# Factors affecting mosquito populations in created wetlands

THESIS

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By

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# Abstract

Constructed, created and restored wetlands are gaining popularity due to multiple benefits they provide. However, there is a concern that wetlands increase mosquito breeding in urban areas. This is especially due to the recent concern regarding mosquito borne viral encephalitis and other diseases. Published studies to quantify mosquito population in constructed and natural wetlands are inconclusive. This study quantified the population of mosquitoes from two experimental flow-through created wetlands and two stormwater fed wetland at the Olentangy River Wetland Research Park (ORWRP) in Columbus, Ohio in summer. Sampled mosquitoes were identified to species level to investigate their disease vector potential. The study also compared mono specific and multispecies small (1 $m^2$) mesocosms being used for another experiment. The flow-through created wetlands were less conducive to mosquito breeding compared to the pond ($p<0.00001$) and stormwater wetland ($p=0.002$). Outflow regions and emergent vegetation sites in the flow-through wetlands were most conducive to mosquito breeding than were inflows ($p=0.009$) and floating vegetation sites ($p=0.023$). Mixed vegetation communities (*Sparganium eurycarpum, Juncus effusus,* and *Schoenoplectus tabernaemontani*) rather than mono specific *Typha* communities provided most conducive environment for mosquito breeding ($p<0.0001$). Mesocosm plots with steady inflow (10 cm depth) and with deep water (20 cm) in summer and shallow water (5 cm) in spring had higher mosquito densities than did mesocosm plots with pulsed flow (10 cm depth with inflow rate according to the river stage) and deep water (20 cm) in spring and shallow water (5 cm) in fall. Among water quality parameters, conductivity (p=0.004) and, to a lesser extent, dissolved oxygen (p=0.052) correlated with mosquito larval density (adjusted $R^2$ of 0.67). Six mosquito species identified in all water bodies were *Cx. pipiens, Cx. salinarius, Cx. restuans, Ur. sapphirina*, *An. quadrimaculatus, and An. punctipennis.* Among these *Cx. pipiens* and *Cx. salinarius* are both avian and mammalian blood feeders and hence are potential bridge vectors of the encephalitis viruses. *An. quadrimaculatus,* one of the most potent vector of malaria in U.S. and also a major host of the nematode that causes dog heartworm, was present in all the water bodies. *Cx. pipiens* was the dominant mosquito species in all the water bodies sampled. The information obtained can be incorporated in construction design of wetlands, can be used in the future to target mosquito control tactics and can provide baseline at the ORWRP for future surveillance.

Dedication

Dedicated to my family

# Acknowledgements

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# Chapter 1: Introduction

## *1.1 Background of the Problem*

Created wetlands are now gaining popularity in urban landscapes (IWA, 2000). Wetlands are a crucial part of the ecological chain. They provide many ecosystem services such as stormwater run off, erosion and flood control, improvement of water quality, maintenance of plant and animal communities, and carbon sequestration (Mitsch et al., 2009). However, along with their beneficial aspects, it has been argued that wetlands have the potential to cause public health hazards in urban areas by providing harborage, food, and moisture for some nuisance species like mosquitoes (Vymazal et al., 1998; Russell, 1999; Walton et al., 1999, Knight et al., 2003).

Wetland creation refers to the "conversion of a persistent upland or shallow water area into a wetland by human activity" (Mitsch and Gosselink, 2007). Created wetlands are categorized according to the function they are intended to perform. Constructed wetland, also referred to as treatment wetland, is a type of created wetland developed for contaminant and pollutant removal from wastewater or runoff (Mitsch and Gosselink, 2007). Most of the previous studies have concentrated on mosquito population in constructed wetlands receiving urban, agricultural, and industrial run off, or a combination of these. Literature regarding other forms of created wetlands apart from constructed wetlands is scarce. For constructed wetlands, it has been argued that they may not act as natural wetlands, and so their mosquito production should not be considered simply as another natural function of the habitat. Natural predators are often not capable of controlling mosquito populations in constructed wetlands. The problem gets worse if maintenance in these wetlands is neglected (Russel, 1999).

Mosquito breeding differs with geographic regions. As studies pertaining to mosquito production in created wetlands are limited, the results of these studies can not be generalized. This is also true as most of the studies have been done on constructed wetlands which are different from other types of created and restored wetlands. Further, no studies on created wetlands have been carried out in Ohio. There are 67 different species of mosquitoes known to occur in Ohio however only few species are known to be carriers of pathogens like arboviruses, West Nile Virus (WNV), St. Louis encephalitis (SLE) and eastern equine encephalomyelitis (EEE) viruses (Ohio Health Department, 2008). Most of the species are harmless and, infact, form a crucial component of wetland's ecological food-web. Hence, it is critical to identify mosquitoes to species level to estimate their vector potential. Our study will provide insights into the extent of mosquito production from the wetlands and will estimate the risk of arbovirus (like WNV) by identifying the mosquitoes to species level. The main study site was at the Olentangy River Wetland Research Park, created and restored research wetland site in the city of Columbus, Ohio on The Ohio State University campus.

### *1.2 Rationale and Significance*

From a medical perspective, mosquitoes are arguably the most important group of insects in terms of both economic and health costs worldwide to humans and animals. Mosquito vectors serve as obligate intermediate hosts for numerous diseases like arboviral encephalitides, dengue fever, malaria, rift valley fever and yellow fever (CDC, 2007). Arboviral encephalitides in the United States are mainly due to five virus agents: EEE, western equine encephalitis (WEE), SLE, La Crosse (LAC) encephalitis and WNV, all of which are transmitted by mosquitoes (CDC, 2008).

The understanding of the dynamics of mosquitoes in wetland habitats and their identification to species level can provide critical information for targeting mosquito-control efforts and to estimate the potential pathogen activity in the area. Effective mosquito control intervention requires knowledge of the timing of mosquito breeding activity and breeding site preferences. Therefore, this study is aimed at quantifying the mosquito population in created wetlands and the factors favoring breeding of mosquitoes in these habitats.

### *1.2 Objectives*

This research had the following specific objectives:

***Objective 1.*** To quantify mosquito populations in the two experimental created wetlands, a stormwater wetland, and a pond at the Olentangy River Wetland Research Park by sampling immature mosquitoes and to determine the factors (water quality, flow gradient, emergent and floating vegetation) influencing mosquito abundance.

***Objective 2.*** To identify mosquitoes to species level, and

***Objective 3.*** To determine the effect of vegetation communities and hydrology on mosquito breeding in experimental mesocosms.

# Chapter 2: Literature Review

## *2.1 Mosquito Ecology and Diseases*

Mosquitoes belong to the Class Insecta, the Order Diptera, and Family Culicidae. Some of the genera which are important from the medical point of view include *Aedes*, *Culex* and *Anopheles*. Mosquito borne diseases are a cause of concern not only for humans but also for dogs and horses. Some of the mosquito transmitted diseases include protozoan diseases, e.g., malaria; filarial diseases e.g. dog heartworm; and viral diseases such as dengue, viral encephalitis and yellow fever (CDC, 2007).

The following description of mosquito biology is taken from the review article by Curtis (1996). Mosquito genera differ in their habitat requirements and lifecycle timeframe. There are four main stages in the lifecycle of mosquitoes – egg, larva, pupa and adult. The developmental stages (egg to adult) may take as little as 5 days to as long as 1 month depending on species as well as geographic location and temperature. The eggs are laid in water either singly (*Anopheles* spp. & *Aedes* spp.) or in raft of 200 to 300 eggs (*Culex* spp.). Eggs hatch within one week and then go through four instars which feed on small organisms like algae or decaying organic material. Larvae, also known as wigglers, breathe through a breathing tube called a siphon (in *Culex* and *Aedes* spp.), or lie parallel to the water surface in order to get a supply of oxygen through a breathing opening (*Anopheles* spp.), or in some species (*Mansonia* and *Coquilletidida*) attach to plant stems and obtain oxygen directly from the plant tissue. After the fourth instar, larvae pupate and after 1-4 days emerge as adults. Adult female mosquitoes bite and drink blood and feed on flower nectar whereas male mosquitoes only feed on the nectar of flowers. Usually, the newly emerged adult females require a blood meal to produce and lay eggs. It is during the blood meal that the disease causing pathogen is picked up as well as transmitted to and from hosts. Vertical transmission, i.e. passing pathogen directly from the adult female via the egg to the larva, is also possible in some cases.

Source reduction is one of the important aspects of mosquito control. This project is aimed to determine if constructed wetlands serve as a source of mosquito reproduction. From a wetland perspective, it is important to identify the habitats of the immature stages.

### *2.2 Mosquito Larval Habitat and its Attributes*

Mosquito larvae occupy a wide range of habitats in diverse environmental conditions. Aquatic environments differ chiefly in the chemistry of the water (acid or alkaline; fresh, salt or brackish). These environments may be natural or man-made and may also differ in the amount or type of vegetation present and the amount of sun or shade. Abundance of different mosquito species in a water body may differ depending on the geographic location, water level fluctuation as well as perpetual presence of water, size of water body, vegetation, predator abundance and organic composition of the water (Tenesson, 1993; Russel, 1999). Mosquito species exploiting floodwater habitats like rain pools, snow pools, tree holes, rain barrels, and artificial containers like old tires are from *Aedes, Psorophora* and *Culex* genera. Standing water habitats like freshwater marshes, lakes, ponds, drainage ditches etc. are exploited mainly by *Anopheles,* several *Culex* species, *Uranotaenia* and *Coquillettidia* (Public Health Pest Control, 2007). Usually mosquitoes exploit small shallow water bodies which are high in nutrients and salinity and low in dissolved oxygen content (Tenesson, 1993). In such habitats mosquitoes have higher rates of survival due to abundant food source and low predator populations (Tenesson, 1993, Sarneckis, 2002). *Culex* mosquitoes are opportunistic breeders, preferring man-made habitats to the natural ones. In fact man-made habitats are the primary source of *Culex* mosquitoes (Park and Recreational Department, 2007).

Among the abiotic factors, mosquitoes usually prefer high air humidity. Rainfall can be both a limiting factor as it may flush out breeding places and a positive factor as it fills up water bodies and hence providing more potential habitats. Effect of sunlight or shade varies depending on the species (Fritsch, 1997). Physiochemical water quality factors are difficult to quantify with respect to mosquito abundance. Studies have shown that mosquitoes are present in highest density when the average water temperature is between 23°C and 33°C (Fritsch, 1997). Among the chemical factors, orthophosphate, ammonia nitrogen, and dissolved solids are positively correlated with overall mosquito abundance, while chloride and dissolved oxygen appear to be inversely correlated (Bradley and Kutz, 2006; Sanford et al., 2005; Muturi, 2008). *Anopheles* and *Aedes* prefer clean water, whereas *Culex* prefers water with high biological oxygen demand (Pathak et al., 2002). Mosquito larvae in natural waters are usually inhibited by extremes of pH conditions and occur mostly between the pH ranges 5.8 and 8.6 with *Anopheles* having higher range than *Culicines.* Stagnant water species usually tolerate higher alkalinity and moving water species tolerate higher acidity. In artificial water bodies, however, this generalization does not hold (Senior-White, 1926).

Biotic factors like vegetation type and proportion of coverage are implicated as being better predictors of larval abundance than the physicochemical factors (Walton, 1990). The presence of vegetation and floating plants provide optimal breeding conditions by acting as food sources as well as shelter from predators. Vegetation also creates stagnant conditions by decreasing water movement. Greenway et al. (2003) found that dense monospecific stands of *Typha* (cattail) with an accumulation of submerged dead stems and isolated pockets of water are suitable for mosquito breeding. Similarly, dense mats of floating vegetation are also conducive for mosquito breeding. The abundance of a number of mosquito species is linked to the presence of specific plants (Fritsch, 1997). For example, *Coquillettidia perturbans*, *Mansonia dyari* and *Mansonia titillans* are found in association with *Pistia stratiotes*, *Eichornia crassipes* and *Typha*. In general, among the vegetation types, *Typha* and *Phragmites* are found to be most conducive to mosquito breeding and alternatives like *Schoenoplectus*, *Lepironia*, *Baumea*, *Phylidrum*, *Bolboschoenu* have been suggested (Greenway, 2003). A more recent study however pointed out that *Schoenoplectus californicus* (bulrush) was more conducive to mosquito breeding than *Typha* (Jianinno & Walton, 2004.)

Macroinvertebrates have been suggested to be a crucial factor in the control of mosquito larvae, ensuring that natural predation of the early instar prevents or limits the development of pupae and the emergence of adults (Greenway, 2003). Recent studies on mosquitoes in constructed wetlands have shown that the presence of mosquito larvae can be minimized by increasing macroinvertebrate biodiversity, by planting a variety of macrophyte types and species, and maintaining at least 30% open water (Sarneckis, 2002; Greenway et al., 2003). However, detailed information on the immature mosquito stage habitats is still lacking. Further, concerns have been raised regarding mosquito breeding in wetlands.

### *2.3 Wetland Values*

The values of wetlands have widely been acknowledged. Wetlands are an important ecosystem with higher productivity as compared to other ecosystems and potential of maintaining a high biodiversity (Mitsch and Gosselink, 2007). Constanza et al. (1997) recognized these services and affixed a value of 33 trillion US dollars per year to the services provided by the world's wetlands. Wetland functions are the result of various processes taking place within a wetland namely, water storage, transformation of nutrients, and growth and diversity of biota (Novitzki et al., 1997). These functions can be simplified and described as flood control, groundwater replenishment, shoreline stabilization and storm protection, sediment and nutrient retention and export, climate change mitigation, water purification, reservoirs of biodiversity, and recreation and tourism (Ramsar Convention Bureau, 2002). For these functions, wetlands have been termed as "the kidneys of the landscapes" and "ecological supermarkets" (Mitsch and Gosselink, 2007).

### *2.4 Created and Constructed Wetlands and Concern for Mosquites*

Association of mosquitoes and wetlands is controversial. On one hand mosquito larvae have been claimed to be a component of aquatic food web (USEPA, 2000; Greenway and Simpson, 1996) as well as a part of wetland biodiversity (Shaefer, 2004) and on the other hand, increasing concern over mosquito breeding is being raised due to their potential for disease spread and nuisance especially in the constructed wetlands (Vymazal et al., 1998; Russell, 1999; Walton et al., 1999, Sarneckis, 2002; Knight et al., 2003).

Previous studies comparing mosquito reproduction from natural wetlands and constructed wetlands have shown mixed results (Mayhem, 2004; Greenway, 2005; Shaefer, 2004). Depending upon design, construction, and management, mosquito production from constructed systems can be much greater than from natural systems (Mayhew et al., 2004). Some studies supported the idea that natural predators e.g., water boatmen, backswimmers, predacious diving beetles, water striders, salamander larvae, native fish, dragonflies, purple martins, swallows, etc, present in constructed wetlands keep mosquito population under control just as in natural wetlands (Green et al, 2005; Sarneckis, 2002). Others argued that in constructed wetlands even natural populations of predators may not provide sufficient control (Metzger et al., 2002, Rey et al., 2006). This is true for those constructed wetlands which remain largely unsupervised and are ill maintained. As most of the constructed wetlands are not the target of active surveillance and maintenance, most of the constructed wetlands belong to this category. In terms of macroinvertibrate communities, quick colonization of insects including mosquitoes was seen in newly constructed wetlands (D' Amico et al., 2004; Schafer, 2004) but some studies did not find any difference in natural vs constructed wetlands (Streever at al., 1996; Brown et al., 1997).

Mosquito abundance and species diversity has been positively correlated with the size of the wetland (Hanski & Gyllenberg, 1997; Shafer et al., 2004). Mosquito abundance is usually low in wetlands that have higher biodiversity of macrophytes and macro-invertebrates (Greenway et al., 2003). The presence of litter, emergent and floating vegetation, algae and pollutant traps increase the potential breeding habitats for mosquitoes in wetlands. Factors such as water permanence, flow, and depth of the wetland also influence mosquito breeding (Sarneckis, 2002). Apart from this direct effect, hydrology also has indirect effect on mosquito breeding by having a major influence on all wetland communities (Wissinger, 1999). The regular fluctuations in water level may provide breeding opportunities for some West Nile virus potential bridge vector species of mosquitoes (Wallace, 2007). These mosquitoes are often in close contact with disease reservoirs, such as wild birds, that also are attracted to wetland habitats and can contribute to disease amplification (Shaman et al., 2002).

Restoring wetlands is being implicated as a good alternative to floodwaters and woodland pools which pose serious mosquito problems. Wetland restoration is thought to help by decreasing mosquito populations by providing proper habitat for the natural enemies of mosquitoes, and by reducing flooding. For example, a wetland restoration project in Massachusetts showed a 90 percent drop in mosquito population (IDNR Fact Sheet).

While some of the above studies suggest that restoring wetlands may actually reduce the mosquito production, many other studies indicate otherwise. Mosquito problems led to closure of around half of the pilot water treatment wetlands between 1974 and 1988 (Martin & Eldridge, 1989). Mosquitoes increased around 100-fold when a surface-flow, wastewater treatment constructed wetland was started in Tucson, Arizona (Karpiscak et al., 2004). Irrigated rice fields which make up the largest man-made wetland environment in the world have been associated with many mosquito-borne diseases in many countries (WHO, 1996). Russell (1999) studied constructed wetlands of Australian region and attributed outbreaks of Ross River virus and other arboviruses in Australia to local increases in mosquito populations because of these wetlands. It was observed that areas of increasing arbovirus activity have more wetland habitat and larger mosquito populations than did other areas and these wetlands are often close to residential areas. So, increasing development of constructed waste water wetlands in or near urban communities is a concern. Construction of new wetlands in an area may also lead to enhancement of cycles of zoonotic pathogens (Russell, 1999). In areas where some vector borne disease was endemic but eliminated by reducing the vector habitats, new wetlands may lead to reintroduction or resurgence of transmission (Russell, 1999; Gratz, 1999).

These studies highlight the importance of mosquito surveillance and control in wetland design. However, as most of the studies have been done in treatment wetlands, further studies are warranted to study created wetlands and clearly recognize the factors affecting mosquito breeding in these wetlands.

# Chapter 3: Research Methods and Data Analysis

## *3.1 Study Area*

The study area was the Olentangy River Wetland Research Park (ORWRP), a 20-ha site owned by The Ohio State University, immediately north of the Columbus campus (Mitsch, 2005). Sampling for mosquitoes was carried out in 5 locations: the two experimental wetlands, a stormwater wetland, Odum pond and 40 mesocosm plots (Figure 1).

***3.1.1 Two experimental wetlands.*** Both the experimental wetlands are 2.5-acre deepwater marshes built in 1994 (Mitsch et al., 1998) (Figure 1). Water from Olentangy River is pumped into these wetlands continuously. It then flows by gravity back to the Olentangy River through a swale and constructed stream system. The only difference between the two wetlands is the initial vegetation introduction. Wetland 1 was originally planted with 13 species typical of Midwestern marshes and wetland 2 was left as an unplanted control for natural vegetation to colonize (Mitsch et al., 1998). The two wetlands were similar ecologically until 3-5 years (Mitsch et al., 1998), but started diverging afterwards. After 14 years, the planted wetland had higher macrophyte diversity but lower productivity than naturally colonizing wetland (Mitsch et al., 2005, Mitsch et al., 2009). Water depths in the major portions of the wetland have been maintained generally at 20-40 cm in the shallow areas and 50-80 cm in the deepwater areas (Mitsch et al., 2009.

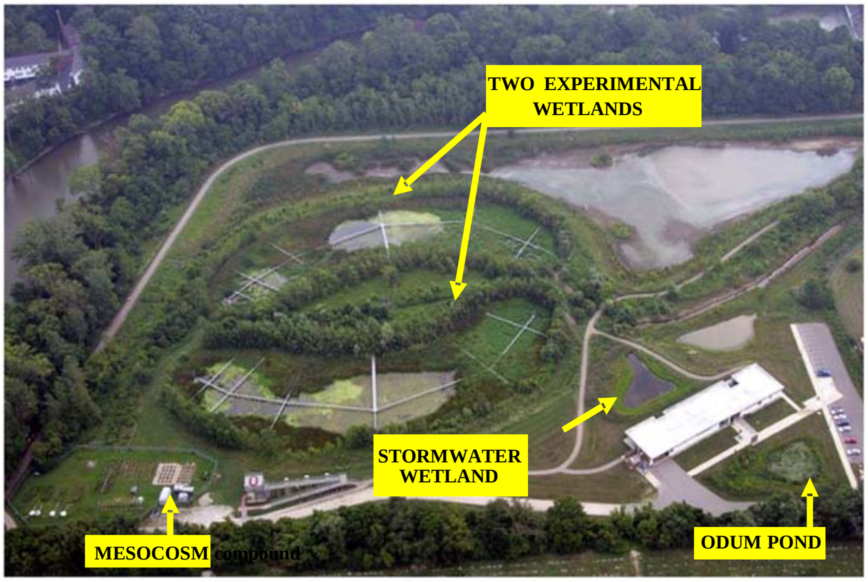


Figure 1. Schematic overview of Olentangy River Wetland Research Park highlighting the study sites.

***3.1.2 Stormwater wetland.*** A 0.13-ha stormwater wetland was constructed in 2002 adjacent to the new Heffner wetland research building (Figure 1). It was designed as a water garden to collect precipitation that falls on the roof of the building and desynchronize the flow to the Olentangy River. It has very rarely overflowed in its 7 years of operation (Mitsch, pers comm.). It was lined with a bentonite liner to minimize subsurface seepage.

***3.1.3 Odum pond***. Similar in area to the stormwater wetlands, Odum pond is located at the entrance to the Heffner wetland research building (Figure 1). It is an isolated pond that overflows only during extreme precipitation events to the swale flowing from the experimental wetland

***3.1.4 Mesocosm wetlands.*** A 40-mesocom experiment already underway in the ORWRP mesocosm compound (Keljo, 2009) was used for mesocosm sampling. Each mesocosm represented a mini experimental wetland (= 0.8 m × 1.3 m × 0.6 m polyethylene tubs) (Figure 1). During the summer of 2008, 20 of these plots were planted with *Typha* and 20 with mixed communities consisting of *Sparganium eurycarpum*, *Juncus effusus*, and *Schoenoplectus tabernaemontani*. These planted plots (cattail or mixed) were further divided into 4 categories each according to their hydrology. The hydrology patterns were designated as ‘WS’, ‘WSP’, ‘P’ and ‘S’ according to their depths and inflows. ‘WS’ is short for "Wet Summer." This means that these mesocosms were kept deep (20 cm with steady inflow) in the late summer and shallow (5 cm with steady flow) in spring. ‘WSP’ is short for "Wet Spring." In these mesocosms, the water depth was kept deep (20 cm with steady inflow) in the spring and shallow (5 cm with steady flow) in fall. ‘P’ is short for "Pulsing” (10 cm with inflow rate varied according to the river stage). ‘S’ is short for "Steady." In the last two hydrology categories, the water depth was maintained around 10 cm and the inflow stayed constant.

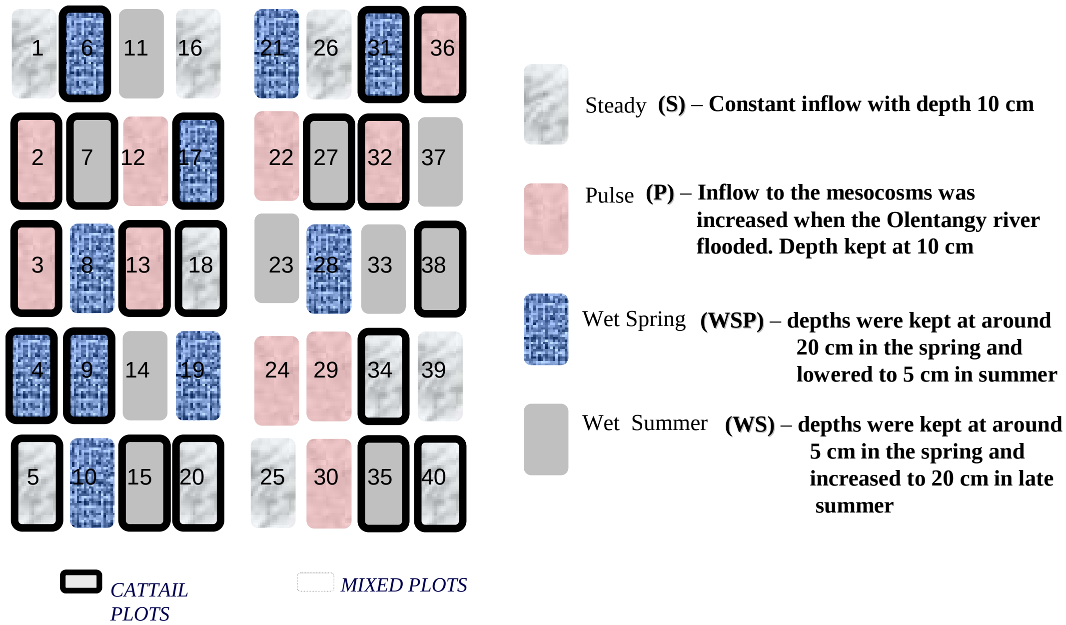



Figure 2. The two vegetation treatments and four hydrological regimes mesocosm schematic (From Kurt Keljo, reprinted with permission)

### *3.2 Sampling of mosquitoes*

The two experimental wetlands, stormwater wetland and Odum pond in ORWRP were sampled. A dipper, having a white plastic container (11 cm diameter and 350 ml capacity) and an adjustable plastic handle, was used to sample larval-stage mosquitoes. Dipping was done weekly during the months of June, July and August, 2008.

***3.2.1 Sampling sites in experimental wetlands.*** For sampling larval stages of mosquitoes, the two experimental wetlands were divided into three major sites each according to the inflow-outflow gradient (Figure 3). Each of the 3 major sites was further divided into 2 subsites according to the vegetation characteristics and proximity. These subsites were floating vegetation (FV) and emergent vegetation (EV) (Figure 3). At each subsite, 10 dips were taken randomly every week for 3 months. The mosquitoes from the 10 dips from each subsite were counted separately and then were pooled to form a composite sample. Thus, each of the two subsites had two representative samples – EV and FV samples. Data were represented as both total numbers of mosquitoes in each representative sample as well as numbers of mosquitoes per dip.

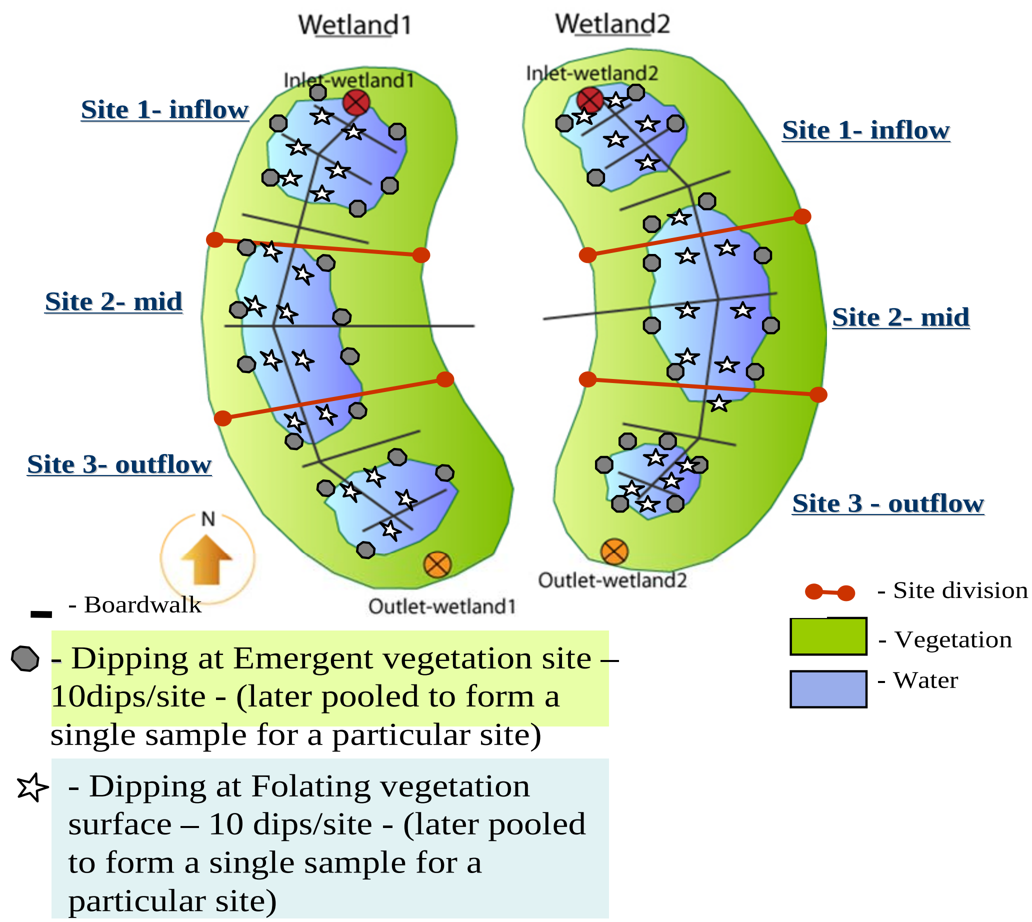



Figure 3. Sampling sites in the experimental wetlands at the Olentangy River Wetland Research Park: Inflow, mid and outflow regions with dipping sites at emergent vegetation and floating vegetation

***3.2.2 Sampling sites in the stormwater wetlands.*** Sampling was done at 15 sites in the periphery of stormwater wetland at water vegetation interface once a week for 10 weeks. Floating vegetation was present in the middle portion but it was at the depth (>30 cm) not usually conducive to mosquito breeding. Sampling was attempted in this deep zone for first two weeks but due to scarcity of mosquito larvae, sampling was restricted to the perimeter water vegetation interfaces only. All samples collected during a week were pooled to represent a composite weekly sample. Data were represented as both total numbers of mosquitoes as well as numbers of mosquitoes per dip.

***3.2.3 Sampling sites in the Odum pond.*** Similar to the stormwater wetland, sampling in the Odum pond was done at 15 sites evenly throughout the periphery of pond at water vegetation interface. Sampling was done for 10 weeks. All the samples colleted during a week were pooled to represent a single sample. The data were represented as both total numbers of mosquitoes as well as numbers of mosquitoes per dip.

***3.2.4 Sampling sites in the mesocosms.*** From the 40 mesocosm plots (differing in vegetation as well as hydrology), 16 mesocosms were sampled each week – 8 randomly chosen from those with cattail stands with 2 plots each having a different hydrology (Pulsed, Steady, Wet Summer and Wet Spring) and 8 from those with mixed stands with 2 plots each having a different hydrology, for 5 weeks. Four dips were taken at each site. The site samples representing similar vegetation and hydrology were pooled. The data were presented as total numbers of mosquitoes in four dips in each of the sampled plots.

### *3.3 Identification of mosquitoes*

All mosquito larvae sampled from various sites were identified to genus level using the pictoral key obtained from training branch of Communicable Disease Center (CDC) in U.S. Department of Health, Education, and Welfare (Pratt, 1959). Sub-sampling was done from all the stored samples to identify the mosquitoes further to the species level using illustrated key to the mosquitoes (Restifo, 1982). For sub-sampling, ten mosquitoes were identified randomly from each weekly sample from both the experimental wetland outflow regions, Odum pond and stormwater wetland. A total of 478 mosquitoes were identified to the species level.

### *3.4 Water quality measurements*

YSI sonde was used for determining water quality parameters including pH, temperature, dissolved oxygen, conductivity, and oxidation reduction potential (ORP) of all the major sites of the two experimental wetlands. Data were taken every alternate day for 10 weeks and weekly averages were recorded for all the quality parameters. Some of the missing data were retrieved from ORWRP data record sheet.

### *3.5 Weather measurements*

Temperature, dew point, precipitation, wind speed and atmospheric pressure were recorded daily in the morning from the meteorological station located between the two experimental wetlands. Only weekly averages for all the parameters were used.

### *3.6 Statistical analysis*

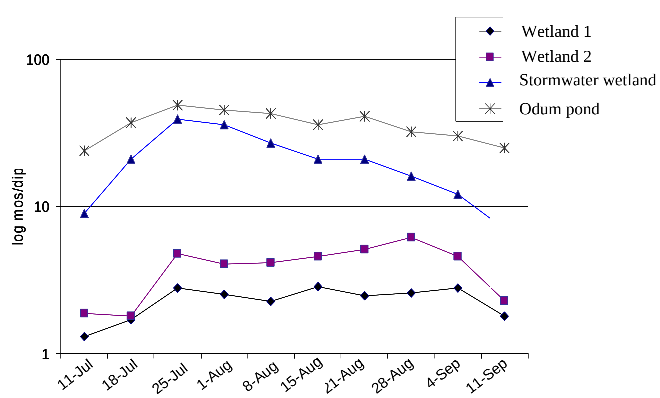
Mosquito larval density data of the 4 locations (two eperimental wetlands, stormwater wetland and Odum pond) were log transformed to normalize the variation and then analyzed by Student's two tailed t-test. Mosquito counts and genera along the flow gradient (i.e., outflow region, mid region and inflow region) and proximity to vegetation (EV vs FV) over 10 weeks in both experimental wetlands (as replicates) were analyzed using general linear models of repeated measures analysis of variance and means were separated by Tukeys Multiple Comparison test. A p-value $< 0.05$ was considered significant. Abundance of mosquito species over time was determined from the subsampling data using similar analysis of repeated measures. Mosquito counts and genera in mesocosm plots of different hydrology and vegetation type (cattail vs mixed) were also analyzed using repeated measures ANOVA. Regression model was built to analyze contributions of water quality parameters (pH, temperature, dissolved oxygen, conductivity, and ORP) in predicting mosquito density along the flow gradient of the experimental wetlands. Repeated measures analysis was also conducted for all the water quality parameters individually along the flow gradient in the two experimental wetlands.

# Chapter 4: Results

## *4.1 Mosquito density in different wetlands and Odum pond*

Data for all the locations are presented in appendix section (Table 1, 2 and 3). Log counts per dip are presented for all locations over ten week period (Figure 4a). Two tailed t-tests comparing different locations showed that mosquito counts varied significantly between the various locations (2 experimental wetlands, stormwater wetland, and Odum pond) (Figure 5). Mosquito populations were significantly high in Odum pond compared to the 2 experimental wetlands ($p<0.00001$) and stormwater wetland ($p=0.002$). Also, stormwater wetland mosquito population was higher than that from the 2 experimental wetlands ($p<0.00004$). Mosquito populations in experimental wetland 2 was significantly higher than that of Wetland 1 ($p=0.004$). For the two experimental wetlands, mosquito counts also varied significantly with time. Counts on week 1, 2 and 10 (July 11$^{th}$ and July 18$^{th}$ and September 11$^{th}$ ) were significantly lower than counts on weeks 3 to 9 (July 28$^{th}$ to September 4$^{th}$ ) ($p<0.03$) (Figure 4b).

(a)



(b)

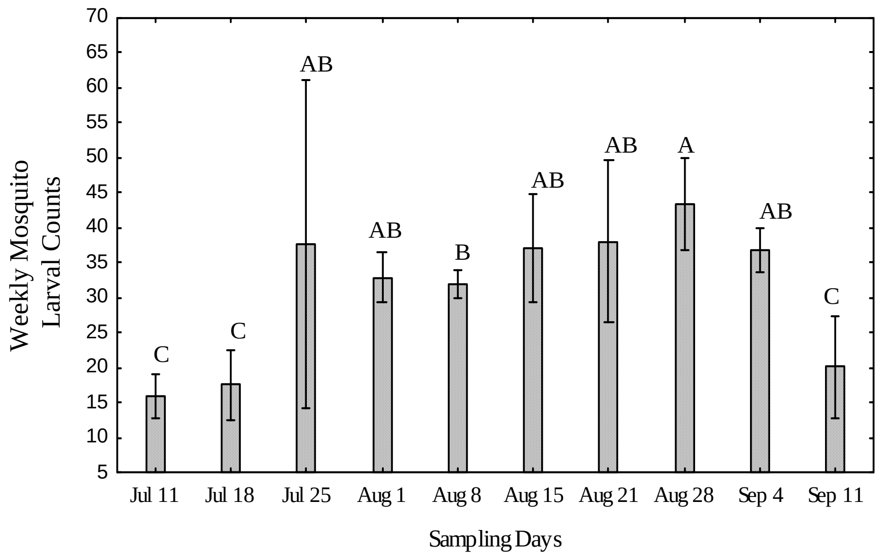



Figure 4. (a) Mosquito counts as per dip log counts at various study sites over 10 weeks. (b) Mosquito counts in two experimental wetlands at different time points. Mean larval counts denoted with different letters are signficantly different (Tukey's HSD, p <0.05).

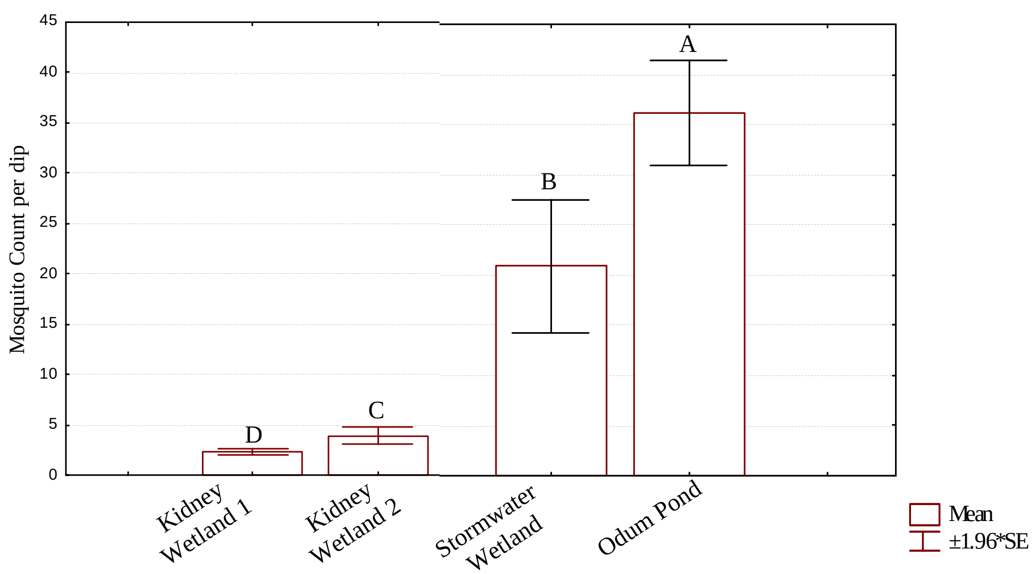



Figure 5. Mean mosquito counts (per dip) at various locations (experimetal wetland 1, experimetal wetland 2, Odum pond and stormwater wetland) over 10 weeks in 2008. Mean larval counts denoted with different letters are signficantly different (p <0.05).

### *4.2 Dominant mosquito genera and species*

Data on mosquito genera and species identified in the two experimental wetlands are presented in appendix Tables 4, 5 and 6. For the experimental wetlands, repeated measures analysis of variance showed significant differences [F (2, 4) =16.86, p value = 0.011] in the relative dominance of mosquito genera. By Tukeys post hoc analysis, it was found that *Anopheles* were present in significantly lower numbers than *Culex* (p value = 0.01) and *Uranotaenia* (p value = 0.047) (Figure 6). *Culex* constituted larger population than *Uranotaenia* but the difference was not significant. There was a difference among different genera in different flow gradient regions. Outflow region had significantly higher numbers of *Culex* than *Anopheles* (p=0.014) (Figure 7). However, any specific mosquito genus was not found to differ significantly between the two wetlands (Figure 8) or along the flow gradient (Figure 7). In stormwater wetlands, Odum pond and the mesocosm wetlands, the dominant genus was *Culex (p<0.05).*

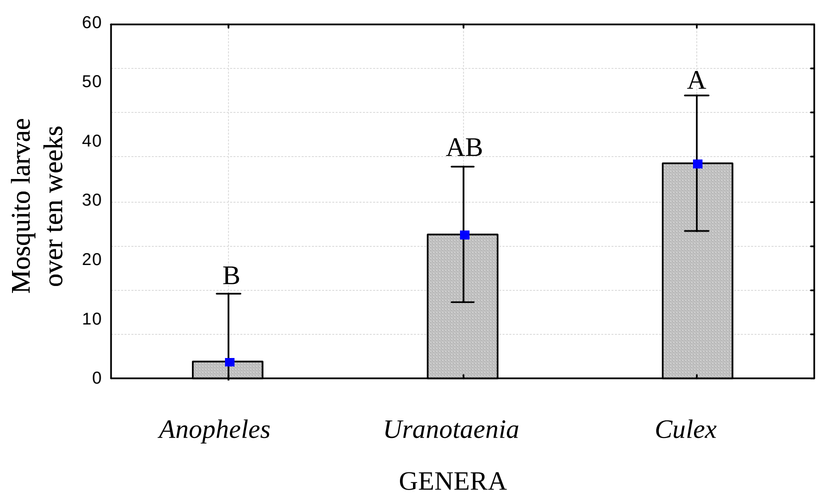



Figure 6. Abundance of different mosquito genera in experimental wetlands. Mean larval counts denoted with different letters are signficantly different within each genus (Tukey's HSD, p <0.05).

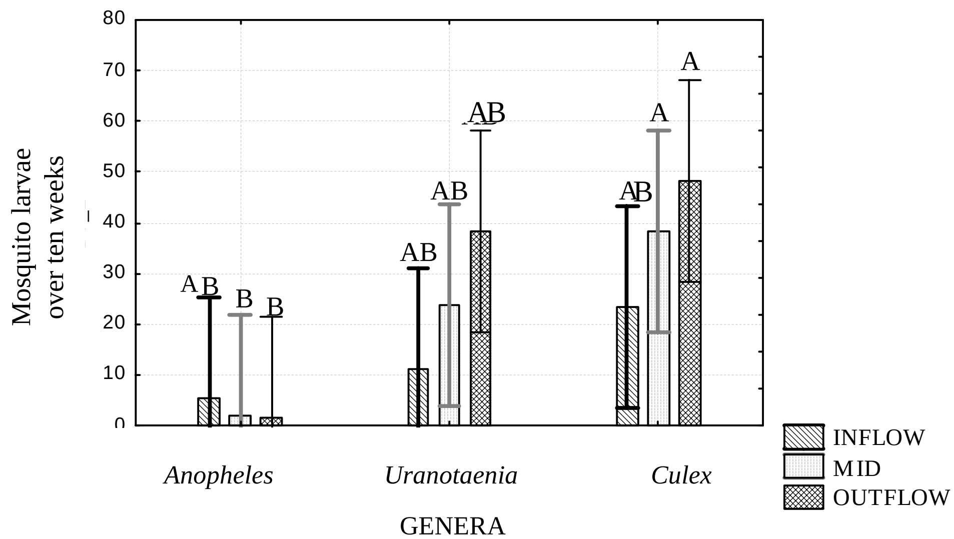



Figure 7. Abundance of different mosquito genera along flow gradient (inflow, mid and outflow). Mean larval counts denoted with different letters are signficantly different within each genus (Tukey's HSD, p <0.05).

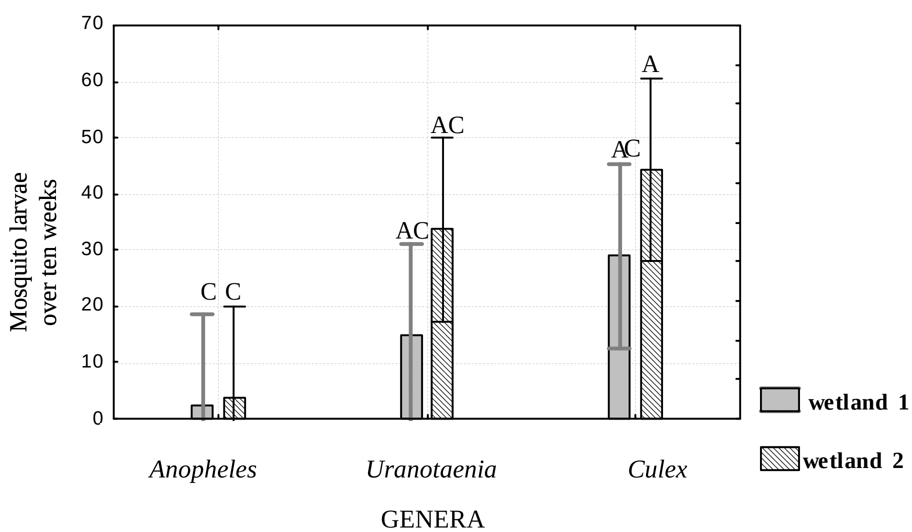



Figure 8. Abundance of different mosquito genera in wetland 1 and 2. Mean larval counts denoted with different letters are signficantly different within each genus (Tukey's HSD, p <0.05).

Subsample analysis for species identification indicated that relative species abundance differed significantly (p = 0.00034) and among the six species identified (*Cx. pipiens, Cx. salinarius, Cx. restuans, Ur. sapphirina, An. quadrimaculatus, and An. punctipennis), Cx. pipiens* was the dominant species (p<0.042) (Figure 9). Diversity analysis over time could not be performed due to sparse species richness.

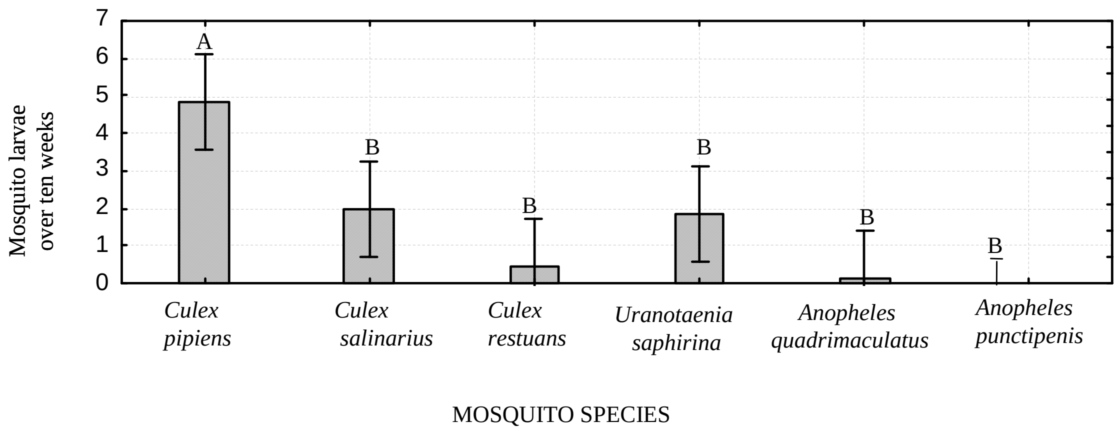



Figure 9. Abundance of different mosquito species in all locations (2 experimental wetlands, stormwater wetland and Odum pond). Mean larval counts denoted with different letters are signficantly different within each species (Tukey's HSD, p <0.05).

### *4.3 Mosquito population and habitat characteristics*

***4.3.1 Flow gradient.*** Analysis using repeated measures model showed significant differences in mosquito counts along the flow gradient (p=0.009). Outflow region had significantly higher number of mosquitoes as compared to the inflow region (p=0.009) and midregion (p=0.003) (Figure 10). But, there was no difference between the inflow and middle of the wetlands (p>0.05).

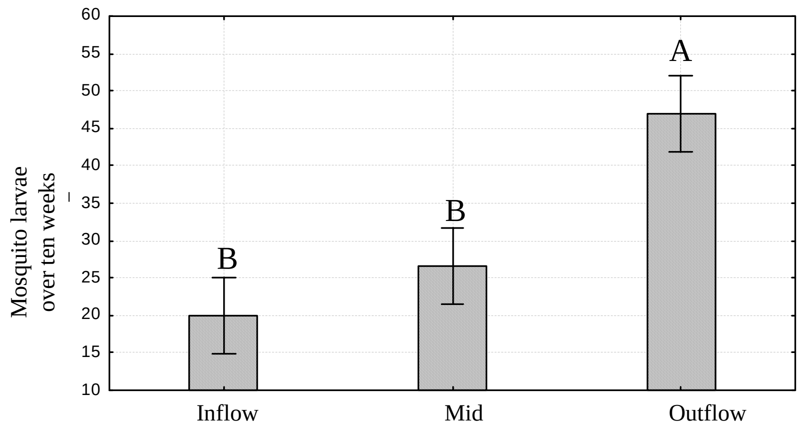



Figure 10. Mosquito count with flow gradient (inflow; mid & outflow region) in the experimental wetlands. Mean larval counts denoted with different letters are signficantly different (Tukey's HSD, p <0.05).

***4.3.2 Floating vegetation vs emergent vegetation.*** Significant difference was found between the mosquito counts in floating vegetation (FV) versus water emergent vegetation interface (EV) with higher mosquito count in EV sites than FV sites (p=0.023) (Figure 11). This trend was most prominent in outflow regions of the wetlands (Figure 12). Open waters were not found to act as a breeding habitat for mosquitoes, so were not included in the analysis.

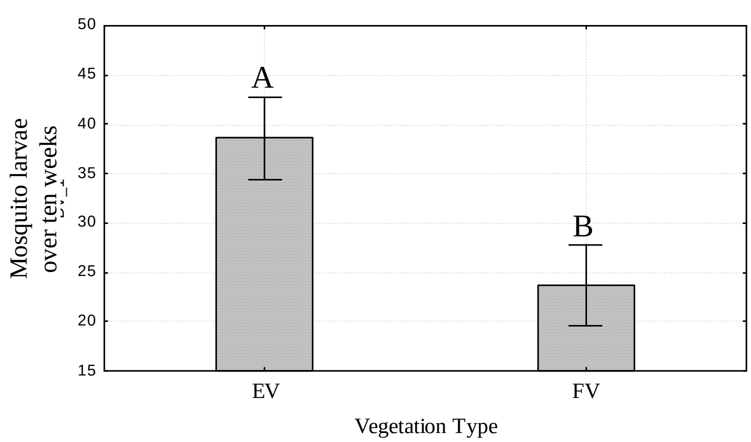



Figure 11. Mosquito density in emergent vegetation (EV) vs floating vegetation(FV). Mean larval counts denoted with different letters are signficantly different (Tukey's HSD, p <0.05).

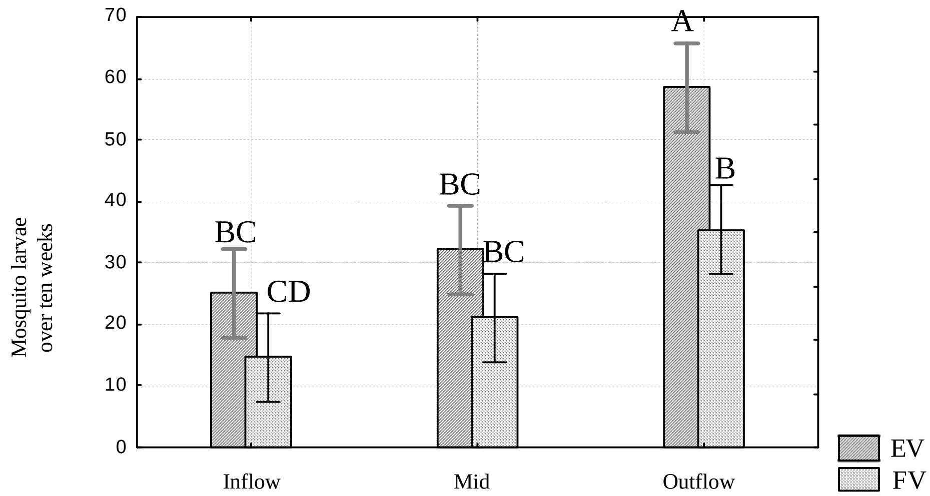



Figure 12. Mosquito density in EV (emergent vegetation) and FV (floating vegetation) along the fow gradient (inflow, mid and outflow). Mean larval counts denoted with different letters are signficantly different (Tukey's HSD, $p < 0.05$).

***4.3.3 Water quality parameters*.** Water quality parameters (pH, DO, ORP, and turbidity) from major sites of both the experimental wetlands were compared along the flow gradient over 10 weeks duration using repeated measures general linear model. Data are presented in appendix section (Table 7). Significant differences in DO and ORP were found with lower DO ($p=0.040$) and ORP ($p=0.037$) in outflow regions than inflow regions of the wetlands. Regression model built taking into account the repeated measures over 10 weeks along the flow gradient in both wetlands produced an adjusted R2 of 0.67 [$F(17, 42) = 7.97$, and $p = .000$] for the prediction of mosquito population density. The strongest predictor among the water quality parameters was conductivity ($p=0.004$) followed by DO ($p=0.052$).

***4.3.4 Vegetation type and hydrology in mesocosms*.** Data for mesocosm plots are presented in Table 8. Mixed stand mesocosm plots with *Sparganium eurycarpum, Juncus effusus,* and *Schoenoplectus tabernaemontani* had significantly ($p<0.0001$) higher mosquito counts than mesocosm plots with *Typha* monoculture (Figure 13). Mosquito counts also differed significantly with hydrology (p=0.001) with steady flow hydrology (S) having higher counts than pulsed (P) hydrology (p=0.001) and Wet Spring (WSP) hydrology (p=0.008) (Figure 14). Wet summer hydrology also exceeded in mosquito counts than Wet Spring (WSP) hydrology (p=0.024) (Figure 14). This difference in larval density with hydrology was more marked in mesocosms planted with mixed vegetation (Figure 15).

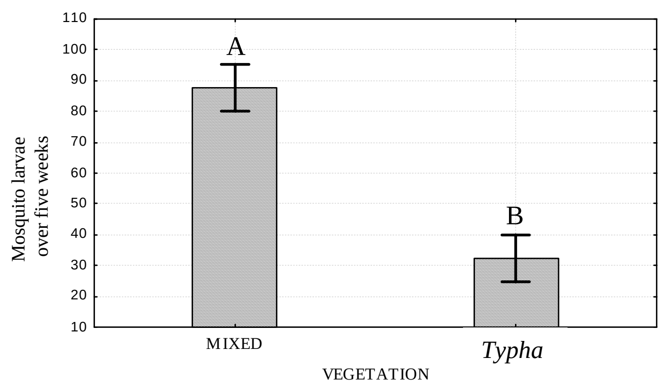



Figure 13. Mosquito counts in mesocosm plots differing in vegetation types (mixed vs *Typha*). Mean larval counts denoted with different letters are signficantly different (p <0.05).

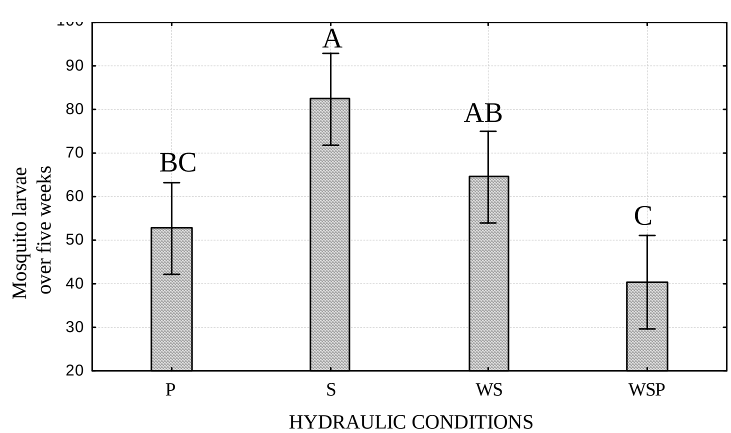



Figure 14. Mosquito counts in mesocosm plots with different hydrologies: Wet Summer (WS), Wet Spring (WSP), Pulsed (P), Steady (S). Mean larval counts denoted with different letters are signficantly different (Tukey's HSD, p <0.05).

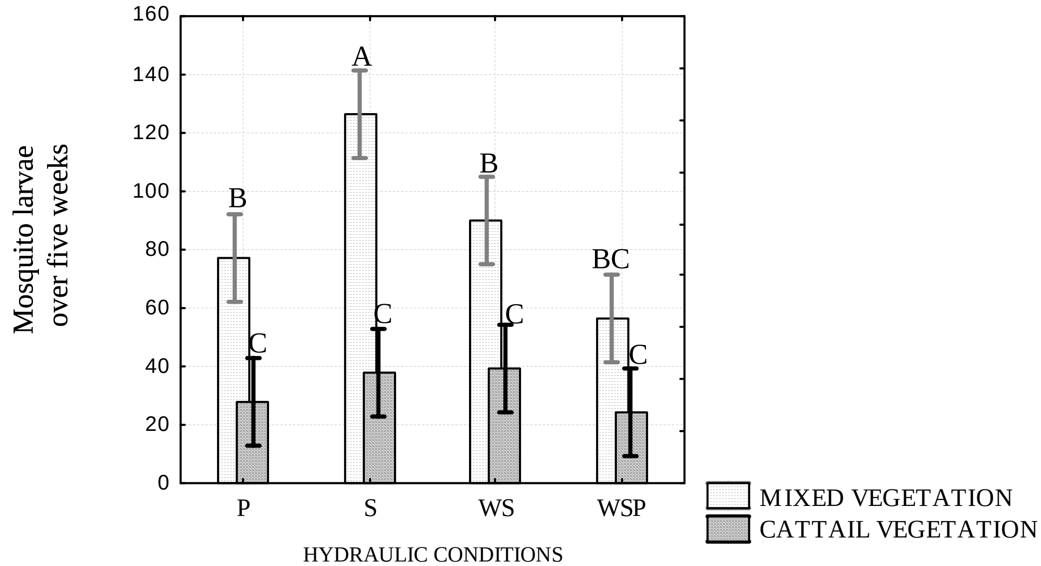



Figure 15. Mosquito counts in mixed vegetation and cattail vegetation mesocosm plots with different hydrologies: Wet Summer (WS), Wet Spring (WSP), Pulsed (P), Steady (S). Mean larval counts denoted with different letters are signficantly different (Tukey's HSD, $p < 0.05$).

# Chapter 5: Discussion

Analysis of mosquito population from all water bodies showed that the ornamental pond, Odum pond, was most conducive to mosquito breeding. Factors that can be implicated for higher mosquito breeding in the pond are the presence of stagnant water, a dense vegetation cover throughout the periphery and deep water table which does not support many of the natural predators of mosquito larvae. The stormwater wetland had few shallow pools of water formed in the periphery intermittently which were highly conducive to mosquito breeding. Also, the stormwater wetland is comparatively a new construction (6 years old) than the two experimental wetlands (14 years old). So, it may not have developed a complex network of natural predators yet which can control mosquito population. This brings forth the issue of integration of mosquito control measures in construction design of wetlands atleast for the first few years after the construction till the natural predator prey cycle is established. However, as the stormwater is filled only intermittently, it is hard to predict if it could develop predator population as this condition is not favorable for the survival of many predators. Therefore, such water bodies need continuous surveillance. Though there were some mosquitoes in the two experimental wetlands, the numbers were an order of 3-6 magnitude lower than mosquitoes in the two stormwater filled ponds. The experimental wetlands were designed for low mosquito populations (Mitsch, pers comm.). They were made flow through and have 3 distinct deeper cells of water to allow overwintering of the fish (Mitsch et al, 2009). At the beginning of this year, biologically friendly pumps were installed which allow fish to pump through. This will further help in controlling mosquito population by providing for adequate predator abundance.

Average larval numbers ranged from 0-6 per dip in experimental wetland sites. Previous studies have reported much higher mosquito larval densities per dip of sample in many constructed, restored and natural wetlands (Table 10).

| LOCATION | AVERAGE MOSQUITO LARVAE PER DIP | CITATION |
|---|---|---|
| ORWRP<br>Experimental wetlands<br>Stormwater wetlands | <br>upto 6/dip<br>upto 40/dip | Current study |
| Tres Rios Demonstration Wetland basins (Arizona) | upto 30/ dip | Karpiscak et al., 2004 |
| Wastewater treatment plant in Cameroon (Central Africa) | upto 15 culex /dip | Kengne et al. 2003 |
| Treatment wetland in Santa Rosa de Copa´n, Honduras | upto 20/ dip | Diemont, 2006 |
| Beaver Valley restored Wetland, Iowa | upto 50/dip | Mercer et al., 2005 |
| Greater Accra Region, Ghana<br>Rice fields<br>Storm drains<br>Small pools/drains | <br>upto 200/ dip<br>upto 25/dip<br>upto 100/dip | Opoku et al., 2005 |
| Wastewater treatment wetlands | usually upto 200/dip | Walton, 2003 |

Table 10. Previous studies with findings on mosquito larval density per dip in various constructed, created and natural wetland sites

Review of findings from other wetlands (Table 10) suggests that created experimental wetlands are far less conducive to mosquito breeding than the constructed wetlands. The high populations of mosquitoes seen in previous studies may be due to the steady water levels and nutrient-rich wastewater present in most of the treatment wetlands providing favorable conditions for the bacteria, alga and protozoa, which are chief food sources for mosquito larvae (Walton, 2002; Keiper et al., 2003; Opoku, 2005).

Six species of mosquito larvae were identified from both the experimental wetlands: *Cx. pipiens, Cx. salinarius, Cx. restuans, Ur. sapphirina, An. quadrimaculatus, and An. punctipennis*. *Cx.* spp. is the main vectors of many arboviruses including WNV, SLE and EEE. Hence, increased *Culex* population is always a cause for concern especially due to recent emergence of diseases like West Nile fever. All the *Culex* species found in the wetland sites, particularly *Cx. pipiens* and *Cx. salinarius* are potential bridge vectors of arboviruses (CDC, 1999; CDC, 2000; Sardelis et al., 2001). Among the *Anopheles* species in the wetlands, *An. quadrimaculatus* is a dominant vector of malaria in North America (O'Malley, 1992). Though the abundance of *Culex* was expected, presence of *Uranotaenia* genus was unexpectedly high. *Uranotaenia* is found from southeastern Canada to Florida and extends into the central states west to North Dakota and south into Mexico (Hinman, 1935). *Ur. spp* larvae tend to remain close to mats of floating vegetation especially duckweed (Hinman, 1935). Presence of duckweed in our wetlands may be a reason for abundance of *Uranotaenia*. *Uranotaenia* have, however, no known medical or economic importance (Hinman, 1935). Many species of mosquitoes (e.g. *Culiseta*, *Coquillettidia*, etc.) breed in the roots of emergent vegetation. So, these species may have escaped from being sampled as we did not sample the roots.

Mosquito larval density in experimental wetland 2 was higher than experimental wetland 1. Water quality parameters between the two wetland sites were not found to differ significantly. The difference in vegetation may be contributing to this difference in larval density. Further study taking into account the quantitative estimation of vegetation (emergent as well as floating) in the wetlands will clarify if vegetation is a good predictor of mosquito density in the two experimental wetlands.

The outflow region of the wetlands was found to be most conducive to mosquitoes. This correlates with higher gross primary productivity at outflow regions than inflow regions (Spieles and Mitsch, 2000) which translates to better food source for mosquito larvae. Further analysis of water quality parameters along the flow gradient indicated lower dissolved oxygen in outflow areas which are favorable to mosquito breeding. Also the minimal flow of water, shallow depth and higher vegetation cover especially dense floating vegetation may be contributing to successful mosquito breeding. Regression model looking at water quality parameters as predictors of mosquito density indicated that conductivity and dissolved oxygen were the strongest factors. This is in accordance with the literature that mosquito breeding negatively correlates with dissolved oxygen, electrical conductivity and higher suspended solids which are related to conductivity (Adebode, 2008). Dissolved oxygen and conductivity are also positively correlated with macroinvertibrate community structure as a whole (Spieles and Mitsch, 2000). This suggests that such sites will also have an adequate mosquito predator population and hence lower mosquito densities. Previous studies have also established correlations with temperature and pH (Adebode, 2008; Opoku, 2005). However, we failed to see such a correlation though we did see differences in oxidation and reduction potential between inflow and outflow sites. A longer duration of measurement is warranted to further determine other water quality parameters as predictors of mosquito density.

Analysis of mosquito density in floating vegetation and emergent vegetation indicated that emergent vegetation is more conducive to mosquitoes; hence maintenance to check their overgrowth will be beneficial in keeping mosquito populations under control (Thullen, 2002). High density of emergent vegetation not only provides favorable oviposition sites but also protects mosquito larvae from predacious insects and fish (Mulrennan, 1970). Emergent vegetation grows in shallow water and floating vegetation in deep water. Shallow water bodies are more conducive to mosquito than deeper water bodies. This may be another reason of higher mosquito density in emergent vegetation.

Some previous studies have suggested *Typha* as being the most conducive to mosquito breeding among the emergent vegetation types (Collins and Resh, 1989). But the mesocosm study (Keljo, 2009) indicated mixed vegetation as more favorable for mosquitoes than cattail. Among the hydrology types, steady inflow (10 cm depth) and wet summer hydrology were more conducive to mosquitoes. This indicates that both vegetation and hydrology may be manipulated in order to control mosquito populations. The information can be integrated in the management of constructed wetlands to render them less conducive to mosquito breeding. Also, information regarding plant communities which favor mosquito breeding can be utilized during the establishment of plant communities while constructing wetlands.

## *Overall Conclusions*

Our study assessed the mosquito population and characterized various mosquito habitat characteristics in experimental wetlands and other water bodies in Olentangy River Wetland Research Park in Columbus, Ohio. We found that the created wetlands have low populations of mosquitoes compared to more traditional treatment wetlands and were less conducive to mosquitoes than newly constructed stormwater-fed wetlands at the ORWRP in 2008.

*Cx. pipiens* was the dominant mosquito species in all the water bodies sampled thus creating a big pool of potential vectors for viruses like West Nile Virus (WNV), St. Louis encephalitis (SLE) and eastern equine encephalomyelitis (EEE). Among the other species found in our study sites, *Cx. salinarius, An. quadrimaculatus* and, to a lesser extent, *An. punctipennis* are considered pest species due to their vector potential.

Mosquitoes do act as an important component of the food chain in the wetlands. Hence, specific targeting of potentially harmful species needs to be implemented rather than targeting all the species. This issue can be well addressed by the use biological control agents for specific mosquito species sparing the harmless species and hence keeping the ecological food wed undisturbed. The outflow region of the experimental wetlands provides most conducive environment for mosquito breeding. This is probably because of higher vegetation cover and lower DO in the outflow region which favors mosquito breeding. Emergent vegetation had higher propensity to support mosquitoes than floating vegetation surface. This implies that management of dense vegetation may be needed particularly in constructed wetlands which usually have higher density of emergent vegetation. Mixed vegetation containing *Sparganium eurycarpum, Juncus* *effusus,* and *Schoenoplectus tabernaemontani* is more favorable to mosquitoes than Cattail (*Typha spp*). Hydrology may also influence mosquito population. Hydrologic conditions with steady inflow (10 cm depth) followed by conditions with deep water levels in summer and low in spring was found to be more favorable to mosquitoes.

The information regarding vegetation type, hydrology and other habitat characteristics will be crucial for the design phase of the constructed wetlands. Further investigations are however warranted to study weather and water quality parameters as predictors of mosquito breeding in created and constructed wetlands.

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# Appendix A – Raw data tables

| | WETLAND 1 | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | INFLOW | | | | MID | | | | OUTFLOW | | | | TOTAL | |
| Sampling days | FV | EV | total | | FV | EV | total | | FV | EV | total | | No. | No./dip |
| 11-Jul | 8 | 15 | 23 | | 11 | 16 | 27 | | 12 | 16 | 28 | | 78 | 1.30 |
| 18-Jul | 9 | 14 | 23 | | 14 | 17 | 31 | | 10 | 38 | 48 | | 102 | 1.70 |
| 25-Jul | 16 | 22 | 38 | | 17 | 27 | 44 | | 27 | 58 | 85 | | 167 | 2.78 |
| 1-Aug | 26 | 33 | 59 | | 13 | 12 | 25 | | 23 | 45 | 68 | | 152 | 2.53 |
| 8-Aug | 15 | 30 | 45 | | 11 | 25 | 36 | | 23 | 32 | 55 | | 136 | 2.27 |
| 15-Aug | 13 | 40 | 53 | | 12 | 35 | 47 | | 15 | 56 | 71 | | 171 | 2.85 |
| 21-Aug | 16 | 28 | 44 | | 15 | 26 | 41 | | 14 | 49 | 63 | | 148 | 2.47 |
| 28-Aug | 18 | 24 | 42 | | 9 | 18 | 29 | | 22 | 61 | 83 | | 154 | 2.57 |
| 4-Sep | 16 | 38 | 54 | | 11 | 25 | 36 | | 26 | 52 | 78 | | 168 | 2.80 |
| 11-Sep | 7 | 9 | 20 | | 9 | 11 | 20 | | 22 | 46 | 68 | | 108 | 1.80 |

Table 1. Mosquito counts in experimental wetland 1 at major sites (inflow. mid and outflow) with site subdivisions - Floating vegetation (FV) and Emergent vegetation (EV)

| | WETLAND 2 | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | INFLOW | | | | MID | | | | OUTFLOW | | | | TOTAL | |
| Sampling days | FV | EV | total | | FV | EV | total | | FV | EV | total | | No. | No./dip |
| 11-Jul | 7 | 16 | 23 | | 15 | 18 | 33 | | 22 | 34 | 56 | | 112 | 1.87 |
| 18-Jul | 12 | 15 | 27 | | 14 | 22 | 36 | | 14 | 31 | 45 | | 108 | 1.80 |
| 25-Jul | 18 | 21 | 39 | | 25 | 82 | 107 | | 68 | 72 | 140 | | 286 | 4.77 |
| 1-Aug | 12 | 23 | 35 | | 32 | 46 | 78 | | 48 | 82 | 130 | | 243 | 4.05 |
| 8-Aug | 19 | 25 | 44 | | 49 | 58 | 107 | | 45 | 51 | 96 | | 247 | 4.12 |
| 15-Aug | 16 | 33 | 49 | | 55 | 46 | 101 | | 47 | 77 | 124 | | 274 | 4.57 |
| 21-Aug | 22 | 36 | 58 | | 43 | 52 | 95 | | 76 | 79 | 155 | | 308 | 5.13 |
| 28-Aug | 25 | 35 | 60 | | 46 | 55 | 101 | | 75 | 134 | 209 | | 370 | 6.17 |
| 4-Sep | 12 | 28 | 40 | | 11 | 29 | 40 | | 84 | 110 | 194 | | 274 | 4.57 |
| 11-Sep | 6 | 18 | 24 | | 9 | 22 | 31 | | 34 | 48 | 82 | | 137 | 2.28 |

Table 2. Mosquito counts in experimental wetland 1 at major sites (inflow. mid and outflow) with site subdivisions - Floating vegetation (FV) and Emergent vegetation (EV)

| | STORMWATER WETLAND | | | POND | |
|---|---|---|---|---|---|
| Sampling days | total no. (15 dips) | no./dip | | total no. (15 dips) | no./dip |
| **9-10 Jul** | 128 | 9 | | 360 | 24 |
| **16-17 Jul** | 312 | 21 | | 560 | 37 |
| **23-24 Jul** | 580 | 39 | | 740 | 49 |
| **30-31 Jul** | 540 | 36 | | 680 | 45 |
| **6-7 Aug** | 410 | 27 | | 650 | 43 |
| **13-14Aug** | 320 | 21 | | 540 | 36 |
| **19-20 Aug** | 315 | 21 | | 620 | 41 |
| **26-27Aug** | DRY | DRY | | 480 | 32 |
| **2-3 Sep** | DRY | DRY | | 450 | 30 |
| **9-10 Sep** | DRY | DRY | | 380 | 25 |

Table 3. Mosquito counts in stormwater wetland and Odum pond through 10 weeks

| | **WETLAND 1 mosquito genera count** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **in INFLOW** | | | **in MID** | | | **in OUTFLOW** | | |
| **ANO** | **URO** | **CUL.** | **ANO** | **URO** | **CUL.** | **ANO** | **URO** | **CUL.** |
| 2 | 7 | 14 | 0 | 11 | 16 | 0 | 11 | 17 |
| 3 | 9 | 11 | 0 | 13 | 18 | 0 | 14 | 34 |
| 4 | 14 | 29 | 3 | 8 | 33 | 1 | 16 | 68 |
| 4 | 11 | 36 | 2 | 12 | 11 | 2 | 13 | 42 |
| 7 | 13 | 25 | 2 | 11 | 23 | 4 | 22 | 29 |
| 4 | 11 | 38 | 0 | 16 | 31 | 2 | 26 | 43 |
| 5 | 12 | 27 | 1 | 21 | 19 | 3 | 25 | 35 |
| 6 | 13 | 23 | 2 | 15 | 12 | 3 | 28 | 52 |
| 2 | 15 | 37 | 1 | 18 | 27 | 2 | 35 | 41 |
| 1 | 7 | 12 | 1 | 10 | 9 | 0 | 12 | 56 |

(a).

| | **WETLAND 2 mosquito species count** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **in INFLOW** | | | **in MID** | | | **in OUTFLOW** | | |
| **ANO** | **URO** | **CUL.** | **ANO** | **URO** | **CUL.** | **ANO** | **URO** | **CUL.** |
| 6 | 7 | 10 | 3 | 14 | 16 | 0 | 25 | 29 |
| 7 | 6 | 14 | 4 | 16 | 16 | 2 | 28 | 33 |
| 12 | 12 | 15 | 4 | 42 | 61 | 2 | 62 | 82 |
| 7 | 10 | 18 | 2 | 39 | 37 | 0 | 66 | 47 |
| 10 | 12 | 22 | 3 | 54 | 50 | 2 | 80 | 71 |
| 8 | 11 | 30 | 2 | 51 | 48 | 2 | 87 | 63 |
| 9 | 13 | 36 | 3 | 43 | 49 | 2 | 81 | 69 |
| 5 | 16 | 39 | 1 | 49 | 51 | 1 | 76 | 84 |
| 5 | 15 | 20 | 2 | 17 | 221 | 1 | 38 | 41 |
| 3 | 9 | 12 | 0 | 14 | 17 | 1 | 18 | 26 |

(b).

Table 4. Mosquito counts of specific genera (ANO - *Anopheles;* URO – *Uranotaenia*; and CUL. – *Culex*) in **(a)** Experimental wetland 1; and **(b)** Experimental wetland 2 at major sites (inflow, mid and outflow).

| | | | WETLAND 1 (outflow) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **wk 1** | **wk 2** | **wk 3** | **wk 4** | **wk 5** | **wk 6** | **wk 7** | **wk 8** | **wk 9** | **wk 10** |
| ***Cx pipiens*** | 3 | 4 | 2 | 2 | 3 | 4 | 3 | 3 | 3 | 6 |
| ***Cx salinarius*** | 0 | 2 | 5 | 3 | 0 | 0 | 0 | 2 | 3 | 1 |
| ***Cx restuans*** | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 1 |
| ***Ur. Sapphirina*** | 7 | 4 | 3 | 3 | 7 | 5 | 5 | 5 | 4 | 2 |
| *An*.***quadrimaculatus*** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| ***An. punctipennis*** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 5. Mosquito species sub-sampled from experimental wetland 1.

| | | | WETLAND2 (outflow) | | | | | | | | | | STORMWATER WETLAND | | | | | | | | | | ODUM POND | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wk 1 | wk 2 | wk 3 | wk 4 | wk 5 | wk 6 | wk 7 | wk 8 | wk 9 | wk 10 | | wk 1 | wk 2 | wk 3 | wk 4 | wk 5 | wk 6 | wk 7 | | wk 1 | wk 2 | wk 3 | wk 4 | wk 5 | wk 6 | wk 7 | wk 8 | wk 9 | wk 10 |
| ***Cx pipiens*** | 3 | 4 | 4 | 7 | 3 | 3 | 5 | 5 | 3 | 5 | | 7 | 7 | 8 | 8 | 9 | 9 | 6 | | 6 | 6 | 6 | 7 | 5 | 8 | 8 | 7 | 5 | 6 |
| ***Cx salinarius*** | 4 | 2 | 3 | 1 | 3 | 0 | 1 | 1 | 4 | 1 | | 3 | 2 | 2 | 1 | 0 | 1 | 4 | | 4 | 2 | 3 | 3 | 4 | 1 | 2 | 3 | 5 | 4 |
| ***Cx restuans*** | 2 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | | 0 | 0 | 0 | 1 | 1 | 0 | 0 | | 0 | 2 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| ***Ur. Sapphirina*** | 1 | 3 | 2 | 2 | 4 | 5 | 3 | 4 | 3 | 2 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *An*.***quadrimaculatus*** | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***An. punctipennis*** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 6. Mosquito species subsampled from experimental wetland 1, stormwater wetland and Odum pond

| TEMP. ( c ) | MID WETLAND 1<br>Cond (yS/cm) | DO (MG/l) | pH | ORP (Mv) |
|---|---|---|---|---|
| 23.240 | 478.332 | 2.640 | 6.656 | 467.400 |
| 23.125 | 639.340 | 5.260 | 6.910 | 284.300 |
| 24.110 | 643.220 | 6.130 | 6.820 | 89.300 |
| 22.340 | 613.400 | 5.270 | 7.914 | 258.500 |
| 21.032 | 598.230 | 5.590 | 7.780 | 409.120 |
| 21.923 | 625.670 | 5.320 | 8.125 | 87.200 |
| 21.920 | 640.320 | 4.120 | 8.156 | -82.140 |
| 22.100 | 559.230 | 3.980 | 8.720 | 216.700 |
| 18.240 | 548.650 | 4.112 | 9.382 | 428.500 |
| 19.650 | 698.230 | 4.143 | 8.370 | 489.700 |

| TEMP. ( c ) | MID WETLAND 2<br>Cond (yS/cm) | DO (MG/l) | pH | ORP (Mv) |
|---|---|---|---|---|
| 503.000 | 499.420 | 2.790 | 6.510 | 508.200 |
| 23.250 | 647.500 | 4.520 | 7.110 | 287.200 |
| 25.130 | 661.370 | 6.310 | 7.110 | 113.600 |
| 24.110 | 622.400 | 7.260 | 7.960 | 259.240 |
| 21.150 | 617.370 | 5.890 | 8.310 | 426.300 |
| 22.123 | 633.410 | 6.420 | 8.414 | 80.120 |
| 22.780 | 642.460 | 3.720 | 8.112 | -83.390 |
| 20.950 | 573.500 | 3.820 | 9.260 | 227.100 |
| 19.690 | 568.180 | 4.620 | 9.620 | 422.340 |
| 22.240 | 673.400 | 4.990 | 8.890 | 501.220 |

**(a)**

| OUTFLOW WETLAND 1 | | | | | |
|---|---|---|---|---|---|
| TEMP. ( c ) | Cond (yS/cm) | DO (MG/l) | pH | ORP (Mv) | Turbidity (NTU+) |
| 22.170 | 466.333 | 1.973 | 6.367 | 509.000 | 9.310 |
| 22.125 | 626.500 | 3.425 | 6.755 | 174.800 | 39.700 |
| 24.217 | 632.000 | 4.357 | 6.920 | 76.667 | 18.600 |
| 21.883 | 607.333 | 4.023 | 7.323 | 268.667 | 21.600 |
| 19.173 | 589.333 | 4.783 | 7.520 | 416.667 | 12.700 |
| 21.500 | 619.667 | 3.887 | 7.700 | 94.000 | 19.450 |
| 21.800 | 654.000 | 2.915 | 8.050 | -83.000 | 8.900 |
| 23.000 | 553.500 | 3.850 | 8.450 | 187.150 | 12.200 |
| 17.400 | 534.000 | 3.450 | 8.350 | 440.000 | 14.100 |
| 18.757 | 685.667 | 3.833 | 7.567 | 474.667 | 7.680 |

| | OUTFLOW WETLAND 2 | | | | |
|---|---|---|---|---|---|
| TEMP. ( c ) | Cond (yS/cm) | DO (MG/l) | pH | ORP (Mv) | Turbidity (NTU+) |
| 21.930 | 493.000 | 2.343 | 6.443 | 512.000 | 3.100 |
| 21.725 | 637.500 | 2.315 | 6.695 | 182.500 | 4.300 |
| 24.867 | 659.667 | 5.927 | 7.177 | 106.000 | 3.250 |
| 22.400 | 621.000 | 5.093 | 7.743 | 269.333 | 12.500 |
| 19.833 | 613.333 | 5.117 | 7.600 | 444.000 | 4.833 |
| 21.733 | 628.000 | 5.567 | 7.900 | 82.800 | 7.450 |
| 21.800 | 656.500 | 2.420 | 7.835 | -84.350 | 14.300 |
| 20.300 | 581.500 | 3.100 | 8.550 | 175.500 | 4.900 |
| 18.000 | 568.000 | 4.000 | 9.050 | 425.500 | 18.700 |
| 23.833 | 644.333 | 3.503 | 9.167 | 420.000 | 6.667 |

**(b)**

**Continued**

Table 7. Water quality parameters for **(a)** mid region of experimental wetland 1 and 2; **(b)** outflow region of experimental wetland 1 and 2; and **(c)** inflow region of experimental wetland 1 & 2 :TEMP – Temperature; Cond.- Conductivity; DO – Dissolved oxygen; ORP- Oxidation reduction potential.

**Table 7 continued**

| | INFLOW WETLAND 1and 2 | | | | |
|---|---|---|---|---|---|
| TEMP. ( c ) | Cond (yS/cm) | DO (MG/l) | pH | ORP (Mv) | Turbidity (NTU+) |
| 24.600 | 508.000 | 3.993 | 7.777 | 439.333 | 16.100 |
| 25.100 | 669.000 | 6.225 | 7.085 | 384.250 | 5.800 |
| 25.757 | 667.000 | 7.233 | 6.613 | 122.233 | 5.700 |
| 25.273 | 623.000 | 8.097 | 8.143 | 252.000 | 8.167 |
| 22.337 | 628.667 | 6.400 | 8.337 | 400.000 | 7.910 |
| 23.767 | 633.667 | 7.727 | 8.433 | 72.000 | 11.950 |
| 23.125 | 636.500 | 5.375 | 8.200 | -83.200 | 14.700 |
| 22.767 | 568.000 | 5.190 | 9.410 | 256.400 | 18.400 |
| 21.500 | 569.500 | 5.195 | 9.870 | 415.000 | 16.400 |
| 20.850 | 717.000 | 5.465 | 8.670 | 503.000 | 4.793 |

**(c)**

| | | | **Mosquito Counts (4 dips) on following SAMPLING DAYS** | | | | |
|---|---|---|---|---|---|---|---|
| | | | **28-Jul** | **5-Aug** | **12-Aug** | **19-Aug** | **25-Aug** |
| **VEG + HYDRO** | **Replicates** | | | | | | |
| **MWS** | 1 | | 34 | 96 | 120 | 105 | 96 |
| | 2 | | 52 | 63 | 95 | 116 | 122 |
| **CHF** | 1 | | 10 | 23 | 28 | 68 | 64 |
| | 2 | | 28 | 116 | 19 | 19 | 17 |
| **MWSP** | 1 | | 31 | 28 | 34 | 63 | 64 |
| | 2 | | 48 | 62 | 105 | 72 | 58 |
| **CWSP** | 1 | | 18 | 62 | 17 | 19 | 19 |
| | 2 | | 15 | 16 | 25 | 27 | 24 |
| **CP** | 1 | | 32 | 68 | 21 | 14 | 23 |
| | 2 | | 30 | 19 | 22 | 30 | 21 |
| **MP** | 1 | | 55 | 94 | 26 | 130 | 68 |
| | 2 | | 44 | 64 | 62 | 134 | 98 |
| **CS** | 1 | | 54 | 27 | 68 | 61 | 34 |
| | 2 | | 15 | 14 | 19 | 49 | 40 |
| **MS** | 1 | | 123 | 260 | 140 | 38 | 42 |
| | 2 | | 98 | 140 | 120 | 144 | 162 |

Table 8. Mosquito counts (4 dips) in mesocosm plots differing in vegetation and hydrology – Mixed Wet Summer (MWS), Mixed Wet Spring (MWSP), Mixed Pulsed (MP), Mixed Steady (MS), Cattail Wet Summer (MWS), Cattail Wet Spring (MWSP), Cattail Pulsed (CP) & Cattail Steady (CS).

| **Weekly average** | **TEMP(°F)** | **DEW PT(°F)** | **HUMIDITY(%)** | **PRESSURE (in.)** | **PPT.(in.)** |
|---|---|---|---|---|---|
| 6-12 July | 76.571 | 61.143 | 61.000 | 30.02 | 0.003 |
| 13-19 July | 76.714 | 62.286 | 64.000 | 29.97 | 0.051 |
| 20-26 July | 77.429 | 61.857 | 61.000 | 29.84 | 0.002 |
| 27 Jul-2 Aug | 74.571 | 59.429 | 62.429 | 29.95 | 0.052 |
| 3-9 Aug | 70.429 | 54.000 | 58.571 | 29.92 | 0.018 |
| 10-16 Aug | 76.429 | 56.429 | 54.286 | 30.08 | 0.110 |
| 17-23 Aug | 73.429 | 59.571 | 68.571 | 29.95 | 0.481 |
| 23-30 Aug | 76.429 | 60.571 | 60.143 | 29.99 | 0.139 |
| 31 Aug-6 Sept. | 69.429 | 58.286 | 68.714 | 30.07 | 0.321 |
| 7-13 Sept. | 68.429 | 54.857 | 62.286 | 30.10 | 0.004 |

Table 9. Weekly averages of various weather parameters recorded at the Heffner Research Building