel et al. 2000; Kirkman, Smith et al. 2012). Wetlands typically fill in late February and dry down in early July, depending on rainfall (Battle and Golladay 2001). In Missouri, mosquito densities increased following a natural drought in semi-permanent wetlands and an experimental drought in mesocosms, possibly linked to the elimination of mosquito predators and competitors (Chase and Knight 2003). In Carolina wetlands, mosquito community composition was influenced by seasonal changes in hydroperiod, where wetlands with a flooding-drying regime had higher species diversity and evenness, than permanently inundated basins (Ortiz, Wozniak et al. 2005). Agricultural irrigation also influences the wetland hydroperiod, and has been linked with greater incidences of WNV cases (Gates and Boston 2009). Because the hydrologic regime of isolated wetlands plays an important role in determining the mosquito species composition, the effects of agricultural runoff were predicted to influence mosquito community composition in this study.

Understanding the landscape distribution and ecological requirements of larval mosquito populations is important for managing potential breeding habitats and also for predicting vector-borne disease prevalence (Reiter and Lapointe 2007; DeGroote, Sugumaran et al. 2008). Several studies have examined the relationships between agricultural landscape level factors and the abundance and distribution of mosquito assemblages. For example, agriculture (e.g., cattle ranching, farming, and horticulture) and forest fragmentation increased the probability of capturing adult *Culex quinquefasciatus* in a mixed residential-agricultural landscape in Hawai'i (Reiter and Lapointe 2007). Similarly, pasture and urban lands in temperate, lowland areas had higher larval mosquito densities compared with native forests (Leisnham, Slaney et al. 2005). In Mwea, Kenya, adult mosquitoes that vector important arboviruses were more abundant, but species diversity was lower in rice agroecosystems compared with non-irrigated agroecosystems (Muturi, Shililu et al. 2006). In the Gulf Coastal Plain of Georgia, *Coquillettidia perturbans* and *Psorophora ferox* were associated with natural land cover such as wetlands and forested land, whereas *Aedes albopictus*, *Culex coronator*, *Culex quinquefasciatus*, and *Culex salinarius* were associated with sites that had the greatest human activity (Buckner, Blackmore et al. 2011).

Physical habitat features are also predictive of wetland mosquito species assemblages. For example, larval habitats dominated by filamentous algae were positively associated with *Anopheles* spp. in northern Belize (Rejmankova, Roberts et al. 1993). In California wetlands dominated by *Myrophyllum aquaticum*, oviposition rates of *Anopheles* females increased as stem density approached 2000 stems m^{-2} (Orr and Resh 1992). Similarly, the number of emerged

Culex erythrothorax was positively correlated with the density of emergent vegetation and nearly absent from open water California wetland areas (Workman and Walton 2000).

The key landscape and environmental habitat variables that determine larval mosquito assemblages in isolated wetlands of the Gulf Coastal Plain remain largely undocumented. This is the first regional study to investigate the relationships between immature mosquitoes and landscape and environmental habitat characteristics in wetlands associated with row crop agriculture during wet and dry hydrologic regimes. Most mosquito surveillance focuses on urban areas and few observational data of mosquito assemblages are available from rural areas of the southeastern U.S.A. (Buckner et al. 2011). The expansion of mosquito-borne disease, and outbreak of WNV during the summer of 2012 (CDC 2012) has underscored the need to determine how altered wetland conditions influence mosquito ecology. The objectives of this research were to 1) compare immature mosquito abundance, diversity, and species composition in reference and agricultural wetlands during wet and dry years, and to 2) compare immature mosquito abundance, diversity, and species composition with selected landscape and environmental habitat variables to determine the effects of agricultural land use on immature mosquitoes in wetlands of the Gulf Coastal Plain of Georgia, U.S.A.

MATERIALS AND METHODS

Study area

The study was conducted in the Gulf Coastal Plain of Georgia, U.S.A., a region dominated by peanut, corn, and cotton row crop agriculture. Surveys sites were chosen based on land use and access. Ten reference wetlands (five cypress-gum swamps and five grass-sedge marshes) and ten agricultural wetlands (center-pivot irrigation, row crops and pasture) were used in the landscape analysis comparing wetland type and hydrologic regime (Figure 2.1). Because of an extended drought (NOAA 2012), additional sites were used in the environmental habitat analysis, and included fourteen agricultural sites and eight reference sites (five cypress-gum swamps and three grass-sedge marshes) (Figure 2.1). Cypress-gum swamps are dominated by a closed canopy of pond cypress (*Taxodium ascendens*) and black gum (*Nyssa biflora*), while the grass-sedge marshes lack trees and are dominated by emergent grasses (Kirkman, Goebel et al. 2000). The ten reference wetlands are located on the J. W. Jones Ecological Research Center at Ichauway, a 12,000 ha reserve that has been managed for several decades with prescribed fire to maintain and restore the longleaf pine (*Pinus palustris*) forest and wiregrass (*Aristida stricta*) dominated groundcover. The reserve contains more than 30 seasonal wetlands and several ephemeral pools (Smith, Steen et al. 2006), which are surrounded by second growth longleaf pine forest and native groundcover. The seasonal variability of hydrology and diverse vegetation are representative of reference conditions.

Immature mosquito sampling

Immature mosquitoes (larvae and pupae) were sampled, when water was present, by taking 20 - 40 (350 mL) dip samples following a standardized larval sampling protocol at each wetland (Silver 2008). In grass-sedge marshes and agricultural wetlands, representative microhabitats in emergent vegetation, near the shallow edges of the wetland, and in open water were sampled. In cypress-gum swamps, microhabitats around cypress trees and in open water were sampled. Immature mosquito samples were compiled from each wetland and placed in plastic vials for transport to the laboratory. Reliable taxonomic identification (species level) of larval mosquitoes requires fourth-instar larvae. Therefore, < fourth-instar larvae were reared in the laboratory on a diet of homogenized aquarium fish food in containers of filtered wetland water. Fourth-instar larvae were heat-killed and preserved in 95% EtOH for identification.

Because mosquito pupae are difficult to identify to species, they were allowed to eclose to the adult stage and preserved in vials for identification. For the landscape analysis, weekly/monthly mosquito samples collected from March 2009 - June 2010 (48 sample dates), compared with March 2011- June 2012 (65 sample dates), from each wetland (n = 20) were used. For the environmental habitat analysis, weekly mosquito samples collected from January 2012 to June 2012 (26 sample dates) from each wetland (n = 22) were compared.

Landscape analysis

Landscape Development Intensity (LDI) indices for each wetland were determined using growing season 2010 digital aerial imagery obtained from the National Agriculture Imagery Program (NAIP) (1:12,000, 1-m resolution) for Baker, Mitchell, and Dougherty Counties in Georgia. Using ArcGIS (ESRI, Redlands, CA, v. 9.3), land use/cover within a 100-m buffer surrounding each field-delineated wetland was manually digitized at a scale of 1:5,000. Previous studies in Florida wetlands demonstrated that a 100-m radius is the optimum buffer distance for calculating LDI values (Brown and Vivas 2005; Reiss 2006). The land use/cover classification scheme and LDI coefficients follow previous research in Gulf Coastal Plain wetlands (Martin 2010) and Florida wetlands (Reiss and Brown 2007) (Table 2.1). LDI index scores were calculated as follows:

$$LDI_{total}=\sum(\%LU_i*LDI_i)$$

where LDI_{total} is the LDI index value for an each wetland site, $\%LU_i$ is the percent of a land use/cover within the 100-m radius, and LDI_i is the LDI coefficient for each respective land use/cover class (Table 2.1) (Brown and Vivas 2005; Reiss and Brown 2007; Martin 2010).

Environmental habitat sampling

Eighteen environmental habitat variables were characterized at each wetland site (n = 22), when water was present, on February 16-17, March 26-27, and May 8-10, 2012, representing the early, middle and late hydroperiod. The habitat variables included: 1) water temperature, 2) dissolved oxygen, 3) specific conductance, 4) pH levels, 5) alkalinity, 6) suspended solids in the water column, 7) nitrate (NO_3-N), 8) phosphate (PO_4-P), 9) ammonium nitrogen (NH_4-N), 10) water surface area, 11) proportion of canopy cover, 12) hydroperiod, 13) stem density of emergent vegetation, 14) vegetation height, 15) proportion of emergent aquatic vegetation, 16) proportion of floating aquatic vegetation, 17) proportion of submerged aquatic vegetation, and 18) water depth.

Water temperature, specific conductivity, pH levels, and dissolved oxygen measurements were taken in the field using a portable meter (Quanta Hydrolab, Yellow Springs Instrument Co., Inc., Yellow Springs, OH). On each sample date, three replicate 500 mL water samples were obtained from each wetland, held on ice, and processed within 24 hours in the laboratory at the J. W. Jones Ecological Research Center. These samples were analyzed for pH, alkalinity, and filtered for suspended solids (ash-free dry mass) using standardized methods (Eaton, Clesceri et al. 2005). Nitrate (NO_3-N), phosphate (PO_4-P), and ammonium nitrogen (NH_4-N) were determined using a Lachat Quickem 8000 flow-injection colormetric method (Lachat Instruments, Milwaukee, WI). Alkalinity and pH were assessed with a Mettler DL15 titrator (Mettler-Toledo Inc., Columbus, OH). Water surface area was measured by taking points around the wetted perimeter of the wetland using a GPS unit (Nomad 900L, Trimble Inc., Sunnyvale, CA). The proportion of canopy cover was averaged at three locations per wetland using a spherical densiometer. Hydroperiod was evaluated as the number of days the wetland was inundated during the sampling period (March 2011 - June 2012), based on weekly visits. The proportion of aquatic vegetation, stem density, plant height, and water depth were evaluated by establishing three transects perpendicular to a baseline transect, and three quadrats were sampled at each transect. Each 0.5 m^2 quadrat was measured for the proportion of emergent, floating or submerged vegetation, stem density, plant height, and water depth.

Statistical analysis

Immature mosquito species abundance was expressed as the number of individuals per 350 mL dip sample**.** Shannon-Wiener diversity indices were used to characterize mosquito species diversity in each wetland. The Shannon-Wiener diversity index (H) accounts for both abundance and evenness of the species present. The proportion of species i relative to the total number of species (pi) is calculated and then multiplied by the natural logarithm of this proportion (ln pi). The resulting product is summed across species and multiplied by -1 to give the Shannon-Wiener diversity index.

$$H = -\sum pi \ln pi$$

Wetland hydroperiod, mosquito abundance, and Shannon-Weiner diversity indices in reference (n = 10) and agricultural wetlands (n = 10) were compared at two sampling periods (February 2009 - June 2010 and February 2011 - June 2012) representing different hydrologic regimes, using a Mann-Whitney Rank Sum Test (Sigma Stat, Systat Software Inc., v. 12.0), since the majority of the data violated the assumption of normality. Dunn's test was used for pairwise comparisons.

Landscape Development Intensity indices were used to group the wetland study sites (n = 20) into low (LDI < 2) and high (LDI > 2) LDI groups (Martin 2010). These groups were used to test for associations among mosquito species and groups of wetland sites with similar LDI values using Indicator Species Analyses (ISA) (PC-ORD, MjM Software Design, v. 5.10). The ISA was performed on 2009 - 2010 and 2011 - 2012 data separately. The ISA produces indicator values for each species based on the relative abundance and frequency of the species to a group (McCune, Grace et al. 2002).

Mosquito abundance, species diversity, and the abundance of mosquito species for each wetland (n = 22) from January 2012 - June 2012, were used to construct the mosquito habitat models. This subset of mosquito data corresponded to the environmental habitat sampling period. Mosquito species were selected based on their significance in the ISA during the 2011 - 2012 sampling period. In order to reduce the number of variables included in model construction, the strongest environmental habitat variables were selected from the results of Principle Components Analysis ordination (PC-ORD, MjM Software Design, v. 5.10) and averaged over the three sample periods. The matrix was further reduced by eliminating those variables that were highly correlated with each other ($|r| > 0.7$) (Brennan, Brock et al. 1986). Fourteen models were developed to describe mosquito habitat preference using seven descriptor variables: % emergent vegetation (EmgVeg), % bare ground (BareGrd), % canopy (Canopy), dissolved oxygen (DO), ash-free dry mass (AFDM), ammonium nitrogen (NH_4-N), and Landscape Development Intensity (LDI) (Table 2.2). Response variables were analyzed as a function of physical habitat (H) (EmgVeg, BareGrd, and Canopy), water chemistry (W) (DO, AFDM, and NH_4-N), and the LDI variables using a Generalized Linear Model in R (R ver. 2.12.2). Competing models were ranked using Akaike's Information Criterion adjusted for small sample size (AIC_c), and a Hosmer and Lemeshow (2000) test was used to evaluate goodness-of-fit for the global model (Conner, Rutledge et al. 2010). The model with the lowest AIC_c score was considered the best model, but models with $AIC_c < 2.0$ were included as the best set of candidate models in predicting variable importance (Conner, Rutledge et al. 2010). Variable weights were calculated by summing model weights for all models containing the variable. Model averaging was used to calculate parameter estimates and unconditional standard errors for all variables (Conner, Rutledge et al. 2010). Predictor variables were considered useful based on their variable weight ($w_i > 0.80$) and if the 95% confidence interval did not contain zero (Conner, Rutledge et al. 2010).

RESULTS

Wetland hydroperiod

Wetland hydroperiods from the 20 wetland sites were significantly greater during the March 2009 - June 2010 sampling period compared with the March 2011- June 2012 sampling period ($p < 0.05$). Reference and agricultural wetland hydroperiods did not differ significantly within the March 2009 – June 2010 sampling period or within the March 2011 - June 2012 sampling period ($p > 0.05$) (Table 2.3). Therefore, subsequent analyses allowed comparison of mosquito species composition across land use types in two disparate hydrologic regimes, i.e. 2009 - 2010 (wet) vs. 2011 - 2012 (dry).

Mosquito species

From March 2009 - June 2010, approximately 5,500 immature mosquitoes were collected from the 20 wetland sites, over 48 sample dates. Thirty-two mosquito species were collected within the following genera: *Aedes*, *Anopheles*, *Culiseta*, *Culex*, *Ochlerotatus*, *Psorophora*, and *Uranotaenia*. Nine species (*Culex territans* (23%), *Culiseta melanura* (21%), *Aedes vexans* (20%), *Anopheles crucians* (6%), *Culex restuans* (6%), *Culex erraticus* (5%), *Ochlerotatus canadensis* (5%), *Psorophora columbiae* (3%), *Anopheles punctipennis* (2%)), comprised over 90% of the total mosquitoes collected (Table 2.4).

From March 2011- June 2012, approximately 13,100 immature mosquitoes were collected from 20 wetland sites, over 68 sampling dates. Twenty-four mosquito species were collected within the following genera: *Aedes*, *Anopheles*, *Culiseta*, *Culex*, *Ochlerotatus*, *Psorophora*, and *Uranotaenia*. Five species (*Aedes vexans* (70%), *Culex territans* (10%), *Psorophora columbiae* (5%), *Culex restuans* (4%), *Anopheles crucians* (3%)) comprised over 90% of the total mosquitoes collected (Table 2.4).

Immature mosquito abundance across all sample dates was significantly greater in the reference wetlands compared with the agricultural wetlands ($p < 0.05$). During the 2009 - 2010 sampling period, mosquito abundance was greater in the reference wetlands compared with the agricultural wetlands ($p < 0.05$), however, during the 2011 - 2012 sampling period, there was no significant difference in mosquito abundance in the reference wetlands compared with the agricultural wetlands ($p > 0.05$). Although it was not significant, mosquito abundance was generally greater in agricultural wetlands from mid-May 2011 through February 2012, when reference wetlands had dried (Table 2.3, Figure 2.2).

Mosquito species diversity was greater in reference wetlands compared with agricultural wetlands across all sample dates ($p < 0.05$). During the 2009 - 2010 sampling period, mosquito communities were more diverse in reference wetlands compared with agricultural wetlands ($p < 0.05$). However, during the 2011 - 2012 sampling period, diversity was not significantly different in reference wetlands compared with agricultural wetlands ($p > 0.05$). (Table 2.3)

Landscape distribution

Two groups of wetland sites were used based on the Landscape Development Intensity indices: reference sites (LDI < 2.0) (n = 10) (W15, W2, W21, W3, W4, W42, W46, W52, W53, W58) and agricultural sites (LDI > 2.0) (n = 10) (DS2, OS1, OS2, OS3, OS4, OS5, OS6, OS7, OS8, OS9). The sites in the reference wetland group had LDI values that ranged from 1.0 - 1.8, and were dominated by forested land use/cover. The sites in the agricultural wetland group had LDI values that ranged from 2.2 - 4.6 and were dominated by row crop and pasture land use/cover (Table 2.5, Figure 2.3).

Of the 16 mosquito species considered, the ISA identified seven species with significant indicator values (5% level of significance) for either agricultural or reference wetlands during March 2009 - June 2010. Nine species were significant indicators during March 2011- June 2012. During March 2009 - June 2010, *Anopheles crucians* (IV = 22.4, $p = 0.023$), *Culiseta melanura* (IV = 23.7, $p < 0.001$), and *Culex territans* (IV = 34.4, $p < 0.001$) were associated with reference wetlands, and *Anopheles punctipennis* (IV = 16.9, $p = 0.002$), *Anopheles quadrimaculatus* (IV = 23.6, $p < 0.001$) *Culex erraticus* (IV = 12.3, $p = 0.030$), and *Psorophora columbiae* (IV = 7.0, $p < 0.001$) were associated with agricultural wetlands. During March 2011 - June 2012, *Ochlerotatus mitchellae* (IV = 5.9, $p = 0.021$), *Anopheles crucians* (IV = 43.8, $p < 0.001$), *Culex territans* (IV = 58.8, $p < 0.001$), and *Psorophora ferox* (IV = 7.9, $p = 0.024$) were associated with reference wetlands, and *Anopheles quadrimaculatus* (IV = 38.3, $p < 0.001$), *Culex coronator* (IV = 7.9, $p < 0.001$), *Culex erraticus* (IV = 12.3, $p = 0.004$), *Culex salinarius* (IV = 5.7, $p = 0.012$), and *Psorophora columbiae* (IV = 7.0, $p < 0.001$) were associated with agricultural wetlands (Table 2.6).

Environmental habitat variables

Of the eighteen variables sampled, seven variables from the PCA ordination results (Figure 2.4) were included in model construction. The proportion of canopy cover in wetlands ranged from 0% in agricultural wetland to 100% in reference wetlands, emergent vegetation ranged from 0% in agricultural wetlands to 34% in reference wetlands, and bare ground ranged from 0% in reference wetlands to 100% in agricultural wetlands. Water column AFDM ranged from 2.3 mg/L to 234.4 mg/L, NH_4-N ranged from 9.6 µg/L to 5352.3 µg/L, and dissolved oxygen ranged from 1.9 mg/L to 10.1 mg/L (Table 2.7). The best model for predicting overall mosquito abundance ($w = 0.60$) and species diversity ($w = 0.83$) contained all physical habitat variables and the LDI indices (Table 2.8). The best models for predicting the abundance of individual mosquito species varied by species (Table 2.8). The results of Hosmer and Lemshow (2000) tests suggested that all global models fit the data.

Model-averaged parameter estimates suggested that mosquito abundance was best predicted by the proportion of bare ground and LDI indices (Table 2.9). Species diversity was best predicted by the proportion of emergent vegetation, the proportion of bare ground, and LDI indices. The abundance of *Ochlerotatus mitchellae* and *Culex coronator* were best predicted by LDI indices. The abundance of *Culex territans* was best predicted by dissolved oxygen concentration. The abundance of *Culex salinarius* and *Psorophora columbiae* were best predicted by the proportion of emergent vegetation and LDI indices. Finally, the abundance of *Anopheles quadrimaculatus*, *Anopheles crucians*, *Culex erraticus*, and *Psorophora ferox* were not successfully predicted using the environmental habitat or landscape variables.

DISCUSSION

This study confirmed certain mosquito species have distinct habitat preferences for larval development, and these preferences persist across wet or dry hydrologic regimes. The ISA results indicated, in both wet and dry years, *Anopheles quadrimaculatus*, *Culex erraticus*, and *Psorophora columbiae* were associated with agricultural wetlands, whereas *Anopheles crucians* and *Culex territans* were associated with reference wetlands. The species fidelity to wetland type, regardless of the hydrologic regime, demonstrates these species are robust indicators of wetland condition. The associations between the selected mosquito species and groups of reference and agricultural sites in the ISA are consistent with known ecological preferences of each species, and are further validated by AIC modeling results which tested the importance of environmental and landscape variables to the selected mosquito species.

Anopheles quadrimaculatus and *Culex erraticus* were associated with agricultural wetlands in wet and dry years. These species are primarily found in permanent fresh water pools containing aquatic vegetation or floating debris (Carpenter and LaCasse 1955). *Psorophora columbiae* was also associated with agricultural wetlands in wet and dry years (Table 2.6) and was positively associated with LDI indices (Table 2.9). This species prefers irrigated croplands, ricefields, roadside ditches, and open fields (Meisch 1994; Bolling, Kennedy et al. 2005). *Cx. erraticus* and *Ps. columbiae* are both typically found in the same habitat as *An. quadrimaculatus* (McNelly and Crans 1989; Meisch 1994), which is consistent with our results.

Culex coronator, which has a wide geographic distribution, including the American tropics and the U.S.A. (Arizona, New Mexico, and Texas), has recently expanded its range to the Gulf Coastal Plain of Georgia (Kelly, Mead et al. 2008). *Culex salinarius* has the ability to occupy undisturbed fresh and saltwater margins, lakes, and ponds but can also tolerate nutrient-rich conditions (Rochlin, Dempsey et al. 2008). *Cx. salinarius* was positively associated with emergent vegetation, and previous studies have shown this species to be associated with densely vegetated areas (Rochlin, Dempsey et al. 2008). *Cx. coronator* and *Cx. salinarius* were both associated with agricultural wetlands in dry years (Table 2.6) and positively associated with LDI indices (Table 2.9). These results are consistent with previous studies that linked these species with agricultural wetlands in southwestern Georgia (Buckner, Blackmore et al. 2011). *Cx. coronator* and *Cx. salinarius* are late season breeders that typically reach peak population densities in mid- to late summer (Andreadis, Anderson et al. 2001; Goddard, Varnado et al. 2006; Buckner, Blackmore et al. 2011). This late season breeding could explain the significance of these species as indicators in agricultural wetlands during dry years, when agricultural sites remained ponded through the late summer, whereas reference wetlands dried in the spring.

Studies in Belize showed *Anopheles crucians* occurred most often in heavily shaded areas of marshes and was also negatively associated with cyanobacteria mats (Grieco, Johnson et al. 2006). The research is consistent with our results, which showed *An. crucians* abundance was associated with reference wetlands that provide more shade than agricultural wetlands, and where cyanobacteria mats were uncommon (personal observation). *Culex territans* is commonly found in clear-water habitats with grassy stands of emergent vegetation (Joy and Clay 2002), where females prefer to oviposit (McIver 1969). *Cx. territans* prefers the cooler waters of swamps and large wetland pools (Carpenter and LaCasse 1955), which is consistent with its association at reference wetland sites in wet and dry years. The abundance of *Cx. territans* was negatively associated with dissolved oxygen concentration. *Ochlerotatus mitchellae* was associated with reference wetlands, particularly in dry years (Table 2.6), and was negatively associated with LDI indices (Table 2.9). This species is most commonly found in clear, partly shaded, rain-filled pools, which contain grasses and emergent vegetation (Carpenter and LaCasse 1955). *Psorophora ferox* was associated with reference wetlands, particularly in dry years, and has been previously associated with forested wetlands dominated by coniferous forest and little cultivated land within a 1-km radius around the trap site in southwestern Georgia (Buckner, Blackmore et al. 2011). *Culiseta melanura* prefers more acidic waters associated with shaded swamps (Carpenter and LaCasse 1955) and was associated with lower pH reference wetlands during wet years in this study.

The well documented and species-specific larval habitat requirements make mosquitoes valuable bioindicators of wetland condition that reflect land use and hydrologic variability. Aquatic macroinvertebrates are frequently used for monitoring habitat quality in lotic systems, but macroinvertebrates are less commonly used as indicators in lentic systems (Rosenberg and Resh 1993; Foote and Rice Hornung 2005). Mosquitoes meet many of the criteria previously described as ideal biomonitoring organisms (Rosenberg and Resh 1993; Bonada, Prat et al. 2006), including: 1) immature mosquitoes are ubiquitous and found in a variety of habitats, ranging from small collections of water in artificial containers and tree holes to large areas of water such as wetland complexes (Silver 2008), 2) mosquitoes are diverse, with approximately 60 species found in Georgia alone (Carpenter and LaCasse 1955), offering a range of responses to environmental stress, 3) sampling and sorting mosquitoes is simple and inexpensive (Silver 2008), 4) the taxonomy of mosquito species is well-developed (Carpenter and LaCasse 1955), and 5) methods of collecting and surveying mosquito populations are standardized (Silver 2008).

These results also underscore the importance of land use changes on the potential spread of vector-borne diseases. Knowledge of the suitability of different wetland habitats types for disease vectors is essential in developing wetland management strategies that improve wetland ecosystem services in agricultural lands. These results indicate landscape screening tools like GIS and LDI indices are useful in predicting the distributions of disease vector or other nuisance mosquito species across broad geographic areas. Additionally, the habitat preferences of several common nuisance species (i.e. *Anopheles quadrimaculatus* and *Culex erraticus*) are not well known in agricultural areas, but because of their link with human and livestock pathogens, future research should focus on the habitat preferences of these species.

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Table 2.1: Land use/cover classification scheme and LDI coefficients following Martin, et al. (2010) and Reiss and Brown (2007).

Land use	**LDI coefficients**
Natural system	1.00
Natural open water	1.00
Pine plantation	1.58
Recreational/open space - low intensity	1.83
Woodland pasture (with livestock)	2.02
Improved pasture (without livestock)	2.77
Improved pasture - low intensity (with livestock)	3.41
Citrus/Orchard	3.68
Improved pasture - high intensity (with livestock)	3.74
Recreational/open space - medium intensity	4.38
Row crops	4.54
Single family residential - low density	6.79
Recreational/open space - high intensity	6.92
Single family residential- high intensity	7.55
Highway (≥ 2 lane)	7.81
Industrial	8.32
Business district (≥ 2 stories)	9.42

Table 2.2: Models used in the second-order Akaike's Information Criteria (AIC_c) analysis.

Model	Environmental Variables	Notation
1) Null	Response variable	N
2) Effect of Physical Habitat, Water Chemistry, and Landscape Development Intensity	% Emergent vegetation + % Bare ground + % Canopy, + Dissolved Oxygen + AFDM + NH_4-N + Landscape Development Intensity	H, W, LDI
3) Effect of Physical Habitat and Water Chemistry	% Emergent vegetation + % Bare ground + % Canopy, + Dissolved Oxygen + AFDM + NH_4-N	H, W
4) Effect of Physical Habitat and Landscape Development Intensity	% Emergent vegetation + % Bare ground + % Canopy + Landscape Development Intensity	H, LDI
5) Effect of Physical Habitat	% Emergent vegetation + % Bare ground + % Canopy	H
6) Effect of Water Chemistry and Landscape Development Intensity	Dissolved Oxygen + AFDM + NH_4-N + Landscape Development Intensity	W, LDI
7) Effect of Water Chemistry	Dissolved Oxygen + AFDM + NH_4-N	W
8) Effect of Landscape Development Intensity	Landscape Development Intensity	LDI
9) Effect of Emergent Vegetation	% Emergent Vegetation	EmgVeg
10) Effect of Bare Ground	% Bare Ground	BareGrd
11) Effect of Canopy	% Canopy	Canopy
12) Effect of Dissolved Oxygen	Dissolved Oxygen	DO
13) Effect of AFDM	AFDM	AFDM
14) Effect of NH_4-N	NH_4-N	NH_4-N

Table 2.3: Mean hydroperiod, mosquito diversity, and abundance in ten reference and ten agricultural wetland sites by sampling period. Dunn's test used for multiple comparisons of wetland type and sampling period.

Wetland Type/Sampling Period	**Mean Hydroperiod**	**Mean Diversity**	**Mean Abundance**
All Wetlands: March 2009 - June 2010	488.3	33.7	16.3
All Wetlands: March 2011 - June 2012	206.5	31.3	23.6
Reference: 2009 - 2012	326.7	38.1	4.4
Agricultural: 2009 - 2012	357.0	26.9	2.5
Reference: 2009 - 2010	495.4	45.5	5.3
Agricultural: 2009 - 2010	481.2	21.9	1.2
Reference: 2011 - 2012	158.0	30.7	3.9
Agricultural: 2011 - 2012	255.0	31.9	3.4
Multiple Comparisons	**Hydroperiod $p < 0.05$**	**Diversity $p < 0.05$**	**Abundance $p < 0.05$**
All Wetlands: March 2009 - June 2010 vs. All Wetlands: March 2011 - June 2012	Yes	No	No
Reference 2009 - 2012 vs. Agricultural 2009 - 2012	No	Yes	Yes
Reference 2009 - 2010 vs. Agricultural 2009 - 2010	Yes	Yes	Yes
Agricultural 2009 - 2010 vs. Reference 2011 - 2012	Yes	No	No
Agricultural 2009 - 2010 vs. Agricultural 2011 - 2012	Yes	No	No
Reference 2009 - 2010 vs. Agricultural 2011 - 2012	Yes	No	Yes
Reference 2009 - 2010 vs. Reference 2011 - 2012	No	No	Yes
Reference 2011 - 2012 vs. Agricultural 2011 - 2012	No	No	No

Table 2.4: Number of mosquitoes per species collected during March 2009 – June 2010 compared with March 2011 – June 2012 in ten agricultural and ten reference wetland sites in the Gulf Coastal Plain of Georgia, U.S.A.

	March 2009- June 2010				**March 2011- June 2012**			
Species	**Agricultural Wetlands n = 10**	**Reference Wetlands n = 10**	**Total**	**%**	**Agricultural Wetlands n = 27**	**Reference Wetlands n = 10**	**Total**	**%**
Ae. albopictus	0.0	1.6	1.6	0.5	0.0	0.0	0.0	0.0
Ae. vexans	13.6	52.1	65.7	20.2	160.7	171.2	331.8	70.2
Oc. atlanticus	0.0	0.2	0.2	0.1	0.0	0.1	0.1	0.0
Oc. canadensis	0.0	14.8	14.8	4.5	0.0	0.9	0.9	0.2
Oc. infirmatus	0.5	1.8	2.3	0.7	0.0	0.4	0.4	0.1
Oc. mitchellae	0.2	0.3	0.5	0.2	0.1	0.9	1.0	0.2
Oc. sticticus	0.1	2.5	2.6	0.8	0.0	0.3	0.3	0.1
Oc. thibaulti	0.1	0.4	0.5	0.1	0.0	0.0	0.0	0.0
Oc. triseriatus	0.0	0.9	0.9	0.3	0.0	0.1	0.1	0.0
An. crucians	3.8	15.8	19.6	6.0	0.4	13.9	14.3	3.0
An. punctipennis	3.4	4.0	7.4	2.3	2.6	5.5	8.0	1.7
An. quadrimaculatus	4.8	0.7	5.5	1.7	9.5	0.3	9.8	2.1
Cs. melanura	0.1	69.2	69.3	21.3	0.0	0.1	0.1	0.0
Cs. inornata	0	0	0	0	0.1	0.0	0.1	0.0
Cx. coronator	1.0	1.6	2.5	0.8	3.5	0.0	3.5	0.7
Cx. erraticus	6.8	10.1	16.9	5.2	0.6	0.1	0.6	0.1
Cx. nigripalpus	0.6	1.1	1.6	0.5	0.0	0.0	0.0	0.0
Cx. peccator	2.2	0.7	2.9	0.9	0.0	0.0	0.0	0.0
Cx. pilosus	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0
Cx. pipiens complex	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Cx. quinquefasciatus	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0
Cx. restuans	0.8	18.4	19.2	5.9	1.4	16.1	17.5	3.7
Cx. salinarius	0.6	0.7	1.3	0.4	5.7	0.1	5.8	1.2
Cx. tarsalis	0.1	0.4	0.5	0.1	0.0	0.0	0.0	0.0
Cx. territans	8.9	66.9	75.8	23.3	0.4	44.4	44.8	9.5
Ps. ciliata	0.2	0.2	0.4	0.1	0.2	1.2	1.4	0.3
Ps. columbiae	9.7	0.0	9.7	3.0	24.0	0.9	24.9	5.3
Ps. cyanescens	0.3	0.0	0.3	0.1	0.0	0.0	0.0	0.0
Ps. discolor	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Ps. ferox	0.0	0.1	0.1	0.0	0.6	6.2	6.8	1.4
Ps. horrida	0.1	0.0	0.1	0.0	0.0	0.2	0.2	0.0
Ps. howardii	0.4	2.1	2.5	0.8	0.0	0.0	0.0	0.0
Ur. sapphirina	0.6	0.5	1.1	0.3	0.4	0.4	0.7	0.2
Total	**58.5**	**267.1**	**325.5**	**100**	**210.1**	**262.7**	**472.8**	**100**
Percent	**18.0**	**82.0**	**100**		**44.4**	**55.6**	**100**	

Table 2.5: Landscape Development Intensity (LDI) index values calculated from manually digitized 2010 National Agriculture Imagery Program aerial photographs of twenty wetland study sites in the Gulf Coastal Plain of Georgia, U.S.A.

Wetland	**LDI value**
DS2	2.22
OS1	3.25
OS2	2.78
OS3	3.91
OS4	4.26
OS5	2.81
OS6	3.30
OS7	4.20
OS8	4.54
OS9	4.64
W15	1.00
W2	1.00
W21	1.00
W3	1.00
W4	1.00
W42	1.27
W46	1.78
W52	1.16
W53	1.84
W58	1.66

Table 2.6: Indicator species analyses (ISA) computing indicator value (IV) coefficient of selected mosquito species in reference sites (LDI < 2.0) (n = 10) and agricultural sites (LDI > 2.0) (n = 10) during two sampling periods. An IV closer to 100 represents a strong species association with a group (McCune, Grace et al. 2002).

		March 2009 - June 2010				March 2011 - June 2012			
Species	**Group**	**IV**	**Mean**	**S. Dev**	**p**	**IV**	**Mean**	**S. Dev**	**p**
Ae. vexans	Agriculture	9.1	12.3	2.3	0.940	19.0	22.3	2.8	0.934
Oc. mitchellae	Reference	1.4	1.6	0.6	0.546	5.9	3.4	1.0	0.021*
*An. crucians***	Reference	22.4	17.3	2.1	0.023*	43.8	16.7	2.0	< 0.001*
An. punctipennis	Agriculture	16.9	10.0	1.6	0.002*	13.9	11.5	1.8	0.117
*An. quadrimaculatus***	Agriculture	23.6	6.3	1.3	< 0.001*	38.3	12.1	2.1	< 0.001*
Cs. melanura	Reference	23.7	10.8	1.9	< 0.001*	1.2	0.9	0.5	0.527
Cx. coronator	Agriculture	2.7	1.7	0.6	0.070	7.9	3.2	1.1	< 0.001*
*Cx. erraticus***	Agriculture	12.3	8.6	1.6	0.030*	6.0	2.7	0.9	0.004*
Cx. pipiens	Agriculture	0.8	0.5	0.2	0.301	0.7	0.6	0.1	0.445
Cx. restuans	Reference	4.7	3.9	1.1	0.209	7.4	6.7	1.8	0.338
Cx. salinarius	Agriculture	2.5	2.1	0.7	0.238	5.7	3.3	1.0	0.012*
*Cx. territans***	Reference	34.4	22.2	2.5	< 0.001*	58.8	20.5	2.5	< 0.001*
Ps. ciliata	Reference	0.5	0.9	0.4	1.000	4.8	3.6	1.1	0.146
*Ps. columbiae***	Agriculture	7.0	2.0	0.7	< 0.001*	27.8	10.8	2.3	< 0.001*
Ps. ferox	Reference	0.7	0.7	0.3	0.579	7.9	4.8	1.2	0.024*
Ur. sapphirina	Agriculture	2.3	2.1	0.7	0.284	2.6	3.2	1.0	0.713

*Indicates p is significant at 95% level
**Indicates species that are significant indicators during both sampling periods

Table 2.7: Mean environmental habitat variables used in the AIC analysis.

Wetland	% Canopy	DO mg/L	% Emergent Vegetation	% Bare Ground	AFDM ug/L	NH_4-N µg/L	LDI
DS2	0.2	8.4	27.8	0.0	10.6	46.8	2.2
OS1	8.9	5.1	1.1	98.9	110.3	5352.3	3.2
OS2	81.1	3.3	13.3	86.7	73.9	1715.8	2.8
OS31	0.2	8.0	0.0	100.0	50.7	143.7	4.5
OS32	0.2	6.2	0.9	99.1	117.8	943.8	4.5
OS33	0.2	9.5	0.0	77.8	234.4	3255.8	4.5
OS34	67.6	1.9	12.5	0.0	17.0	5099.8	4.5
OS35	65.9	3.2	5.0	56.7	3.4	1167.0	3.3
OS36	69.0	2.1	26.3	23.7	10.6	9.6	3.6
OS37	11.0	10.1	8.0	82.8	25.6	279.3	3.7
OS4	0.2	6.8	0.0	100.0	16.4	87.4	4.3
OS5	2.6	2.0	6.1	40.0	8.8	15.2	2.8
OS7	0.2	7.7	0.6	87.4	19.9	14.1	4.2
OS8	0.2	6.2	11.1	87.8	24.3	303.0	4.5
W2	100.0	3.4	6.7	0.0	10.4	64.1	1.0
W21	0.2	3.1	21.1	0.0	5.6	1176.9	1.0
W3	100.0	7.6	13.3	0.0	51.8	30.8	1.0
W4	100.0	2.7	12.8	0.0	2.3	21.2	1.0
W42	5.2	3.3	33.7	14.1	11.2	65.2	1.3
W52	100.0	2.0	0.0	0.0	7.9	883.9	1.2
W53	5.7	3.8	13.9	0.0	4.5	48.3	1.8
W58	97.6	2.1	1.1	0.0	40.9	1456.3	1.7

Table 2.8: Best candidate models explaining the effects of environmental habitat and landscape variables on mosquito abundance, species diversity, and selected species abundance. The number of predictor variables (K) in each model included the intercept term. Models with greater Akaike weight (w_i) were more supported with data.

Model	**K**	**Δ AIC$_c$**	**w_i**
Mosquito abundance			
H, LDI	6	0.00	0.60
Species diversity			
H, LDI	6	0.00	0.83
Oc. mitchellae			
LDI	3	0.00	0.87
An. quadrimaculatus			
N	2	0.00	0.28
DO	3	0.83	0.19
An. crucians			
BareGrd	3	0.00	0.41
DO	3	1.66	0.18
Cx. coronator			
W, H, LDI	9	0.00	0.45
W	5	1.35	0.23
Cx. territans			
DO	3	0.00	0.41
BareGrd	3	1.02	0.24
Cx. erraticus			
N	2	0.00	0.24
BareGrd	3	0.59	0.18
DO	3	1.54	0.11
H	5	1.58	0.11
Cx. salinarius			
H, LDI	6	0.00	0.97
Ps. columbiae			
H, LDI	6	0.00	0.97
Ps. ferox			
Canopy	3	0.49	0.49

Table 2.9: Model-averaged parameter estimates (coefficient), unconditional standard errors (SE), 95% confidence interval (CI) and variable weights for parameters used to predict mosquito abundance, species diversity and abundance of selected mosquito species. Data are only presented for those parameters with 95% confidence interval (CI) and with a variable weight > 0.80.

Variable	**Coefficient**	**SE**	**95% CI**	**Variable Weight**
Mosquito abundance				
BareGrd	-0.88	0.27	-1.4- -0.36	0.99
LDI	22.37	7.05	8.54 - 36.19	1.00
Species diversity				
EmgVeg	0.63	0.30	0.04 - 1.23	0.99
BareGrd	-0.32	0.11	-0.53- -0.11	1.00
LDI	9.29	2.80	3.8 - 14.78	1.00
Oc. mitchellae				
LDI	-0.03	0.01	-0.04- -0.01	1.00
Cx. coronator				
LDI	0.25	0.11	0.04 - 0.47	1.00
Cx. territans				
DO	-0.63	0.21	-1.04- -0.22	1.00
Cx. salinarius				
EmgVeg	0.02	0.01	0.01 - 0.03	1.00
LDI	0.28	0.06	0.16 - 0.40	1.00
Ps. columbiae				
EmgVeg	0.02	0.01	0.01 - 0.03	1.00
LDI	0.28	0.06	0.16 - 0.40	1.00

Figure 2.1: Ten reference wetlands and ten wetlands influenced by agricultural land use were sampled for immature mosquitoes and used in the landscape analysis comparing wetland type and hydrologic regime. Based on presence of water, eight reference wetlands and fourteen wetlands influenced by agricultural land use were sampled for immature mosquitoes and used in the environmental habitat analysis. Sites used in both the landscape and environmental habitat analysis are indicated as shared wetlands.

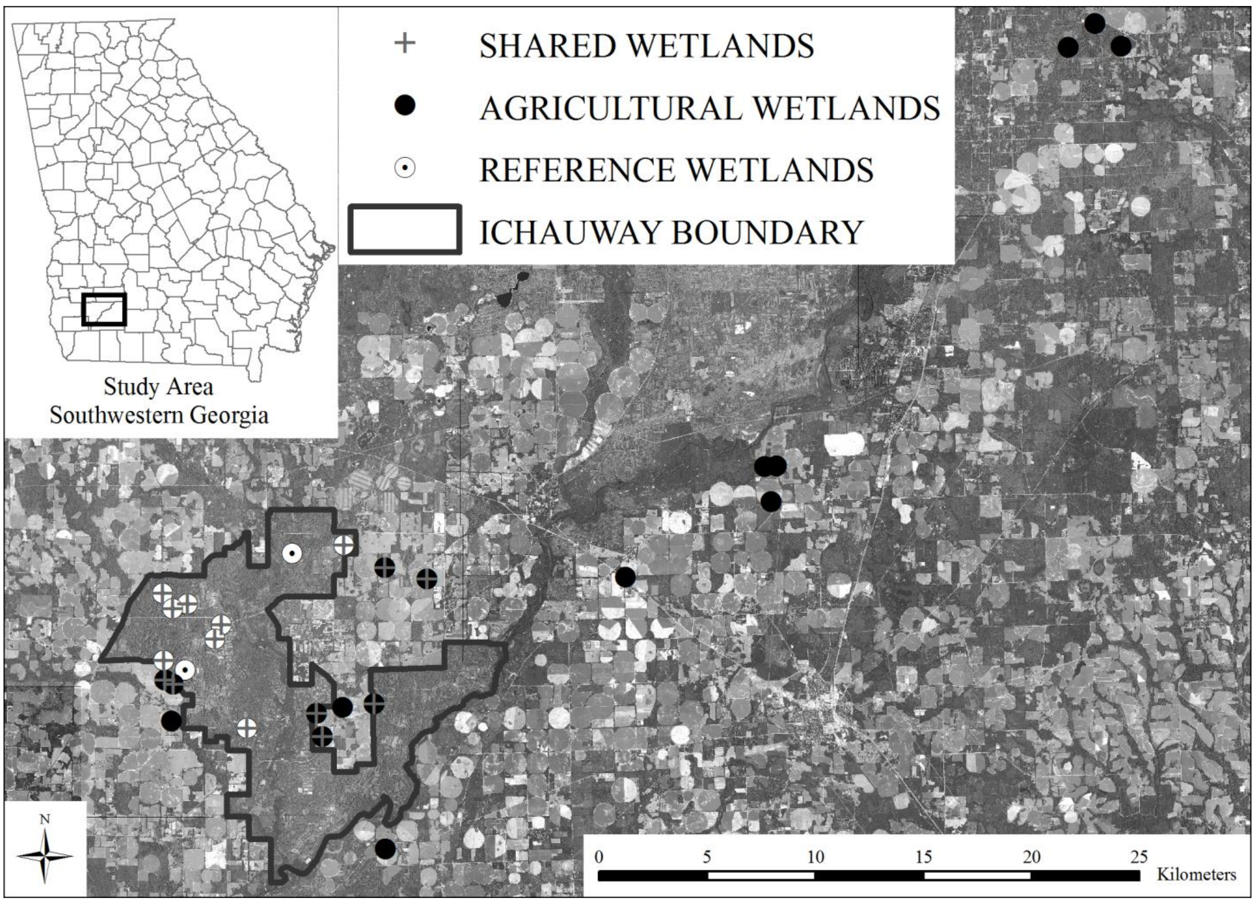


Figure 2.2: Seasonal distribution of mosquitoes sampled in ten reference and ten agricultural wetlands in the Gulf Coastal Plain of Georgia, U.S.A. from March 2009 – June 2012.

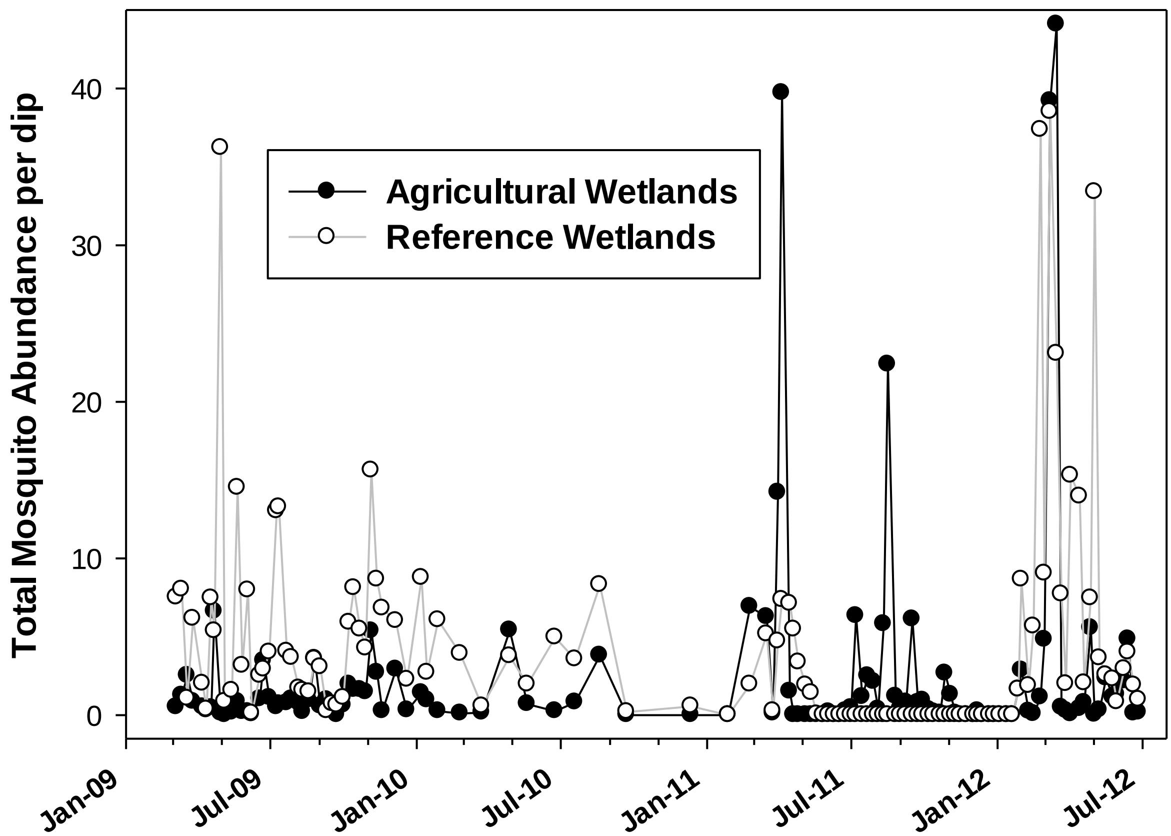


Figure 2.3: Percentage of six dominant land use/cover categories used to calculate Land Development Intensity (LDI) indices within 100-m radius of the twenty wetland study sites in the Gulf Coastal Plain of Georgia, U.S.A.

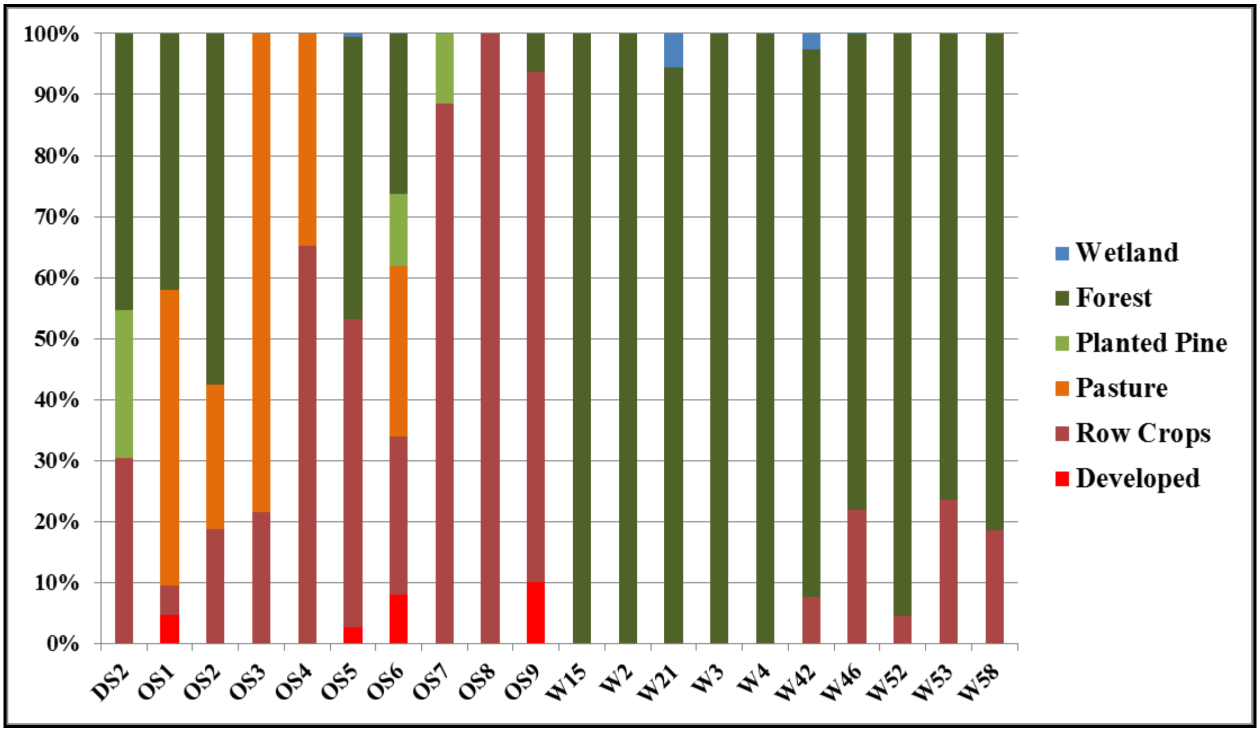
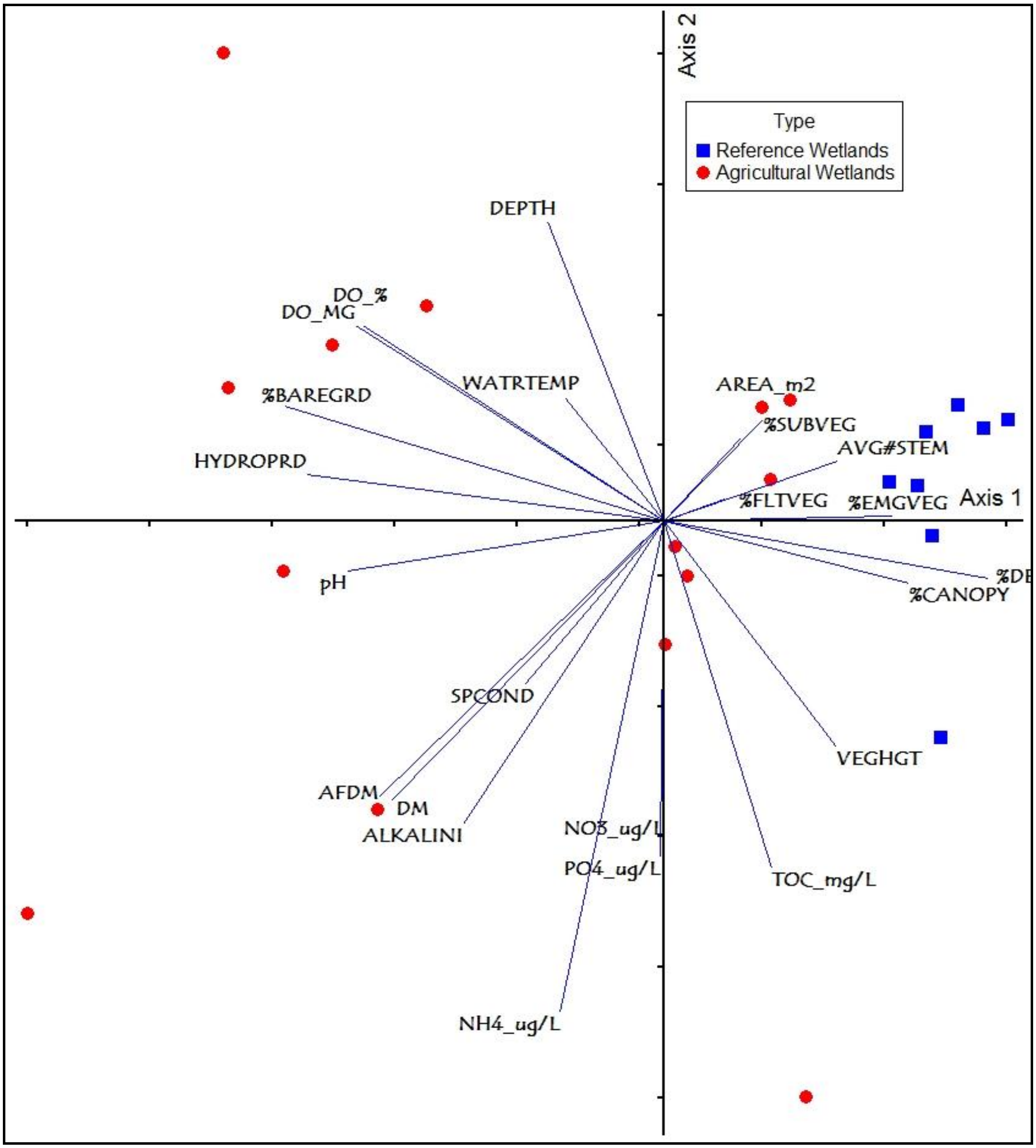


Figure 2.4: The strongest environmental habitat variables were selected from Principle Components Analysis (PC-ORD, MjM Software Design, v. 5.10) ordination results and averaged over the three sample periods.



CHAPTER 3

NUTRIENT ENRICHMENT AFFECTS IMMATURE MOSQUITO ABUNDANCE AND SPECIES COMPOSITION IN FIELD-BASED MESOCOSMS

ABSTRACT

Previous studies have examined relationships between nutrient enrichment and immature mosquito (larvae and pupae) assemblages in mesocosms and constructed water treatment wetlands. This study is the first to examine the relationship between nutrient enrichment and immature mosquito assemblages using field-based mesocosms adjacent to reference and agricultural wetlands in the Gulf Coastal Plain of Georgia, U.S.A. Agricultural wetlands in this region are known to have higher levels of nutrients and suspended sediments compared with forested reference wetlands. ANOVA results indicated overall mosquito abundance, diversity, *Aedes albopictus*, and *Culex restuans* were greater in mesocosms treated with fertilizer compared with non-fertilized mesocosms. Vegetation height, vegetation stem density, and phosphate levels were greater in fertilized mesocosms compared with non-fertilized mesocosms. Nutrient enrichment influenced immature mosquito abundance and assemblage composition, and supported mosquitoes of medical concern. Additionally, these experimental results provide information on the value of using mosquitoes as bioindicators of wetland condition.

INTRODUCTION

In addition to their nuisance feeding behavior, adult mosquitoes in Georgia and other parts of the southeastern U.S.A. serve as vectors of important human and livestock pathogens, including West Nile virus (WNV), Eastern Equine encephalitis virus (EEEV), St. Louis encephalitis virus (SLEV), and LaCrosse encephalitis virus (LACV) (Lance-Parker, Rebmann et al. 2002; Buckner, Blackmore et al. 2011). Human land use has been linked to changes in mosquito community composition (Leisnham, Lester et al. 2004; Muturi, Shililu et al. 2006; Reiter and Lapointe 2007; Johnson, Gómez et al. 2008) and concurrent changes in disease transmission patterns (Patz, Graczyk et al. 2000; Norris 2004; DeGroote, Sugumaran et al. 2008). For example, human WNV incidence was strongly associated with rural agricultural and row crop settings in Iowa (DeGroote, Sugumaran et al. 2008). Understanding the influence of nutrient enrichment on the distribution and abundance of mosquitoes within an agricultural landscape is important in predicting the distributions of disease vector or other nuisance mosquito species.

Altering landscapes through agricultural development can change the physical, chemical, and biological characteristics of larval mosquito habitats, in turn, affecting oviposition and larval survival (Norris 2004; Leisnham, Slaney et al. 2005). The suitability of a mosquito breeding site depends on complex interactions of abiotic factors such as precipitation, temperature, humidity, and soil moisture (Buckner, Blackmore et al. 2011), in addition to biotic factors such as competition (Reiskind and Wilson 2008), predation (Chase and Shulman 2009), and food availability (Blaustein and Kotler 1993; Walker, Merritt et al. 1997; Palik, Batzer et al. 2006; Yee and Juliano 2006). The addition of nutrients from runoff containing chemical fertilizer or livestock waste has been shown to elevate levels of microbial bacteria in the water column in agricultural wetlands in the Gulf Coastal Plain of Georgia (Atkinson, Golladay et al. 2011). This nutrient enrichment can influence mosquito oviposition preference directly by increasing microbes that mosquito larvae feed upon (Walker, Lawson et al. 1991).

Agriculture is an important part of Georgia's economy and is the dominant economic activity in rural areas of southwestern Georgia. Since the 1970s, mechanized agricultural practices such as tilling, leveling of agricultural fields, and in particular, center-pivot irrigation, have shaped the landscape and hydrology of the region. This intensive land use and associated runoff alters the water chemistry of adjacent isolated wetlands, resulting in elevated pH levels, higher nitrate (NO_3-N), phosphate (PO_4-P), ammonium nitrogen (NH_3-N), and suspended sediments compared with reference wetlands (Battle, Golladay et al. 2001; Atkinson, Golladay et al. 2011). Immature mosquito (larvae and pupae) abundance is known to be greater in constructed water treatment wetlands enriched with NH_4-N (Sanford, Chan et al. 2005), and positively associated with dissolved nitrates and phosphates in natural wetlands (Mercer, Sheeley et al. 2005). Nutrient-rich conditions may favor mosquito species that serve as disease vectors (Chaves, Keogh et al. 2009).

Oviposition is an important stage in the mosquito life cycle, and females of different species exploit diverse habitats that promote the growth and survival of their offspring. Identifying individual factors influencing oviposition preference is difficult because of natural variability; but mesocosm studies have proved useful. For example, previous research examining nutrient addition and shade treatments found that mosquito productivity was highest in treatments receiving full sunlight and having medium detrital loads (5 g sheep manure/L of water) (Leisnham, Lester et al. 2004). Several studies indicated that mosquitoes preferentially select oviposition habitats that ensure a reliable food resource. For example, *Anopheles pseudopunctipennis* oviposited exclusively in containers treated with filamentous algae, and algae represented 47% of the gut contents of the larval mosquitoes (Bond, Arredondo-Jiménez et al. 2005). In Israel, *Culiseta longiareolata* oviposition rates were four times greater in artificial pools treated with extra food (ground fish and mouse chow), compared with unsupplemented pools (Blaustein and Kotler 1993). Results from constructed water treatment wetlands in California showed that adult mosquito production was nine times greater in wetlands enriched with NH_4-N, compared with unenriched wetlands (Sanford, Chan et al. 2005). Another mesocosm experiment demonstrated that female *Culex restuans*, a vector of WNV among bird populations, preferred nutrient-enriched containers (Reiskind and Wilson 2004). Similarly, the production of *Culex tarsalis*, another important WNV vector, increased in California rice fields that were nutrient enriched from the incorporation of straw (Lawler and Dritz 2005).

Many studies have reported on the influence of nutrient enrichment on oviposition preferences in container habitats within temperate, tropical, and laboratory settings (Trexler, Apperson et al. 1998; Leisnham, Lester et al. 2004; Reiskind and Wilson 2004; Bond, Arredondo-Jiménez et al. 2005) and also larger scale constructed water treatment wetlands (Sanford, Chan et al. 2005). However, none of these studies have examined the relationship between nutrient enrichment and immature mosquito assemblages in field-based mesocosms adjacent to reference and agricultural wetlands in the Gulf Coastal Plain of Georgia, U.S.A.

MATERIALS AND METHODS

Study sites

Mesocosm sites were located on the J. W. Jones Ecological Research Center at Ichauway (Figure 3.1), a 12,000 ha reserve that has been managed for several decades with prescribed fire to maintain and restore the longleaf pine (*Pinus palustris*) forest and wiregrass (*Aristida stricta*) dominated groundcover. The reserve contains more than 30 seasonal wetlands and several ephemeral pools (Smith, Steen et al. 2006), which are surrounded by second growth longleaf pine forest and native groundcover. A reference mesocosm site was located in second growth longleaf pine forest with native groundcover and adjacent to a forested wetland. An agricultural mesocosm site was located on the border of the J.W. Jones Ecological Research Center and adjacent to an agricultural wetland influenced by row crop agriculture typical of the region (Figure 3.2).

Experimental design and methods

On January 24, 2012, mesocosms (clear plastic storage containers, 67 x 40 cm, 30 cm height) were established to simulate wetland ecosystems. Such mesocosms are within the size ranges used in other non-container breeding mosquito mesocosm studies (Eitam, Blaustein et al. 2002; Silberbush, Blaustein et al. 2005; Stav, Leon et al. 2005; Carver, Storey et al. 2009; Petranka and Doyle 2010). Mesocosms at each site were placed along the outer perimeter of the delineated wetland, to prevent flooding, and were arranged in a 4 x 3 grid with an inter-mesocosm distance of one meter (Figure 3.3). At the beginning of the experiment, each mesocosm was filled with ~10 cm of sand and maintained throughout the study with a depth of ~15 cm of reverse osmosis water. Each mesocosm received a soil core (20 cm diameter x 10 cm height) containing *Panicum hemitomon* plants and rhizomes from a marsh wetland located nearby. Mesocosms were established in a randomized complete block design with two levels of nutrient input (fertilized or non-fertilized). Six mesocosms at each site were randomly selected to receive a fertilization treatment of 36.5g of NPK water soluble fertilizer (20:20:20) on February 28, 2012. Mesocosms remained uncovered throughout the study period, allowing for colonization by aquatic insects.

Immature mosquitoes (larvae and pupae) were sampled weekly in each mesocosm from March 2, 2012 - June 25, 2012 (18 sampling dates). Microhabitats in open water and within emergent vegetation were sampled by taking 10 (350mL) dip samples in each mesocosm. Larval mosquito samples were compiled from each mesocosm and placed in plastic vials for transport to the laboratory. Reliable taxonomic identification (species level) of larval mosquitoes requires fourth-instar larvae. Therefore, < fourth-instar larvae were reared in the laboratory on a diet of homogenized aquarium fish food in containers of filtered wetland water. Fourth-instar larvae were heat-killed and preserved in 95% EtOH for identification. Because mosquito pupae are difficult to identify to species, they were allowed to eclose to the adult stage and preserved in vials for identification.

Eleven environmental habitat variables were characterized in each mesocosm on June 24, 2012, and included: 1) water temperature, 2) dissolved oxygen 3) specific conductance 4) pH levels, 5) alkalinity, 6) suspended solids, 7) nitrate (NO_3-N), 8) phosphate (PO_4-P), 9) ammonium nitrogen (NH_4-N), 10) stem density of emergent vegetation, and 11) vegetation height. The mean of three random plant height measurements was recorded, and vegetation stem density was measured by counting the number of emergent plant stems within each mesocosm. Water temperature, pH levels, specific conductance, and dissolved oxygen measurements were taken in each mesocosm using a portable meter (Quana Hydrolab, Yellow Springs Instrument Co., Inc., Yellow Springs, OH). Water samples from each mesocosm were held on ice, and processed within 24 hours in the laboratory. These samples were analyzed for pH, alkalinity, and filtered for suspended solids (ash-free dry mass) using standardized methods (Eaton, Clesceri et al. 2005). Nitrate (NO_3-N), phosphate (PO_4-P), and ammonium nitrogen (NH_3-N), were determined using a Lachat Quickem 8000 flow-injection colormetric method (Lachat Instruments, Milwaukee, WI). Alkalinity and pH were assessed with a Mettler DL15 titrator (Mettler-Toledo Inc., Columbus, Ohio).

Data analysis

Immature mosquito species abundance was expressed as the number of individuals per 350 mL dip sample, then summed for each mesocosm and used in an ANOVA (Sigma Stat, Systat Software Inc., v. 12.0) where treatment (non-fertilized and fertilized) was the factor of interest, and sites were blocked to control for random variation between sites. Shannon-Wiener diversity indices were used to characterize mosquito species diversity in each mesocosm. The Shannon-Wiener diversity index (H) accounts for both abundance and evenness of the species present. The proportion of species i relative to the total number of species (pi) is calculated and then multiplied by the natural logarithm of this proportion ($\ln pi$). The resulting product is summed across species and multiplied by -1 to give the Shannon-Wiener diversity index.

$$H = -\sum pi \ln pi$$

The relationship between mosquito species and four possible site/treatment combinations was assessed using Indicator Species Analysis (ISA) (PC-ORD, MjM Software Design, v.5.10). ISA was performed on the summed species abundance data collected from each mesocosm across all dates and grouped by each site/treatment combination. The ISA produces indicator values for each species based on the relative abundance and frequency of the species to a group (McCune, Grace et al. 2002).

RESULTS

We collected 8,160 immature mosquitoes from mesocosms over 18 sample dates, comprising nine species. *Culex restuans* (70%), *Anopheles punctipennis* (17%), and *Culex territans* (9%), and *Culex salinarius* (2%), accounted for almost 95% of the total mosquitoes collected in the fertilized mesocosms, whereas *Anopheles punctipennis* (63.0%), *Culex restuans*

(28.0%), and *Culex territans* (5.5%) accounted for over 95% of the total mosquitoes collected from the non-fertilized mesocosms (Figure 3.4).

ANOVA results with blocking showed overall mosquito abundance ($F = 27.9$, $p < 0.001$) (Figure 3.5), diversity ($F = 7.4$, $p = 0.013$), *Aedes albopictus* abundance ($F = 6.8$, $p = 0.016$), and *Culex restuans* abundance ($F = 31.2$, $p < 0.001$) were greater in fertilized mesocosms compared with non-fertilized mesocosms (Table 3.1). Vegetation height ($F = 87.7$, $p < 0.001$), stem density ($F = 59.2$, $p < 0.001$), and PO_4-P ($F = 12.3$, $p = 0.002$) were significantly greater in fertilized compared to non-fertilized mesocosms (Table 3.2).

The ISA identified five species with significant indicator values. *Aedes albopictus* (IV = 90.0, $p < 0.001$), *Culex restuans* (IV = 67.3, $p < 0.001$), and *Culex territans* (IV = 70.1, $p = 0.008$) were associated with fertilized mesocosms at the reference site. *Culex salinarius* (IV = 65.7, $p = 0.005$), was associated with fertilized mesocosms at the agriculture site and *Anopheles crucians* (IV = 48.9, $p = 0.039$) was associated with non-fertilized mesocosms at the agriculture site (Table 3.3).

DISCUSSION

This study confirmed that certain mosquito species have distinct habitat preferences for larval development. Females show oviposition preferences for habitats with structural complexity that provides enhanced food resources, shelter from physical disturbance, and an ideal thermal environment for larval development (Orr and Resh 1992). In California wetlands dominated by *Myrophyllum aquaticum*, oviposition rates of *Anopheles* females increased as stem density approached 2000 stems m^{-2} (Orr and Resh 1992). Similarly, the number of emerged *Culex erythrothorax* was positively correlated with the density of emergent vegetation and nearly absent from open water California wetland areas (Workman and Walton 2000). In this study, vegetation height and stem density were significantly greater in fertilized mesocosms and the corresponding greater overall mosquito abundance, and the abundance of *Culex restuans* and *Aedes albopictus* were likely related to these habitat characteristics. Mosquito diversity was also greater in fertilized mesocosms. Habitat variables in the fertilized treatments could induce a greater number of mosquitoes to oviposit in these treatments, and these conditions might also optimize larval growth, development, and survival.

Overall mosquito abundance was dominated by *Culex restuans* (62%). The abundance of this species was greater in fertilized mesocosms, and was also associated with fertilized mesocosms at the reference site. The wetland adjacent to the reference site had a hydroperiod that was 45 days longer than the wetland adjacent to the agriculture site. This longer hydroperiod provided more opportunities for colonization of mesocosms by mosquitoes emerging from the reference wetland. *Cx. restuans*' association with fertilized treatments is consistent with previous studies showing females preferred to oviposit in nutrient-enriched containers compared with control containers (Reiskind and Wilson 2004; Savage, Aggarwal et al. 2007), and also water containing decaying grass or leaves (Carpenter and LaCasse 1955).

Aedes albopictus, the Asian tiger mosquito, was introduced to the U.S.A. in 1980, and first recorded in Georgia in 1986 (Womack, Thuma et al. 1995). Previous research showed female *Ae. albopictus* oviposited preferentially in containers infused with detritus (Trexler, Apperson et al. 1998; Obenauer, Allan et al. 2010), particularly *Panicum* grasses compared with control containers holding only water (Santana, Roque et al. 2006). Fertilized mesocosms likely have a greater amount of senescing vegetation (personal observation), which could be responsible for this species' association with these treatments.

Anopheles crucians was associated with non-fertilized mesocosms at the agriculture site. Previous studies in Belize showed *An*. *crucians* occurred most often in heavily shaded areas of marshes and was also negatively associated with cyanobacteria mats (Grieco, Johnson et al. 2006). This research is consistent with our results, which showed *An*. *crucians* abundance was greatest in non-fertilized mesocosms along the northern perimeter of the array, which provided the most shade, and where cyanobacteria mats were uncommon (personal observation).

Culex salinarius has the ability to occupy undisturbed fresh and saltwater margins, lakes, and ponds but can also tolerate nutrient-rich conditions (Rochlin, Dempsey et al. 2008), which helps to explain its greater abundance, but not significantly ($p = 0.075$), in fertilized mesocosms. ISA results revealed *Cx*. *salinarius* was associated with fertilized mesocosms at the agriculture site, and previous studies have also linked this species with agricultural wetlands of southwestern Georgia (Buckner, Blackmore et al. 2011).

Culex territans is commonly found in clear-water habitats with grassy stands of emergent vegetation (Joy and Clay 2002), where females prefer to oviposit (McIver 1969). *Cx*. *territans*' greater abundance in fertilized treatments, although not significant ($p = 0.066$), is likely because these treatments had a significantly higher vegetation stem density compared with non-fertilized treatments (Table 3.2). This species prefers the cooler waters of swamps and large wetland pools (Carpenter and LaCasse 1955), which is consistent with its association at the forested reference site, which had a lower average water temperature compared with the open canopy agriculture site (Table 3.2).

This study revealed species-specific site preferences and it is recommended that future research investigate the effects of surrounding land use with greater replication and a particular focus on the interaction between land use and water quality. The well documented and species-specific larval habitat requirements make mosquitoes valuable bioindicators of wetland condition that reflect land use. Aquatic macroinvertebrates are frequently used for monitoring habitat quality in lotic systems, but macroinvertebrates are less commonly used as indicators in lentic systems (Rosenberg and Resh 1993; Foote and Rice Hornung 2005). Mosquitoes meet many of the criteria previously described as ideal biomonitoring tools (Rosenberg and Resh 1993; Bonada, Prat et al. 2006), including: 1) immature mosquitoes are ubiquitous and found in a variety of habitats, ranging from small collections of water in artificial containers and tree holes to large areas of water such as wetland complexes (Silver 2008), 2) mosquitoes are species rich, with approximately 60 species found in Georgia alone (Carpenter and LaCasse 1955), offering a range of responses to environmental stress, 3) sampling and sorting mosquitoes is simple and inexpensive (Silver 2008), 4) the taxonomy of mosquito species is well-developed (Carpenter and LaCasse 1955), and 5) methods of collecting and surveying mosquito populations are standardized (Silver 2008).

This study showed that nutrient enrichment influenced immature mosquito species abundance and supported mosquitoes of medical concern in field-based mesocosms in the Gulf Coastal Plain of Georgia. However, it is important to note that because mesocosms were maintained with water and never allowed to dry, this study selected for mosquito species that oviposit on the water surface, and did not include floodwater species that lay eggs on damp substrata that hatch when subsequently submerged (i.e. *Aedes* and *Psorophora*) (Knight, Walton et al. 2003). It is recommended that wetland management strategies should focus on reducing nutrient enrichment in agricultural areas in order to decrease the abundance of vector-borne diseases in this region.

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Table 3.1: Mean mosquito abundance, diversity, and species abundance by site and treatment are shown. ANOVAs were used in the analysis, blocking by site and testing the effect of treatment (non-fertilized and fertilized).

	Site		**Treatment**		**ANOVA with block**
	Reference	**Agriculture**	**Non-Fertilized**	**Fertilized**	**p value**
Mosquito abundance	40.8	26.0	12.8	53.9	<0.001*
Mosquito diversity	9.7	11.2	7.3	13.6	0.013*
Ae. albopictus	0.5	0.1	0.0	0.6	0.016*
An. crucians	0.0	0.5	0.4	0.1	0.213
An. punctipennis	6.5	10.8	8.1	9.2	0.354
An. quadrimaculatus	0.0	0.0	0.0	0.0	0.329
Cx. coronator	0.1	0.1	0.0	0.1	0.194
Cx. pipiens	0.2	0.34	0.0	0.5	0.198
Cx. restuans	29.8	11.5	3.6	37.6	<0.001*
Cx. salinarius	0.0	1.2	0.0	1.2	0.075
Cx. territans	3.7	1.6	0.7	4.6	0.066

*Indicates p is significant at the 95% level

Table 3.2: Mean habitat and water chemistry variables by site and treatment are shown. ANOVAs were used in the analysis, blocking by site and testing the effect of treatment (non-fertilized and fertilized).

	Site		Treatment		ANOVA with block
Habitat variables	**Reference**	**Agriculture**	**Non-Fertilized**	**Fertilized**	**p value**
Vegetation height (cm)	81.2	67.9	63.7	85.4	< 0.001*
Vegetation stem density	72.8	103.8	63.1	113.4	< 0.001*
Alkalinity (mg $CaCO_3$/L)	1.8	18.6	9.5	10.9	0.371
pH (S.U.)	6.0	6.7	6.3	6.3	0.637
AFDM (mg/L)	30.3	32.9	33.0	30.3	0.818
Water temperature (C°)	23.8	25.0	24.4	24.4	0.765
Specific conductance (μS/m)	0.0	0.1	0.0	0.0	0.610
Dissolved oxygen (mg/L)	4.9	5.8	5.3	5.4	0.774
PO_4-P (μg/L)	63.7	28.2	6.4	85.5	0.002*
NO_3-N (μg/L)	4.9	0.0	0.0	4.9	0.322
NH_4-N (μg/L)	47.5	17.4	38.8	26.0	0.328

*Indicates p is significant at the 95% level

Table 3.3: Indicator species analyses (ISA) computing indicator value (IV) coefficients of weekly mosquito species abundance grouped by site (reference or agriculture) and treatment (non-fertilized or fertilized). An IV closer to 100 represents a strong species association with a group (McCune, Grace et al. 2002).

Species	**Group**	**IV Value**	**Mean**	**S.Dev**	**p value**
Ae. albopictus	Reference, Fertilized	90.0	28.7	11.1	<0.001*
An. crucians	Agricultural, Non-fertilized	48.9	24.7	10.5	0.040*
An. punctipennis	Agricultural, Non-fertilized	31.4	29.4	2.1	0.172
An. quadrimaculatus	Reference, Non-fertilized	16.7	16.7	0.2	1.000
Cx. coronator	Reference, Fertilized	18.4	23.5	10.1	0.547
Cx. pipiens	Agricultural, Fertilized	11.2	18.7	10.7	1.0000
Cx. restuans	Reference, Fertilized	67.3	36.5	6.6	<0.001*
Cx. salinarius	Agricultural, Fertilized	65.7	21.7	11.2	0.005*
Cx. territans	Reference, Fertilized	70.1	35.2	11.0	0.008*

*Indicates p is significant at the 95% level

Figure 3.1: The two mesocosm study sites are representative of wetland sites surrounded by row crop agriculture and forested reference land use and located at the Jones Ecological Research Center at Ichauway in the Gulf Coastal Plain of Georgia, U.S.A.

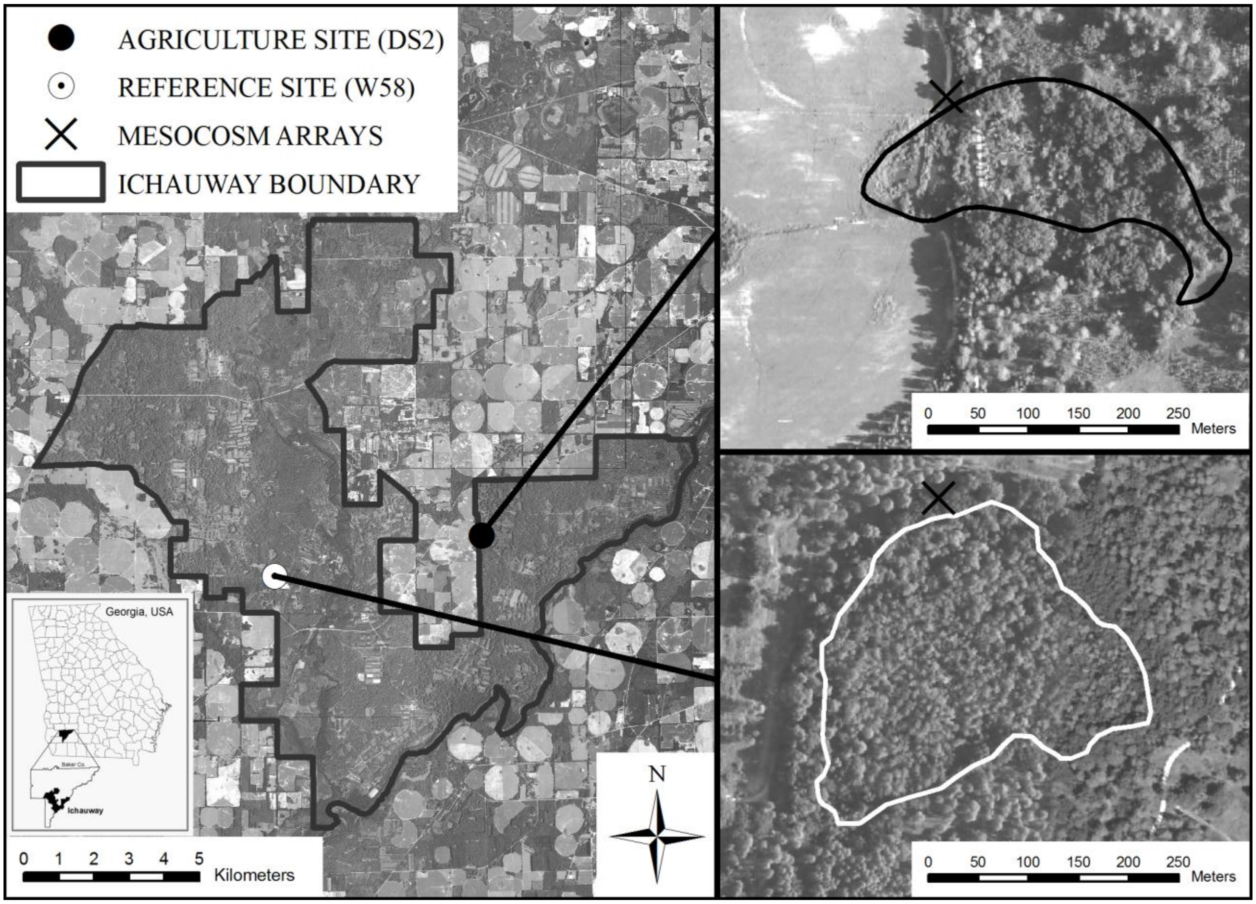


Figure 3.2: Percentage of four dominant land use/cover categories within a 500-m radius around two mesocosm study sites at the Jones Ecological Research Center at Ichauway in the Gulf Coastal Plain of Georgia, U.S.A.

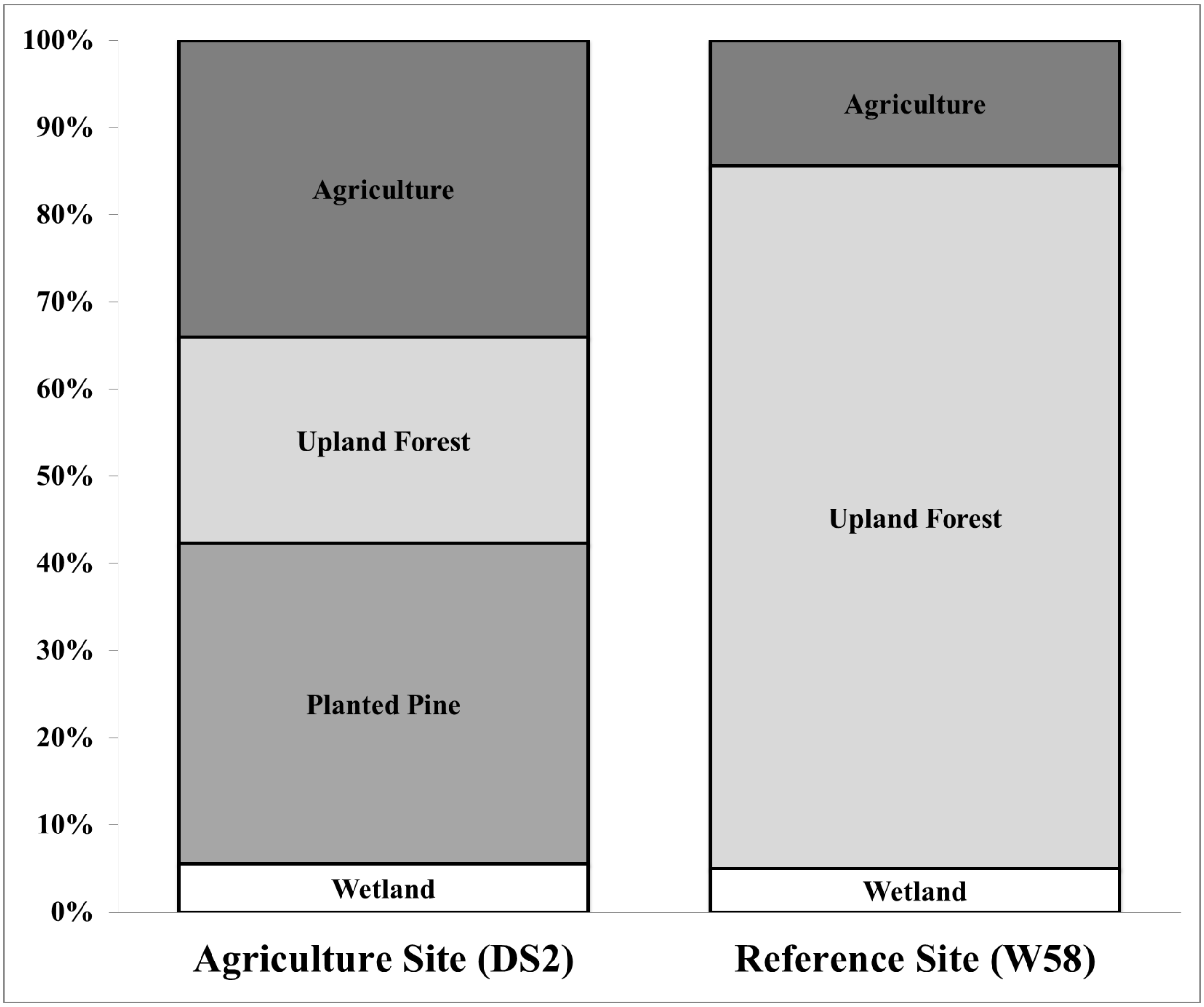


Figure 3.3: Diagrams of mesocosm treatment placements at the forested reference and agriculture sites.

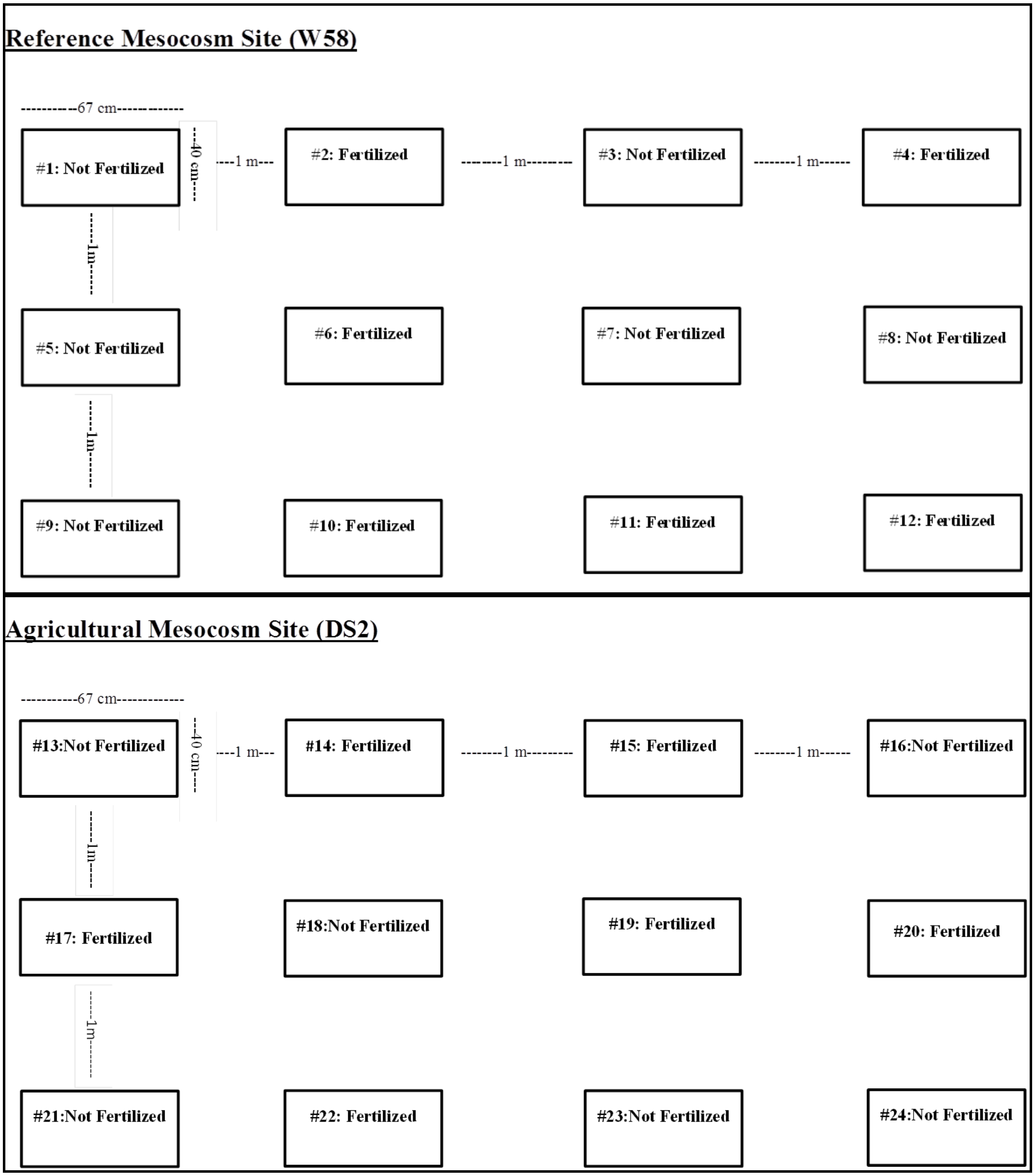


Figure 3.4: Percentage composition of mosquito species collected in fertilized and non-fertilized mesocosms from March 2, 2012 - June 25, 2012.

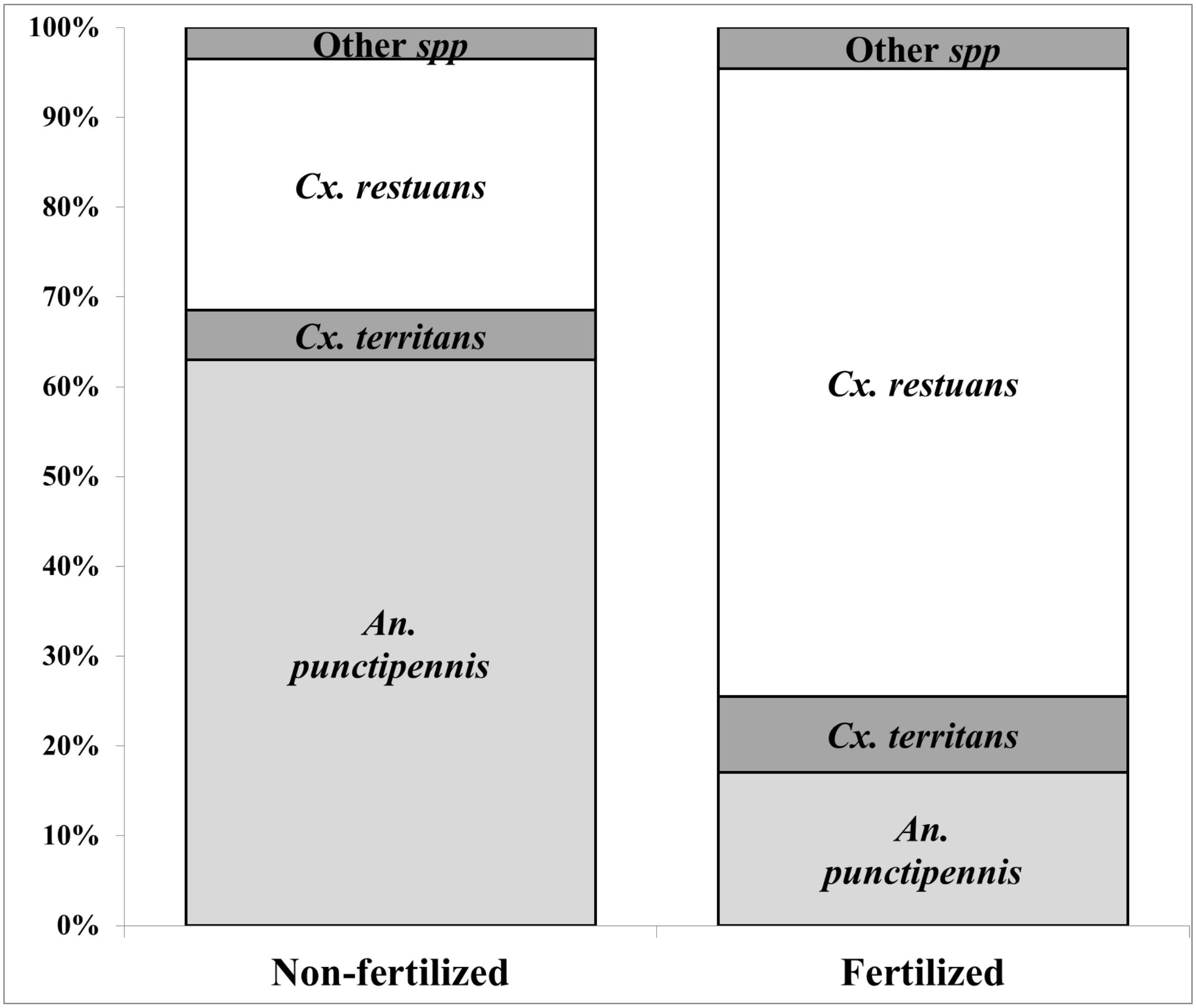
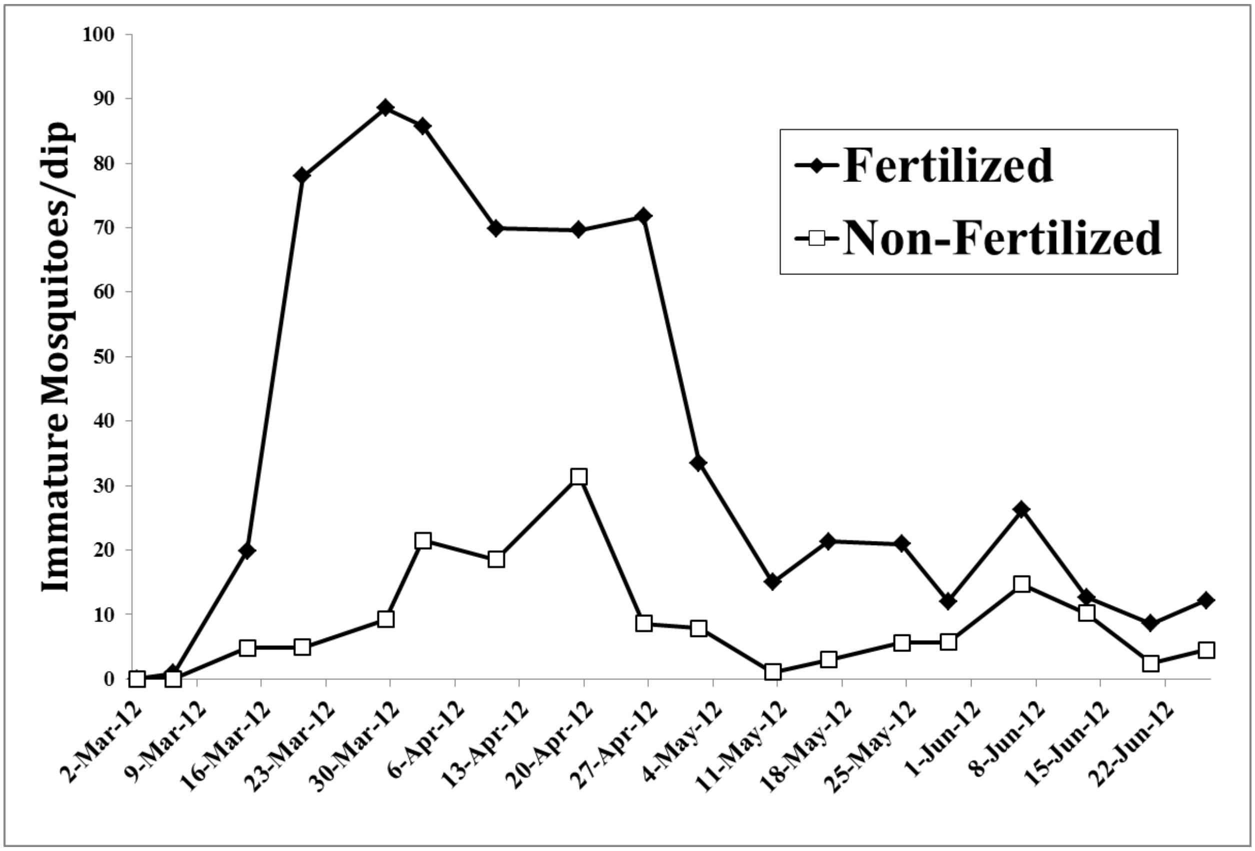


Figure 3.5: The seasonal distribution of immature mosquito abundance in fertilized and non-fertilized mesocosms from March 2, 2012 - June 25, 2012.



CHAPTER 4

STABLE ISOTOPE ANALYSIS OF LARVAL MOSQUITO DIETS IN AGRICULTURAL WETLANDS

ABSTRACT

Previous studies have used $\delta^{13}C$ and $\delta^{15}N$ isotopes to investigate the use of different food resources such as plant and animal detritus by container-breeding mosquitoes. This study is the first to report on the potential food resources assimilated by larval mosquitoes in agricultural and reference wetlands throughout a seasonal hydroperiod. IsoSource mixing model results indicated that in agricultural wetlands, food resources had greater $\delta^{15}N$ isotope values compared with cypress-gum swamps. During the early hydroperiod, *Aedes vexans* and *Culex territans* larvae fed primarily on lower quality food resources (coarse particulate organic matter and sediment), based on C:N. In contrast, higher quality food resources (fine particulate organic matter) were utilized by *Anopheles* spp. and *Psorophora columbiae* throughout the hydroperiod and during the late hydroperiod, respectively. This research contributes to a more comprehensive understanding of the food resources available and assimilated by larval mosquitoes in agricultural wetlands, which is important for predicting mosquito habitat suitability and vector-borne diseases in highly altered landscapes.

INTRODUCTION

In addition to their nuisance feeding behavior, adult mosquitoes in Georgia and other parts of the southeastern U.S.A. serve as vectors of important human and livestock pathogens, including West Nile virus (WNV), Eastern Equine encephalitis virus (EEEV), St. Louis encephalitis virus (SLEV), and LaCrosse encephalitis virus (LACV) (Lance-Parker, Rebmann et al. 2002; Buckner, Blackmore et al. 2011). Human land use has been linked to changes in mosquito community composition (Leisnham, Lester et al. 2004; Muturi, Shililu et al. 2006; Reiter and Lapointe 2007; Johnson, Gómez et al. 2008) and with concurrent disease transmission patterns (Patz, Graczyk et al. 2000; Norris 2004; DeGroote, Sugumaran et al. 2008). For example, human WNV incidence was strongly associated with rural agricultural and row crop land use in Iowa (DeGroote, Sugumaran et al. 2008).

The suitability of a mosquito breeding site depends on complex interactions of abiotic factors such as precipitation, temperature, humidity, and soil moisture (Buckner, Blackmore et al. 2011), in addition to biotic factors such as competition (Reiskind and Wilson 2008) and predation (Chase and Shulman 2009). Food availability and quality are also important factors that influence mosquito oviposition, larval survival, and subsequent adult production (Blaustein and Kotler 1993; Walker, Merritt et al. 1997; Palik, Batzer et al. 2006; Yee and Juliano 2006; Winters and Yee 2012). Altering landscapes through agricultural development can change the physical, chemical, and biological characteristics of larval mosquito habitats, in turn, affecting oviposition and larval survival (Norris 2004; Leisnham, Slaney et al. 2005; Reiter and Lapointe 2007).

Invertebrate communities and food webs vary between forested and marsh wetland habitats (Battle and Golladay 2001; Taylor and Batzer 2010), and may be influenced by differences in the availability and quality of food resources (e.g., plant species composition, detritus, and algae). In the Gulf Coastal Plain, forested cypress-gum swamps typically have longer hydroperiods and large detrital inputs that reduce dissolved oxygen concentrations and lower aquatic invertebrate diversity (Battle and Golladay 2001). Grass-sedge marshes dominated by herbaceous vegetation have greater diversity and density of aquatic invertebrates, which is partially attributed to the availability of both macrophyte detritus and periphyton food resources (Battle and Golladay 2001). In contrast, agricultural wetlands in the region have fewer invertebrate species (Battle, Golladay et al. 2001). Previous studies indicated that wetland habitat type was important in determining larval mosquito diets. For example, *Anopheles quadrimaculatus* larvae consumed more water mites and rotifers in open water habitats compared with vegetated zones (Wallace and Merritt 2004). Different sources and types of detritus can influence oviposition preferences as well as larval mosquito survival and growth (Palik, Batzer et al. 2006; Yee and Juliano 2006; Reiskind, Greene et al. 2009; Ponnusamy, Xu et al. 2010; Winters and Yee 2012).

Understanding the relationship between larval mosquitoes and their food source and quality is essential to understanding the role and species composition of mosquitoes in wetland food webs. Larval mosquitoes are filter-feeders, collector-gatherers, and grazers with diets consisting of organic detritus and microorganisms, such as bacteria and algae. A variety of detrital food resources are suspended in the water column or associated with benthic substrates (Merritt, Craig et al. 1996). Larval *Anopheles*, *Culex*, and *Culiseta*, are considered filter-feeders, extracting suspended fine particulate organic matter (FPOM; size range 0.45 μm to 1 mm) in the water column (*Culex*) or at the air-water interface (*Anopheles*); whereas *Aedes* and *Psorophora* are considered collector-gatherers, feeding in both the water column and on sediments (Merritt, Dadd et al. 1992; Winters and Yee 2012). Knowledge of larval mosquito feeding behavior and diets have important implications for larval abundance, survival, and subsequent disease transmission (Yee, Kesavaraju et al. 2004; Yee and Juliano 2006; Winters and Yee 2012). For example, laboratory studies indicated that decreased food availability led to lower survival and reduced adult biomass of *Culex quinquefasciatus* and *Culex tarsalis* (Peck and Walton 2005). Similarly, containers with some animal detritus versus leaf-only containers produced larger *Aedes albopictus* and *Culex restuans* adults that had higher survival rates (Winters and Yee 2012). The body size of a female mosquito is important, as it can influence fecundity (Ameneshewa and Service 1996; Blackmore and Lord 2000) and vector potential (Hawley 1985; Paulson and Hawley 1991; Nasci and Mitchell 1994; Alto, Reiskind et al. 2008).

Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) is a powerful tool that has been used to identify food resources contributing to higher trophic levels in wetlands (Blaustein, Kiflawi et al. 2004; Opsahl, Golladay et al. 2010; Taylor and Batzer 2010). Primary food resources have consistent differences in the ratio of $\delta^{13}C$ / $\delta^{12}C$, derived from unique isotopic signatures in C_3 and C_4 plants, so that herbivore food preference is reflected in a consumer's tissue (Batzer and Wissinger 1996). Consumers with a diverse food base can be matched to their food supply using a mixing model (e.g., U.S. Environmental Protection Agency's IsoSource), which determines the proportion of diets derived from distinct food sources (Taylor and Batzer 2010). Mixing models have indicated that invertebrates in forested wetlands primarily consume detritus from trees, epiphyton, detrital FPOM, sediment, and macrophyte detritus; whereas, algae, sediment, and macrophyte detritus were the primary food resources for invertebrates in marshes (Taylor and Batzer 2010). In Gulf Coastal Plain marshes, C and N isotope analyses suggested that filter-feeders, such as fairy shrimp and ostracods, used phytoplankton as important food resources (Opsahl, Golladay et al. 2010). Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) has also been used to show that plant detritus contributed up to 80% of mosquito pupal biomass in tree-hole ecosystems, while no more than 30% of tissue was animal detritus (Kaufman, Pelz-Stelinski et al. 2010). Similarly, container experiments indicated that *Aedes albopictus* had higher $\delta^{15}N$

values compared with *Culex restuans,* which is likely the result of higher feeding rates and the direct consumption of detritus-derived microorganisms by browsing feeding behavior (Winters and Yee 2012). Knowledge of larval mosquito feeding behavior and diets have important implications for the success of mosquito biological control agents, such as *Bascillus thuringiensis* (Bt), that must be ingested (Merritt, Craig et al. 1996).

This study used natural stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to examine the relative dietary contributions of food resources to larval mosquitoes in agricultural and reference wetlands in the Gulf Coastal Plain of Georgia throughout a seasonal hydroperiod. This research contributes to a more comprehensive understanding of the food resources available and assimilated by larval mosquitoes in agricultural wetlands, which is important for predicting mosquito habitat suitability and vector-borne diseases in highly altered landscapes.

METHODS AND MATERIALS

Study sites

The study was conducted in the Gulf Coastal Plain of Georgia, U.S.A., a region dominated by peanut, corn, and cotton row crop agriculture. Based on presence of water, seven reference sites (four cypress-gum swamps and three grass-sedge marshes) and five wetlands affected by agricultural land use (center-pivot irrigation, row crops and pasture) were used to collect food resources and larval mosquitoes for isotope analysis (Figure 4.1). Cypress-gum swamps are dominated by a closed canopy of pond cypress (*Taxodium ascendens*) and black gum (*Nyssa biflora*), while the grass-sedge marshes lack trees and are dominated by emergent C_3 and C_4 grasses (Kirkman, Goebel et al. 2000). The reference wetlands are located at the J. W. Jones Ecological Research Center at Ichauway, a 12,000 ha reserve that has been managed for several decades with prescribed fire to maintain and restore the longleaf pine (*Pinus palustris*) forest and wiregrass (*Aristida stricta*) dominated groundcover. The reserve contains more than 30 seasonal wetlands and several ephemeral pools (Smith, Steen et al. 2006), which are surrounded by second growth longleaf pine forest and native groundcover

Sample collecting and processing

Study site wetlands were sampled for larval mosquitoes and food resources, when water was present, on February 17, April 9 - 10, and May 16 - 17, 2012, representing the early, middle, and late hydroperiod. Because of an extended drought (NOAA 2012), sampling was limited to 1 - 3 sites from each wetland type (grass-sedge marsh, cypress-gum swamp, and agriculture) during each sampling period. Food resources that were assumed to be important in larval mosquitoes diets included: 1) coarse particulate organic matter (CPOM > 1 mm), 2) 125 µm fine particulate organic matter (125 µm < FPOM < 250 µm), 3) 45 µm fine particulate organic matter (45 µm < FPOM < 125 µm), and 4) sediment. Larval mosquito samples were compiled from each wetland and placed in plastic vials for transport to the laboratory. In the field, fine particulate organic matter (FPOM) was separated into two particle sizes using a series of sieves (250µm, 125µm, and 45µm), and then filtered on to glass fiber filters (Type AFFA, EMD Millipore, Inc., Billerica, MA), in the laboratory. Sediment samples were collected from beneath benthic litter.

Upon returning to the laboratory, mosquito larvae were identified to species. Mosquito larvae, vegetation, CPOM, FPOM, and sediment samples were rinsed with deionized water, dried at 45°C for 48 hours, and pulverized to a fine powder using a ball-mill grinder or mortar and pestle (Opsahl, Golladay et al. 2010). All samples were weighed into tin capsules and carbon and nitrogen isotope content was determined using an elemental analyzer coupled with a mass spectrometer (Analytical Chemistry Laboratory, Odum School of Ecology, University of Georgia, Athens, GA). C:N ratios were also determined for each sample.

Isotope and larval mosquito diet analyses

A Kruskal-Wallis one way analysis of variance (ANOVA) was used to test for differences in $\delta^{13}C$ and $\delta^{15}N$ content of food resource and mosquito samples among wetlands during each sample date, because the majority of the data violated the assumption of normality (Sigma Stat, Systat Software Inc., v. 12.0). Dunn's test was used for pairwise comparisons. Bi-plots were used to graph average $\delta^{13}C$ and $\delta^{15}N$ values from individual wetlands from each wetland type.

The contributions of potential food resources assimilated by mosquitoes were determined using the IsoSource (Microsoft Visual Basic, v. 1.3.1) mixing model, developed by the U.S. Environmental Protection Agency. This software calculates ranges of possible food resource proportions contributing to a consumer, when the number of sources is too large to permit a unique solution (> number of isotope systems + 1) (Phillips and Gregg 2003). IsoSource has been used in previous studies to investigate wetland invertebrate diets (Taylor and Batzer 2010).

$\delta^{13}C$ and $\delta^{15}N$ mean values for food resources and mosquito species samples in grass-sedge marshes, cypress-gum swamps, and agriculture wetlands were provided to the model for each sample period. In grass-sedge marshes, *Aedes vexans*, *Culex territans,* and *Psorophora columbiae* were used in the analysis. In cypress-gum swamps, *Culex territans* and *Anopheles punctipennis* were used in the analysis. In agricultural wetlands, *Aedes vexans*, *Anopheles punctipennis, Anopheles quadrimaculatus,* and *Psorophora columbiae* were used in the analysis. In order to account for trophic isotope fractionation from consumer assimilation of source material, 2.0‰ was subtracted from the $\delta^{15}N$ mean value and 1.0‰ was subtracted from the $\delta^{13}C$

mean value of each mosquito sample (McCutchan, Lewis et al. 2003; Taylor and Batzer 2010). All possible combinations of source contributions to mosquito diet from 0-100% were examined at increments of 2.0%, and the mass balance tolerance was set to 0.1 – 1.0‰. If statistics could not be generated within these limits, it was concluded that the model could not be used to analyze the mosquito data set.

RESULTS

Carbon and Nitrogen analyses

Mosquitoes in grass-sedge marshes had a $\delta^{13}C$ range from -28.0 to -25.8‰ and $\delta^{15}N$ range from 2.3 to 11.4. Potential basal food resources from grass-sedge marshes had a $\delta^{13}C$ range from -28.4 to -14.3‰ and $\delta^{15}N$ range from -0.6 to 10.8 (Figure 4.2). These basal food resources had C:N ratios between 5.6 and 22.4 (Table 4.1).

Mosquitoes in cypress-gum swamps had a $\delta^{13}C$ range from -33.3 to -26.2‰ and $\delta^{15}N$ range from -1.3 to 2.4. Potential food resources from cypress-gum swamps had a $\delta^{13}C$ range from -30.2 to -26.5‰ and $\delta^{15}N$ range from -3.8 to 3.7 (Figure 4.2). These food resources had a mean C:N ratios between 5.6 and 51.3 (Table 4.1).

Mosquitoes in agricultural wetlands had a $\delta^{13}C$ range from -29.5 to -21.7‰ and $\delta^{15}N$ range from 4.3 to 8.8. Potential food resources from agricultural wetlands had a $\delta^{13}C$ range from -31.9 to -21.1‰ and $\delta^{15}N$ range from 0.1 to 10.9 (Figure 4.2). These food resources had C:N ratios between 5.9 and 45.9 (Table 4.1).

ANOVA on ranks results indicated that median FPOM C:N values in all the wetlands were significantly lower (higher in quality) compared with CPOM ($p < 0.05$) and sediment ($p < 0.05$). Median $\delta^{15}N$ values for mosquitoes and all food resources were significantly greater in agricultural wetlands compared with cypress-gum swamps ($p < 0.05$), but not grass-sedge marshes ($p > 0.05$). Mosquitoes and food resources were not significantly ($p > 0.05$) enriched with $\delta^{15}N$ in grass-sedge marshes compared with cypress-gum swamps, with the exception of FPOM ($p < 0.05$) (Table 4.1). Median $\delta^{13}C$ values and C:N in mosquitoes and food resources were not significantly different in any of the wetland types ($p > 0.05$) (Table 4.1).

Larval mosquito diet analyses

For the grass-sedge marshes, IsoSource analyses in February suggest that *Aedes vexans* larvae primarily consumed CPOM (0-98%: 60.2%), and *Culex territans* larvae primarily consumed 125 µm FPOM (60-74%: 67.4%). During April, *Culex territans* larvae in grass-sedge marshes primarily consumed CPOM (26-40%: 33.6%) and sediment (12-42%: 26.5%). During May, *Psorophora columbiae* larvae in grass-sedge marshes primarily consumed 45 µm FPOM (74-82%: 78.3%) (Table 4.2).

For the cypress-gum swamps, IsoSource analyses in February suggest that *Anopheles punctipennis* larvae primarily consumed 125 µm FPOM (82-100%: 92.2%). During April, *Culex territans* larvae in cypress-gum swamps primarily consumed CPOM (56-84%: 72.1%). During May, *Culex territans* larvae in cypress-gum swamps primarily consumed CPOM (88-94%: 90.3%) (Table 4.2).

For the agricultural wetlands, IsoSource analyses in February suggest that *Aedes vexans* larvae primarily consumed CPOM (40-64%: 54%) and *Anopheles punctipennis* larvae primarily consumed 45 µm FPOM (86-94%: 88.7%). During April, *Anopheles quadrimaculatus* larvae in agricultural wetlands primarily consumed 45 µm FPOM (70-76%: 72.7%). During May, *Psorophora columbiae* larvae in agricultural wetlands primarily consumed 125 µm FPOM (96-100%: 97.3%) (Table 4.2).

DISCUSSION

This study indicated the use of food resources by larval mosquitoes varied by species, wetland type, and date. Results from agricultural wetlands showed food resources and mosquitoes had significantly greater median $\delta^{15}N$ isotope values compared with cypress-gum swamps. Mosquitoes in all wetland types generally fed on lower quality particles (CPOM and sediment) early in the hydroperiod and shifted to higher quality particles (FPOM) later in the hydroperiod. The different feeding behaviors of larval mosquitoes help to explain the observed associations between food resources and mosquito species in different wetland types.

During the early hydroperiod, *Aedes vexans* primarily fed on CPOM, a lower quality food resource, in both grass-sedge marshes and agricultural wetlands. This mosquito is a floodwater species, which oviposits desiccation-resistant eggs on damp substrata that hatch when subsequently submerged (Knight, Walton et al. 2003). This life history strategy allows this species to take advantage of the early hydroperiod when there are fewer mosquito predators (Moorhead, Hall et al. 1998; Lawler and Dritz 2005), but is also a time when FPOM availability may be limited. Previous studies indicated that when suspended particulate food was depleted, *Aedes vexans* moved more frequently to the bottom substrate in habitats to collect food materials using their palatal brushes (Merritt, Dadd et al. 1992). This behavior is consistent with our results which indicated *Aedes vexans* was feeding on bottom substrate food resources.

Throughout the hydroperiod, *Culex territans* fed primarily on CPOM in cypress-gum swamps. In grass-sedge marshes, this species fed primarily on 125 µm FPOM in the early hydroperiod and CPOM and sediment in the mid-hydroperiod. This species is commonly found in clear-water habitats with grassy stands of emergent vegetation (Joy and Clay 2002), and was not found in agricultural wetlands. *Culex* spp. are filter-feeders that feed primarily in the water column, and previous studies indicated that *Culex territans* rarely leave the air-water interface to feed (Merritt, Dadd et al. 1992). In contrast, our research shows *Culex territans* primarily fed on CPOM and sediment from bottom substrata. *Culex territans* may have fed on lower quality food resources because they were the most abundant resource in these habitats.

During the early and middle hydroperiod, *Anopheles punctipennis* and *Anopheles quadrimaculatus* in agricultural wetlands fed primarily on 45 µm FPOM, whereas in cypress-gum swamps, *Anopheles punctipennis* fed primarily on 125 µm FPOM. When feeding, *Anopheles* larvae position their bodies parallel to the water surface, near the air-water interface (Merritt, Dadd et al. 1992). *Anopheles* spp. larvae tend to aggregate near plant stems and algal mats, and previous studies indicated that this surface microlayer is a food-enriched zone, containing greater concentrations of bacteria compared with subsurface water zones (Walker and Merritt 1993). Our results indicated *Anopheles* spp. feeding on FPOM in agricultural and cypress-gum swamps were exploiting a high quality food resource, which was most likely abundant in the surface microlayer zone.

During the late hydroperiod, *Psorophora columbiae* fed primarily on 45 µm FPOM in grass-sedge marshes and 125 µm FPOM in agricultural wetlands. *Psorophora* spp. are considered collector-gatherers, and feed in both the water column and on sediments (Merritt, Dadd et al. 1992). Our results indicate that *Psorophora columbiae* fed on higher quality food resources, which may be more abundant later in the hydroperiod when the microbial community has developed and fine detritus is generated.

This study revealed that FPOM suspended in the water column was an important food resource for mosquito larvae, and was higher in quality in agricultural wetlands. Previous studies in the Gulf Coastal Plain of Georgia showed that the concentration of planktonic microbes was generally greater in agricultural wetlands compared with grass-sedge marshes and cypress-gum swamps (Atkinson, Golladay et al. 2011). Agricultural wetlands with higher quality food resources have implications for mosquito abundance and vector potential. Larval mosquitoes that were fed higher quality animal-based detritus led to greater survival and estimated population growth compared with mosquitoes fed plant-based detritus (Yee and Juliano 2006), which consequently increases the risk of disease transmission. Previous research indicated that containers with more abundant plant and insect detritus produced larger adult mosquitoes (Tun-Lin, Burkot et al. 2000). Adult mosquito size affects fecundity (Ameneshewa and Service 1996; Blackmore and Lord 2000) and vector potential in opposing ways. Larger adult mosquitoes have been shown to have increased longevity, which could enhance vector potential (Hawley 1985), whereas smaller mosquitoes have been shown to blood-feed more frequently which could also enhance vector potential (Paulson and Hawley 1991; Nasci and Mitchell 1994; Alto, Reiskind et al. 2008). It is recommended that future research investigate the influence of food resources in agricultural wetlands on mosquito larval survival, development time, and adult biomass, in order to determine the vector potential of mosquitoes in highly altered agricultural landscapes.

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Table 4.1: Multiple comparisons of mosquito and sampled food resource median $\delta^{15}N$, $\delta^{13}C$, and C:N values in grass-sedge marshes, cypress-gum swamps, and agricultural wetland types during the three sample periods.

Wetland Type	**Source**	**$\delta^{15}N$ Median**	**$\delta^{13}C$ Median**	**C:N Median**
All Wetlands	CPOM	3.13	-28	28.35
All Wetlands	FPOM	3.71	-27.86	8.52
All Wetlands	Sediment	2.35	-25.99	13.79
Marsh	Mosquito	5.02	-26.13	4.33
Swamp	Mosquito	1.02	-30.18	4.17
Agriculture	Mosquito	7.09	-28.19	4.35
Marsh	CPOM	3.58	-27.03	18.42
Swamp	CPOM	-0.25	-27.78	33.19
Agriculture	CPOM	4.98	-26.99	28.31
Marsh	FPOM	3.61	-27.79	8.75
Swamp	FPOM	0.82	-29.64	10.63
Agriculture	FPOM	4.35	-29.26	7.518
Marsh	Sediment	2.02	-26.68	12.98
Swamp	Sediment	-0.56	-27.81	18.49
Agriculture	Sediment	4.85	-24.13	13.49
Source	**Multiple Comparisons**	**$\delta^{15}N$ $p < 0.05$**	**$\delta^{13}C$ $p < 0.05$**	**C:N $p < 0.05$**
	All CPOM vs. All FPOM	No	No	Yes
	All CPOM vs. All Sediment	No	No	No
	All FPOM vs. All Sediment	No	No	Yes
Mosquito	Marsh vs. Swamp	No	No	No
Mosquito	Marsh vs. Agriculture	No	No	No
Mosquito	Swamp vs. Agriculture	Yes	No	No
CPOM	Marsh vs. Swamp	No	No	No
CPOM	Marsh vs. Agriculture	No	No	No
CPOM	Swamp vs. Agriculture	Yes	No	No
FPOM	Marsh vs. Swamp	Yes	No	No
FPOM	Marsh vs. Agriculture	No	No	No
FPOM	Swamp vs. Agriculture	Yes	No	No
Sediment	Marsh vs. Swamp	No	No	No
Sediment	Marsh vs. Agriculture	No	No	No
Sediment	Swamp vs. Agriculture	Yes	No	No

Table 4.2: Food resource contributions to mosquito larvae in grass-sedge marshes, cypress-gum swamps and agricultural wetland types during the three sampling periods.

Wetland Type	**Time**	**Mosquito Species**	**Food Source**	**Mean**	**1%tile**	**99%tile**
Marsh	February	*Aedes vexans*	CPOM	0	0	0
Marsh	February	*Aedes vexans*	45 µm FPOM	2.1	0	6
Marsh	February	*Aedes vexans*	125 µm FPOM	44.5	34	58
Marsh	February	*Aedes vexans*	Sediment*	53.4	42	60
Marsh	February	*Culex territans*	CPOM	0	0	0
Marsh	February	*Culex territans*	45 µm FPOM	7.2	0	16
Marsh	February	*Culex territans*	125 µm FPOM*	67.4	60	74
Marsh	February	*Culex territans*	Sediment	25.4	24	28
Marsh	April	*Culex territans*	CPOM*	33.6	26	40
Marsh	April	*Culex territans*	45 µm FPOM	24.5	0	56
Marsh	April	*Culex territans*	125 µm FPOM	15.4	0	36
Marsh	April	*Culex territans*	Sediment	26.5	12	42
Marsh	May	*Psorophora columbiae*	CPOM	6.5	0	18
Marsh	May	*Psorophora columbiae*	45 µm FPOM*	78.3	74	82
Marsh	May	*Psorophora columbiae*	125 µm FPOM	2	0	8
Marsh	May	*Psorophora columbiae*	Sediment	13.1	0	22
Swamp	February	*Anopheles punctipennis*	CPOM	5.8	0	18
Swamp	February	*Anopheles punctipennis*	45 µm FPOM	1	0	4
Swamp	February	*Anopheles punctipennis*	125 µm FPOM*	92.2	82	100
Swamp	February	*Anopheles punctipennis*	Sediment	1	0	4
Swamp	April	*Culex territans*	CPOM*	72.1	56	84
Swamp	April	*Culex territans*	45 µm FPOM	0.4	0	2
Swamp	April	*Culex territans*	125 µm FPOM	0.5	0	2
Swamp	April	*Culex territans*	Sediment	27	16	44
Swamp	May	*Culex territans*	CPOM*	90.3	88	94
Swamp	May	*Culex territans*	45 µm FPOM	0.7	0	0
Swamp	May	*Culex territans*	125 µm FPOM	8.7	6	8
Swamp	May	*Culex territans*	Sediment	0.3	0	2
Agriculture	February	*Aedes vexans*	CPOM*	54	40	64
Agriculture	February	*Aedes vexans*	45 µm FPOM	44	30	60
Agriculture	February	*Aedes vexans*	125 µm FPOM	2	0	6
Agriculture	February	*Aedes vexans*	Sediment	0	0	0
Agriculture	February	*Anopheles punctipennis*	CPOM	2.1	0	8
Agriculture	February	*Anopheles punctipennis*	45 µm FPOM*	88.7	86	94
Agriculture	February	*Anopheles punctipennis*	125 µm FPOM	2.6	2	10
Agriculture	February	*Anopheles punctipennis*	Sediment	6.7	0	14
Agriculture	April	*Anopheles quadrimaculatus*	CPOM	0.2	0	2
Agriculture	April	*Anopheles quadrimaculatus*	45 µm FPOM*	72.7	70	76
Agriculture	April	*Anopheles quadrimaculatus*	125 µm FPOM	2	0	6
Agriculture	April	*Anopheles quadrimaculatus*	Sediment	25.1	24	28
Agriculture	May	*Psorophora columbiae*	CPOM	13.5	12	18
Agriculture	May	*Psorophora columbiae*	45 µm FPOM	0.7	0	4
Agriculture	May	*Psorophora columbiae*	125 µm FPOM*	83	78	88
Agriculture	May	*Psorophora columbiae*	Sediment	2.8	0	10

*Represents dominant food resource for the mosquito species during the sampling period.

Figure 4.1: Seven reference sites (four cypress-gum swamps and three grass-sedge marshes) and five wetlands affected by agricultural land use in the Gulf Coastal Plain of Georgia, U.S.A. were sampled for immature mosquitoes and food resources used in the isotope analysis comparing wetland type and season.

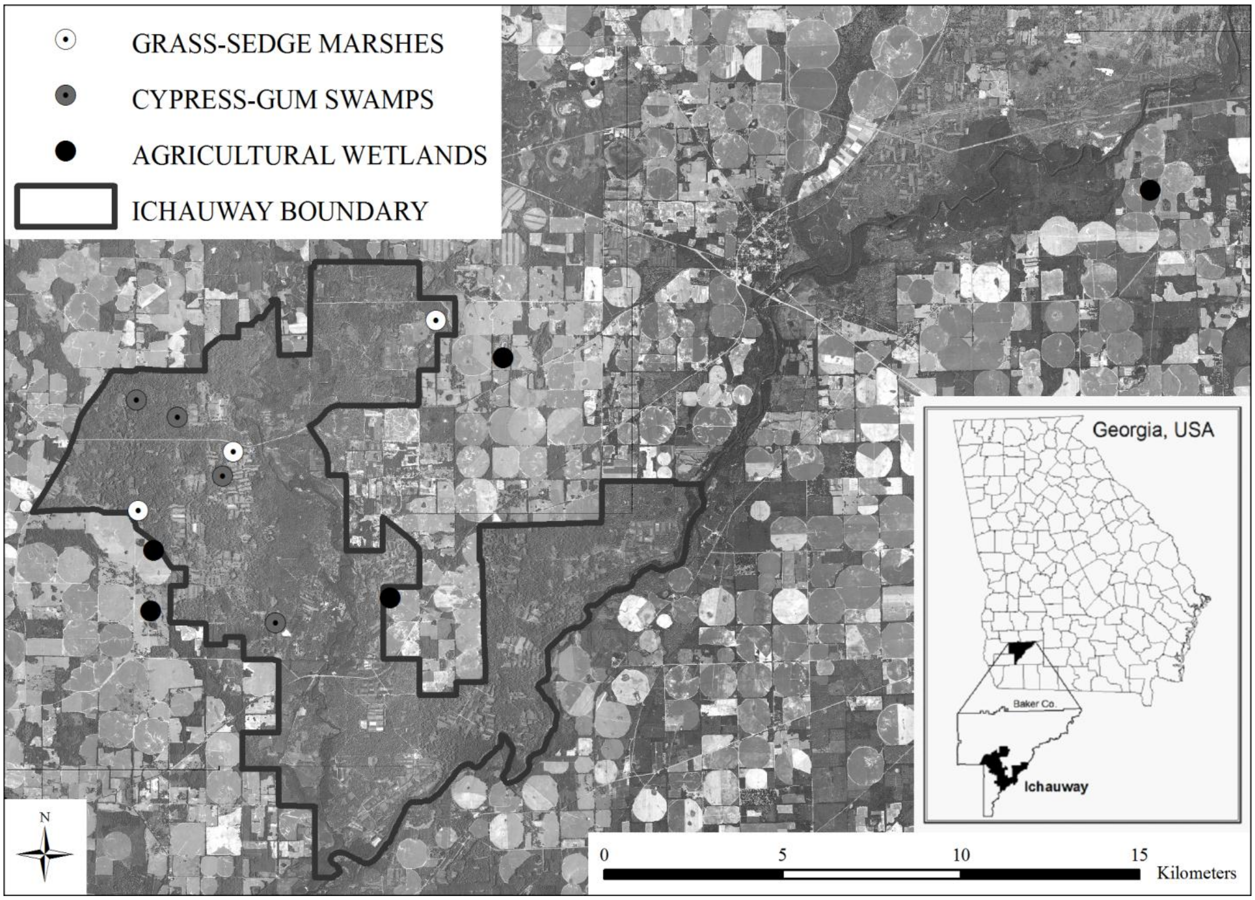
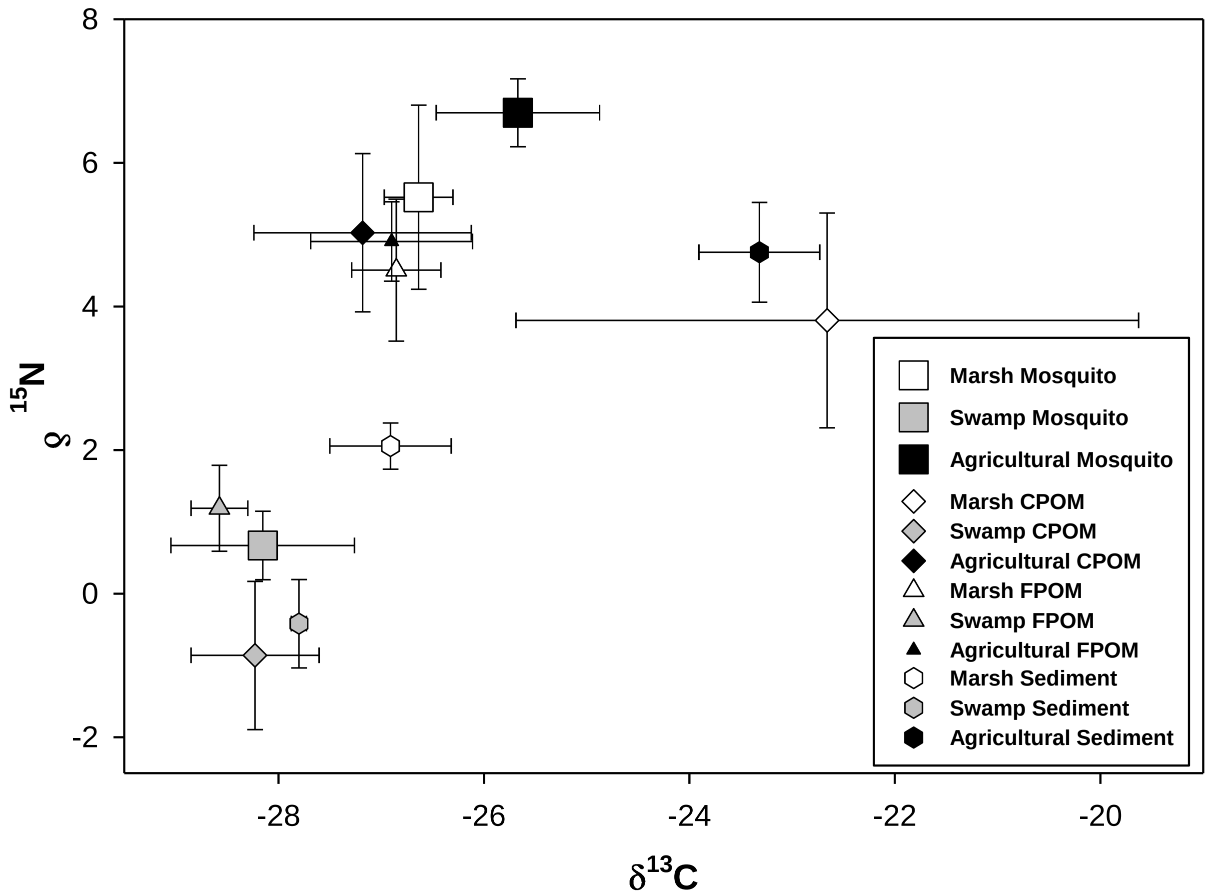


Figure 4.2: Bi-plot of stable isotope composition of larval mosquitoes and food resources in grass-sedge marshes, cypress-gum swamps and agricultural wetland types throughout the hydroperiod. Values are means +- SE from 3-8 replicates.



CHAPTER 5

CONCLUSIONS

Most mosquito surveillance focuses on urban areas and few observational data of mosquito assemblages are available from rural areas of the southeastern U.S.A. (Buckner et al. 2011). The expansion of mosquito-borne disease, and outbreak of WNV during the summer of 2012 (CDC 2012) has increased the need to determine how altered wetland conditions influence mosquito ecology. This research investigated the influence of selected landscape and environmental habitat variables, nutrient enrichment, and food resources on immature mosquito abundance, diversity, and species composition on in agricultural wetlands of the Gulf Coastal Plain of Georgia, U.S.A. (Figure 1.1).

Prior to this study, the key landscape and environmental habitat variables that determine larval mosquito assemblages in isolated wetlands of the Gulf Coastal Plain remain largely undocumented. Previous studies have examined immature mosquitoes in rice and cattle agroecosystems (McLaughlin, Vidrine et al. 1987; Lawler and Dritz 2005; Leisnham, Slaney et al. 2005; Muturi, Shililu et al. 2006) and adult mosquitoes in row crop agricultural areas (Buckner, Blackmore et al. 2011). This is the first regional study to investigate the relationships between immature mosquitoes and landscape and environmental habitat characteristics in row crop agricultural wetlands during wet and dry hydrologic regimes. Indicator Species Analysis (ISA) was used to test for associations among mosquito species and groups of wetland sites with similar Landscape Development Intensity (LDI) values. Results indicated *Anopheles quadrimaculatus, Culex erraticus*, and *Psorophora columbiae* were associated with agricultural wetlands (LDI > 2.0); whereas *Anopheles crucians* and *Culex territans* were associated with forested reference wetlands (LDI < 2.0) in both wet and dry years. The species fidelity to wetland type, regardless of the hydrologic regime, demonstrates these species are robust indicators of wetland condition. Data on immature mosquito assemblages were compared to selected landscape and environmental habitat variables using Akaike's Information Criterion (AIC_c) model selection. LDI indices, dissolved oxygen concentration, the proportion of emergent vegetation, and the proportion of bare ground in wetlands were important factors associated with the selected mosquito species. These results indicate that LDI indices are useful in predicting the distributions of disease vector or other nuisance species across broad geographic areas. Additionally, these results suggest mosquitoes are valuable bioindicators of wetland condition that reflect land use and hydrologic variability. The habitat preferences of several common and nuisance species (i.e. *Anopheles quadrimaculatus* and *Culex erraticus*) are not well known in agricultural areas, and because of their link with human and livestock pathogens, it is recommended that future research should focus on the habitat preferences of these species.

Agricultural wetlands in this region are known to have higher levels of nutrients and suspended sediments compared with reference wetlands (Battle, Golladay et al. 2001; Atkinson, Golladay et al. 2011). Many studies have reported on the influence of nutrient enrichment on oviposition preferences in container habitats within temperate, tropical, and laboratory settings (Trexler, Apperson et al. 1998; Leisnham, Lester et al. 2004; Reiskind and Wilson 2004; Bond, Arredondo-Jiménez et al. 2005) and also larger scale constructed water treatment wetlands (Sanford, Chan et al. 2005). However, none of these studies have examined the relationship between nutrient enrichment and immature mosquito assemblages in field-based mesocosms adjacent to reference and agricultural wetlands in the Gulf Coastal Plain. ANOVA results indicated overall mosquito abundance, diversity, *Aedes albopictus*, and *Culex restuans* were greater in mesocosms treated with fertilizer compared with non-fertilized mesocosms. Vegetation height, vegetation stem density, and phosphate levels were also greater in fertilized mesocosms compared with non-fertilized mesocosms. Nutrient enrichment influenced immature mosquito abundance and assemblage composition, and supported mosquitoes of medical concern. This study revealed species-specific site preferences and it is recommended that future research investigate the effects of surrounding land use with greater replication and a particular focus on the interaction between land use and water quality. Mesocosm results provide additional information consistent with the wetland surveys on the value of using mosquitoes as bioindicators of wetland condition.

Previous studies have used δ^{13}C and δ^{15}N isotopes to investigate the use of different food resources such as plant and animal detritus by container-breeding mosquitoes (Kaufman, Pelz-Stelinski et al. 2010). This study is the first to examine the potential food resources assimilated by larval mosquitoes in agricultural and reference wetlands throughout a seasonal hydroperiod. IsoSource mixing model results indicated that in agricultural wetlands, food resources had greater δ^{15}N isotope values compared with cypress-gum swamps. During the early hydroperiod, *Aedes vexans* and *Culex territans* larvae fed primarily on lower quality food resources (coarse particulate organic matter and sediment), based on C:N. In contrast, higher quality food resources (fine particulate organic matter) were utilized by *Anopheles* spp. and *Psorophora columbiae* throughout the hydroperiod and during the late hydroperiod, respectively. This research contributes to a more comprehensive understanding of the food resources available and assimilated by larval mosquitoes in agricultural wetlands, which is important for predicting mosquito habitat suitability and vector-borne diseases in highly altered landscapes.

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