



# **The Effect of Water Quality and Aquatic Vegetation on West Nile Virus Vector Larval Abundance in Toronto and Region Wetlands and Stormwater Management Ponds**

**February 2011**



Funding and/or in-kind support for the activities outlined in this report has been made available from the following partners:

*Region of Peel  
Region of York  
City of Toronto  
Region of Durham  
Toronto Remedial Action Plan*

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This report may be referenced as:  
Toronto and Region Conservation Authority (TRCA). 2011. The Effect of Water Quality and Aquatic Vegetation on West Nile Virus Vector Larval Abundance in Toronto and Region Wetlands and Stormwater Management Ponds.

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## **Acknowledgements**

TRCA recognizes the valuable contributions toward this report from many staff including Ashley Favaro, Dan Bechard and Cameron Sangster for their technical assistance with the field sampling and larval rearing; Angela Wallace and Claire Holeyton for their advice and assistance in statistical analysis; Ming Guo, and Alberta D'Souza for their help with WNV database and GIS mapping respectively. Art Augenas from the Region of Peel is acknowledged for providing QA/QC on mosquito larval identifications. The West Nile Virus Surveillance and Monitoring Program is a part of TRCA's Regional Watershed Monitoring Program and funded by the Regions of Peel, York, Durham and the City of Toronto.

## Executive summary

West Nile Virus (WNV) is a seasonal epidemic in Canada and mosquitoes are the carriers of the virus. WNV transmission to humans requires a bird - mammal transmission cycle and humans and other animals are incidental or dead end hosts. Two key vector mosquito species namely *Culex pipiens* and *Culex restuans* are primarily responsible for spreading the disease to humans in Ontario. The Toronto and Region Conservation Authority (TRCA) has been actively monitoring for WNV vectors in natural wetlands and stormwater management ponds (SWMPs) since 2003 as a measure of due diligence and at the request of its Regional Health partners (Peel, York, Durham and the City of Toronto) to prevent and manage vector mosquito breeding. The rationale for undertaking vector larval monitoring is that a variety of wetland habitats on TRCA properties such as marshes, woodland pools and ponds have the potential to provide breeding habitats for mosquitoes because of the permanent availability of water. Although monitoring the abundance, distribution and management of vector species is the primary objective for TRCA's activities, data on aquatic vegetation and water quality parameters such as conductivity, dissolved oxygen, pH, temperature and total dissolved solids were also collected during every field visit to assess if any of these factors influence the vector mosquito abundance. Characterizing the waterbodies based on the factors contributing to mosquito breeding will help determine which waterbodies are more conducive for mosquito proliferation and also to determine the preventive measures future mosquito management.

In this respect, monitoring data collected from 2005 to 2009 were analysed with the following objectives: 1) Strengthen some of the conclusions about the influence of water quality on mosquito abundance as suggested in previous reports by TRCA, 2) Investigate the relationship between mosquito abundance, water quality measures and aquatic vegetation, and predict what type of waterbodies in TRCA's jurisdiction pose a threat in terms of WNV vector breeding and, 3) Make recommendations that would help prevent potential vector breeding conditions.

A total of 48 wetlands and 10 SWMPs were monitored from 2005 to 2009 throughout the summer months and each site was sampled up to four times at approximately three week intervals. From the 864 sampling events, 14917 mosquito larvae were identified from the wetlands over the five years. Yearly larval collection data showed that number of larvae collected were the highest in 2005 and lowest in 2007. Species enumeration indicated that among the identified larvae, 60% were non-vectors and 40% were vector larvae in the wetland samples. Fifteen (15) different species identified from the wetlands, among which, the non-vector species *Culex territans* was the predominant species (59%) collected and it was followed by the key vector species *Culex pipiens* (18%).

Larval sampling yielded a lower number of mosquito larvae from the SWMPs with only 4893 larvae identified during the 2005 to 2009 sampling seasons, of which eight percent were non-vectors and 92% were vector larvae. Larval abundance data showed a reverse trend from those seen in the wetlands, in that the percentage of vector larvae ranged from 67% in 2008 to 99% in 2007, while the percentage of non-vector collected was between 1% and 33% for the same years. Species diversity was also lower when compared to the wetlands in that only eight species of mosquito were identified over the years, and the majority of the larvae identified were represented by the key vector *Culex pipiens* (85%). *Culex territans* the most common

non-vector in the wetlands represented only 8% in the SWMPs. *Culex restuans*, the species implicated in WNV along with *Culex pipiens*, only occurred in small proportion (3%) in wetlands as well as the SWMPs.

The effect of abiotic factors on mosquito larval abundance was studied by examining the amount of rainfall received during the summer months and measuring the water quality parameters (conductivity, dissolved oxygen, pH, temperature and total dissolved solids). The rainfall data suggested that while heavy rainfall was detrimental to larval abundance, moderate amounts of rainfall increased the larval abundance in the wetlands. On the contrary, rainfall had negative impact on the number of larvae collected from the SWMPs. Dry summers had the most larvae in the stormwater ponds.

When water quality parameters collected over the five years were analyzed all five parameters (conductivity, total dissolved solids (TDS), dissolved oxygen, pH and temperature) differed significantly between the wetlands and SWMPs. All the five parameters were found to be higher in SWMPs when compared to wetlands. However, detailed temporal analysis of water quality data from 30 ponds that were continuously monitored for five years showed that only the amount of dissolved oxygen was significantly different between the wetlands and SWMPs. Canonical Correspondence Analysis indicated that conductivity and TDS were closely associated with the occurrence of vector species (*Culex pipiens*, *Culex restuans* and *Culex salinarius*) in the stormwater ponds. Further correlation analysis between TDS and total vector numbers did not yield a significant correlation between the two variables. Smaller sample size for the SWMPs, time and frequency of water quality data collection were suggested as possible reasons for these non-significant results.

The influence of biotic factors was investigated by analyzing the percentage of Marginal and Total Emergent vegetation from wetlands and SWMPs. Both Marginal and Total Emergent vegetation were significantly higher in the wetlands than the SWMPs. Ordinal Logistic Regression analysis results indicated that the higher vegetation coverage in the wetlands appear to provide good habitat for larvae: where vegetation coverage (in total or marginal areas) was greater than 75%, there was a significantly higher probability that mosquito larvae (vector or otherwise) were present. However, vector larvae did not occur more frequently at wetland sites (as predicted by the distribution of vegetation); the proportion of larval samples which included vector larvae was higher in SWMPs (93%) than in wetlands (75%).

In conclusion we have empirically proved that although wetlands support more number of mosquito larvae, the majority are non-vectors of WNV. SWMPs had predominantly the vector species and hence these need to be monitored much more carefully. Based on the outcomes of our five year data analyses, we recommend the following for the mosquito and WNV vector management in the SWMPs: proper maintenance and vegetation control should be undertaken in order to prevent mosquito breeding since denser vegetation seems to favour higher numbers of mosquito larvae in general; conductivity and total dissolved solids need to be monitored on a regular basis especially in the SWMPs since they favour the breeding of vector species compared to the wetlands and finally, continuous monitoring of wetlands and stormwater ponds is necessary to detect and prevent the occasional vector hotspots.



# 1. Introduction

West Nile Virus (WNV) is a bird virus and mosquitoes are the vectors of transmission of the virus within the bird population. Humans and other mammals can be incidentally infected with WNV through the bite of an infected mosquito. The mosquitoes *Culex pipiens* and *Culex restuans* are the primary vectors in spreading the disease to humans and animals in Ontario (Ministry of Health and Long Term Care, 2009). The establishment of West Nile Virus as a seasonal epidemic in Canada has greatly increased the importance of preventing or reducing the vector mosquito breeding opportunities. Mosquito production and population dynamics is influenced by several biotic (Durham Regional Health Department, 2003; Greenway, 2005; Gingrich *et al.*, 2006; Dale *et al.*, 2007; Mwangangi *et al.*, 2007; Mwangangi *et al.*, 2008) and abiotic factors (Bolling *et al.*, 2005; Dale *et al.*, 2002; Leishman *et al.*, 2004; Mercer *et al.*, 2005; Rey *et al.*, 2006; Dale *et al.*, 2007; Muturi *et al.*, 2007; Henn *et al.*, 2008; Kwan *et al.*, 2008). Understanding the factors that enhance or deter mosquito breeding would help manage or control the mosquitoes.

Many mosquito genera implicated in WNV such as *Culex* or *Anopheles* require standing water for breeding (stagnant water species). Many shallow aquatic ecosystems such as wetlands (constructed and natural) have the potential to provide such habitats for a variety of mosquito species (Knight *et al.*, 2003; Gingrich *et al.*, 2006; Rey *et al.*, 2006) including those that are capable of transmitting WNV. So when WNV was introduced to Toronto in 2002, wetlands were questioned as a likely source for WNV due to the public perception of wetlands as “mosquito-friendly habitats” for WNV vector mosquitoes. In response to this the Toronto and Region Conservation Authority (TRCA), being the largest landowner in the Toronto region initiated an active WNV vector surveillance and monitoring program in selected natural wetlands and stormwater management ponds (SWMPs) as a measure of due diligence and at the request of its Regional Health partners (Peel, York, Durham and the City of Toronto) to assess the vector production. Since 2003, a variety of wetland habitats on TRCA property such as marshes, woodland pools and ponds have been surveyed for their potential to provide breeding habitats for mosquitoes because of the permanent availability of water and presence of emergent vegetation. The purpose of the TRCA’s monitoring activities is to determine if vector mosquitoes are breeding in these natural areas and if any management actions are required. In addition, the surveillance data have also been used in support of various habitat restoration and wetland creation projects undertaken by TRCA to empirically show that wetlands typically do not support high numbers of vector species under normal circumstances.

In addition to monitoring the abundance and distribution of vector species on TRCA properties, data on aquatic vegetation and water quality parameters such as conductivity, dissolved oxygen, pH, temperature and total dissolved solids were also collected where possible to assess if any of these factors influence the vector mosquito abundance. Assessing the factors contributing to mosquito breeding will help determine which type of waterbodies are more conducive for mosquito proliferation and also to determine the preventive measures to eliminate future mosquito proliferation.

Annual surveillance reports prepared by TRCA (TRCA 2004; 2006a; 2006b; 2008) have indicated that water quality can influence the abundance of mosquitoes in TRCA jurisdiction. However, these results were not statistically significant to clearly depict the relationship between water quality and mosquito abundance; this may, at least in part be due to the small size of the dataset as data from each sampling year was analyzed

separately. Consequently, our objectives for this report were to analyze the data collected over the past five years to:

- Strengthen our conclusions about the influence of water quality on mosquito abundance as suggested in previous reports by combining the data collected from 2005 to 2009, so that a more comprehensive analysis could be used to compare the mosquito vector abundance in wetlands;
- Investigate the relationship between mosquito abundance, water quality measures and aquatic vegetation in order to predict what type of waterbodies in TRCA's jurisdiction pose a threat in terms of WNV vector breeding; and,
- Make recommendations that would help prevent potential vector breeding conditions in wetlands and SWMPs.

Currently, there is only limited literature available that documents empirical evidence from wetlands on vector abundance and the effect of water quality parameters on mosquito species distribution. In this respect, the results from our report will help impart knowledge on mosquito management as well.

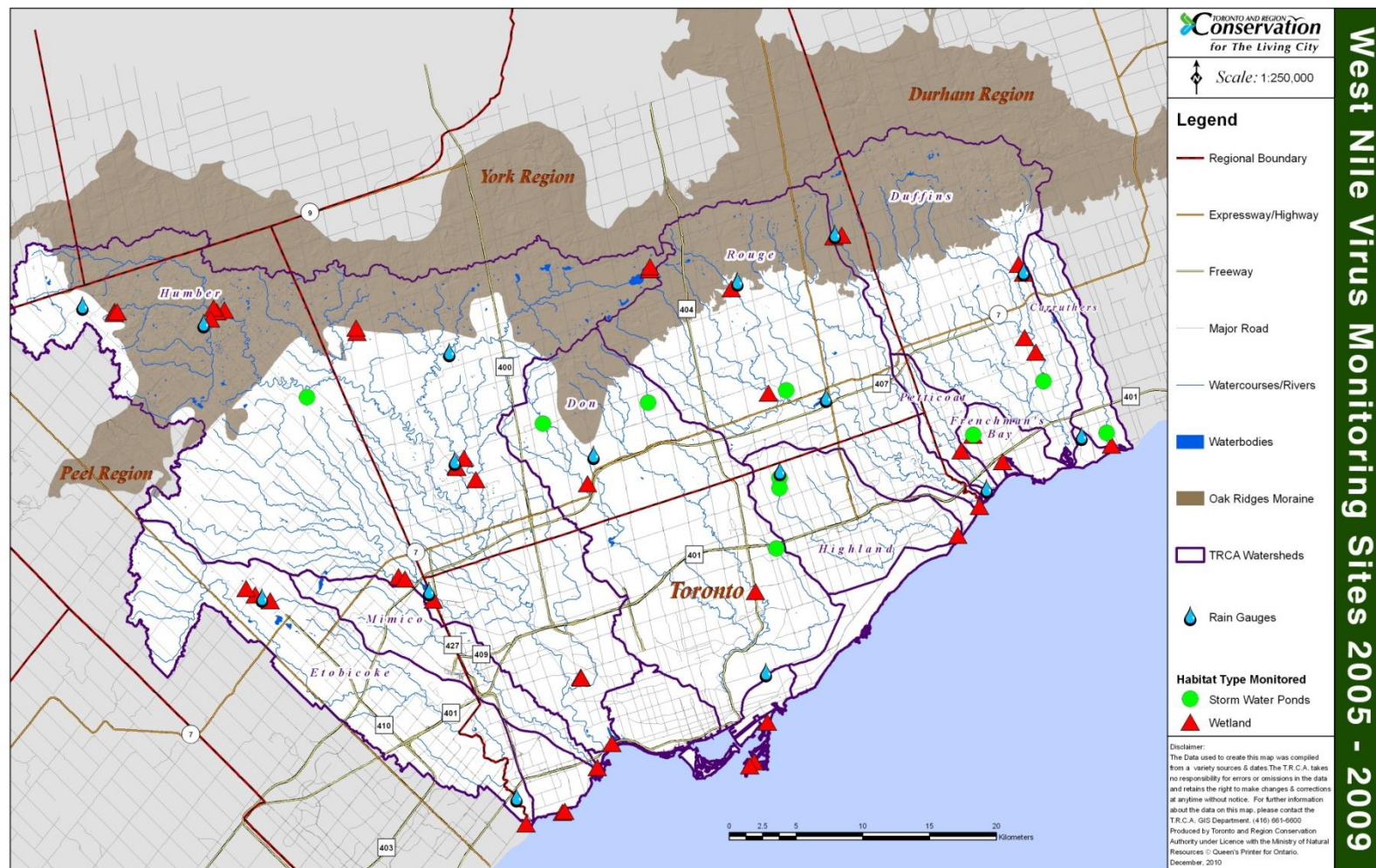
## 2. Methods

### 2.1 Vector Larval Monitoring and Identification

The TRCA larval monitoring and surveillance activities for the 2005 – 2009 field seasons began approximately around the last week of May during each year with an objective to characterize the mosquito species and their abundance in wetlands and SWMPs. Over the five year period, 48 wetlands and 10 SWMPs had been sampled for assessing the vector abundance and species composition (**Figure 1; Appendix 1**). The larval mosquito sampling protocol consisted of each site being sampled up to four times during the summer at approximately three week intervals. Each sample consisted of 10 dips using a standard mosquito dipper. Sites larger than 3m<sup>2</sup> were divided into four equal quadrants with one sample being taken from each quadrant. Upon completion of sampling, the larvae were transported to a processing laboratory in coolers; the mature larvae were killed in boiling water, preserved in 70% ethanol and identified to species using the mosquito identification keys (Wood *et al.*, 1979; Darsie and Ward, 2005). Any mosquito larvae found in 1<sup>st</sup> to 3<sup>rd</sup> instars were reared in the laboratory at room temperature until they had reached the 4<sup>th</sup> instar. The mature larvae (late 3<sup>rd</sup> instars and 4<sup>th</sup> instars) were then killed and preserved to facilitate easy identification. This protocol was repeated every year (TRCA, 2006a; 2006b; 2007; 2008; 2010).

### 2.2 Rainfall, Water Quality and Aquatic Vegetation Assessment

Data on total rainfall received during the summer months (May to September) for 2005 to 2009 were obtained from TRCA's network of rain gauges (**Figure 1; Appendix 2**) that are located close to the WNV



**Figure 1.** Natural wetlands and SWMPs monitored during 2005- 2009 WNV season. Map also shows the location of rain gauges that are located closer to the WNV monitoring sites

monitoring and surveillance sites. Monthly rainfall data from 16 rain gauges were used to get the yearly summer averages for the Toronto region.

During each routine sampling visit, a single in-situ measurement of water temperature, pH, total dissolved solids (TDS), conductivity and dissolved oxygen (DO) was taken at the margin of the wetlands and using an YSI meter (650 MDS). Generally the water quality data was collected at one sampling location per site and data for subsequent visits was collected from the same location each visit.

In order to determine if any association exists between aquatic vegetation density and the mosquito larval communities present in TRCA wetlands and SWMPs visual estimates of emergent vegetation were made for each site during each visit. This included vegetation in an area extending less than 1 meter out from the shoreline – Marginal Emergent vegetation and for the entire wetland/pond – Total Emergent vegetation). The Total and Marginal Emergent vegetation was estimated by visually ranking the percentage of vegetation cover. Sites were ranked as “low” when estimates of each Marginal and Total Emergent vegetation cover fell between 1 - 25%, “moderately low” if each of the vegetation cover was between 25 - 50%, “moderately high” if the vegetation cover was between 50 - 75% and “very high” if estimates fell between 75 - 100%.

## 2.3 Data Analyses

A total of 864 sampling events (visits) from 58 different ponds were conducted during the five year period. However, on a number of occasions data were not collected during each of the four sampling events due to sites being dry, water being too shallow to carry out the dips or inaccessible. Because of this the number of sampling events with data was less than 864 in general for different parameters that were measured during the study period (**Table 1**). In addition, the number of samples used in different analyses also varied depending on the criteria used to select the data.

**Table 1.** Number of sampling events considered in different statistical analyses for the 2005- 2009 monitoring and surveillance study

Study parameters	Sample size (N)
Larval abundance and species composition	864
Larval abundance and composition – five year trend	600
Average rainfall	16
Water quality parameters	743
Water quality parameters – five year trend	600
Water quality parameters and larval abundance (Correlation)	836
Aquatic vegetation and larval abundance	839



### **2.3.1 Mosquito Community Composition**

Data collected on the number of larvae identified per species from all sampling events (N= 864) were used to calculate summary statistics such as the average number of larvae, species abundance and composition for a given species of mosquito for each year.

### **2.3.2 Total Vector Abundance in Wetlands and SWMPs**

All larval samples from 864 sampling events were included in the general analysis to calculate mean number of larvae per sampling event and one-way Analysis of Variance (ANOVA) was used to compare data by waterbody type (SWMP vs. wetland) at  $\alpha = 0.05$ . In order to identify any temporal trends, a more detailed analysis using data from only those ponds that were sampled continuously for five years and had four sampling events was further carried out. Only 30 ponds (22 wetlands and 8 SWMPs) had samples for five consecutive years (2005-2009). Data from these ponds (30ponds \* 5 years\* 4 sampling events; N= 600) were used to test for significance in total vector abundance between the two types of waterbodies using one-way ANOVA.

### **2.3.3 Rainfall and Mosquito Abundance**

Monthly total rainfall data (May 25<sup>th</sup> to September 15<sup>th</sup> for 2005 to 2009) from 16 locations were averaged to get the mean summer rainfall for the TRCA jurisdiction for each year and these yearly summer averages were plotted along with the larval abundance data from the wetlands and SWMPs to determine if the amount of rainfall received in a given summer had any influence on the number of larvae collected from each water body type.

### **2.3.4 Water Quality in Wetlands and SWMPs**

Water quality data was collected only once during each sampling event unlike the mosquito larval collection. However, on a number of sampling occasions (dates) it was not possible to measure in-situ water quality data for a variety of reasons (e.g. water quality probe was not working or not available, water was too shallow to take a reading, pond dried up etc.). Therefore, the number of sampling events per year was not always equal to four. Hence, the number of ponds (N=54) and the number of water quality samples (N=743) is less than the number of samples used in the mosquito community analysis.

Mean water quality parameters per sampling event was calculated from the 743 sampling events and One-way ANOVA was used to test for significance in water quality parameters between the water body types at  $\alpha = 0.05$ . To identify temporal trends, a detailed analysis of water quality data, from the 30 ponds which had been continuously monitored for five years was further carried. However, some of these ponds also had less than four sampling events due to the same reasons mentioned above. Because this would have severely limited the dataset available for the detailed analysis, and the fact large differences were not noted between sampling events at most sites the water quality data was still

pooled together for each year even if there were not four water quality readings. Because of this, the data should be *interpreted with caution* as it may be skewed. All annual averages [including those determined from fewer than 4 sampling events; N= 150 (n=40 for SWMPs and n= 110 wetlands)] were used to carry out an ANOVA of the water quality data by the water body types.

### 2.3.5 Water Quality and Mosquito Species Distribution

Canonical Correspondence Analysis (CCA) was used to investigate associations between water quality parameters and species distribution of the mosquito community at the 30 ponds meeting our selection criteria for larval abundance (continuously monitored, had four sampling events per year). Prior to carrying out CCA, species abundance was log-transformed to reduce the effect of samples with extremely high abundance and data was screened for outliers. Rare species (less than 10% of overall composition) were excluded from the analysis.

For the CCA, the water quality and mosquito community data were averaged as an annual mean per site and also as a 5-year mean (average of the annual means from 2005 - 2009). This resulted in N=30 for the analysis. The results of the CCA were used as an indicator of water quality conditions which were found in association with populations containing high numbers of vector species. Water quality parameters which grouped on the tri-plot close to vector species were chosen for further analysis.

Relationships between the parameters of interest and the abundance of vector species were then further explored using correlation analyses. A non-parametric method (Spearman's rank correlation) was used since the data did not meet the criteria for parametric tests (e.g. normally distributed data). A significant positive correlation between a water quality parameter and the total abundance of all vector species for each site was to be indicative of 'preferred' conditions that support the growth of vector species. Since correlation estimates a relationship (positive/negative) between the water quality parameters of interest regardless of site, data from all 58 sites were used in the analysis whether they were monitored continuously or had 4 sampling events per year. However sampling events with less than 4 replications, where vector larvae were absent, TDS values were missing or had extremely high values (outlier) were omitted from the analysis which resulted in N=836 samples to be used in the correlation analysis.

### 2.3.6 Aquatic Vegetation and Mosquito Abundance

To estimate the effect of Marginal and Total emergent vegetation on the mosquito community, the presence or absence of mosquito larvae in a given habitat (SWMP and wetlands), and the vegetation cover type (Marginal vs. Total Vegetation) was analyzed using the 2005 - 2009 vegetation data. Of the 864 sampling events from 58 sites, only 839 samples had both the larval counts and the vegetation estimated and these were included in the vegetation analysis. Since observations of vegetative cover were measured as categorical ordered data (termed 'ordinal' data), an ordinal logistic regression was used to test for differences in the presence or absence of mosquito larvae between categories of vegetation cover. Fisher's exact test was used to determine if there was a significant difference in frequencies of detecting mosquito larvae between SWMPs and wetlands. This test allows a comparison

between two categories of variable with only two possible values (i.e. presence or absence). All data analyses were completed using Microsoft Excel (Redmond, WA) with the Biplot Macro add (Lipkovich and Smith, 2002) and SAS JMP ver 8.0 for windows (Carrey, North Carolina).

## 3. Results and Discussion

### 3.1 Mosquito Larval Abundance and Species Composition

#### 3.1.1 Wetlands

From the 864 sampling events, a total of 20,313 mosquito larvae were collected from the wetlands over the five years. Of which, 5396 larvae (27%) had died in the lab during rearing process due to a variety of reasons such as cannibalism, over crowding in rearing jars, inadequate lab temperature for rearing etc. This resulted in about 14917 larvae being successfully reared and identified from 2005 to 2009 from the wetlands. Species enumeration indicated that among the identified larvae, 8902 (60%) were non-vectors and 6015 (40%) were vector larvae in the wetland samples. Yearly larval collection data from 2005 to 2009 showed that number of larvae collected were the highest in 2005 and lowest in 2007 (**Figure 2a**). The yearly data also indicated that the percentage of non-vector larvae was higher than the percentage of vectors except for 2005 and 2006; and their percentages ranged from 38% to 81%. This emphasizes the point that although a large number of larvae were collected from the wetlands, they are mostly non-vectors and not necessarily a significant WNV threat.

A total of 15 different species were identified and the species list for the wetlands included six non-vector species and nine vectors (**Table 2**). Among the identified larvae, the non-vector species *Culex territans* was the most commonly collected (59%) mosquito larvae from the wetlands (**Figure 2b**) followed by the vector species *Culex pipiens* (18%).

**Table 2.** List of West Nile Virus vector and non-vector mosquito species identified (in order of decreasing abundance) from the wetlands from 2005 - 2009

Non-vector species	Vector species
<i>Culex territans</i>	<i>Culex pipiens</i>
<i>Psorophora ferox</i>	<i>Anopheles punctipennis</i>
<i>Culiseta inornata</i>	<i>Culex restuans</i>
<i>Anopheles earlei</i>	<i>Aedes vexans</i>
<i>Ochlerotatus mercurator</i>	<i>Anopheles quadrimaculatus</i>
<i>ochlerotatus punctor</i>	<i>Culex salinarius</i>
	<i>Culex tarsalis</i>
	<i>Anopheles walkeri</i>
	<i>Ochlerotatus trivittatus</i>

Figure 2a

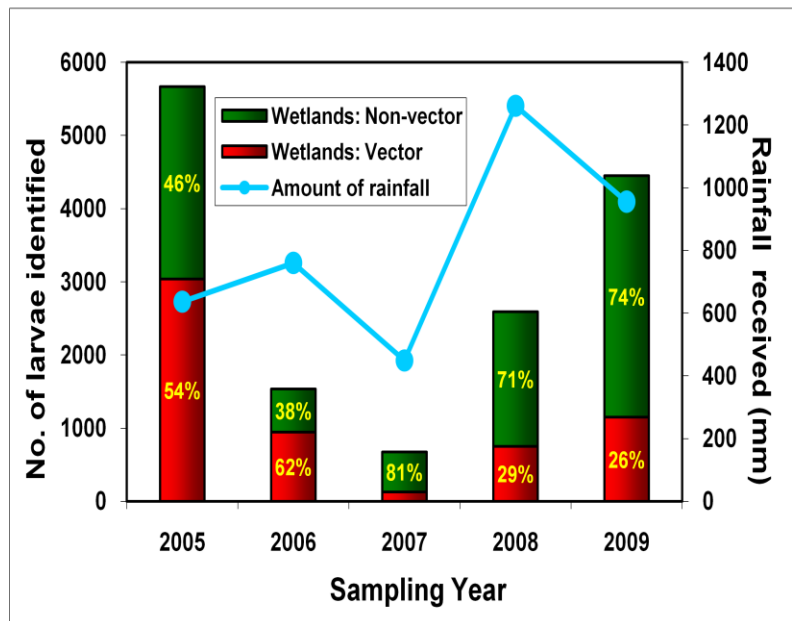
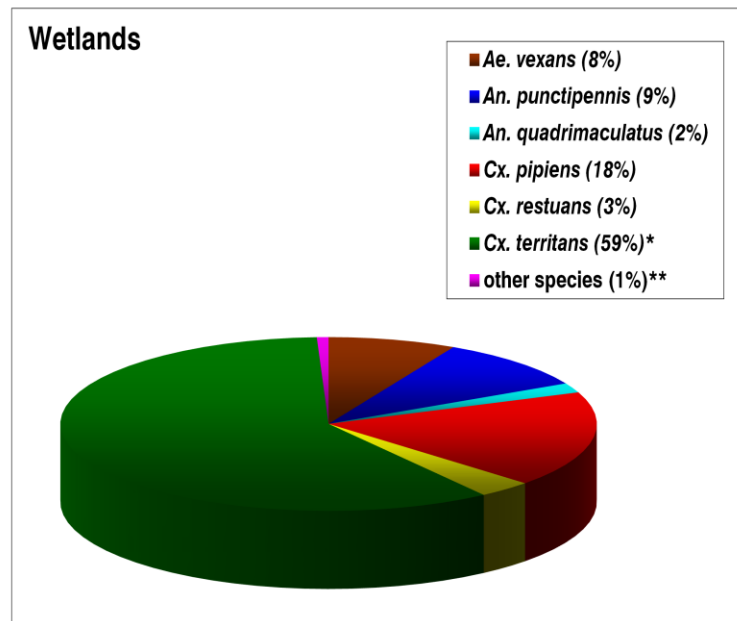


Figure 2b



**Figure 2.** Abundance and species composition of vector and non-vector mosquito larvae in wetlands during 2005 – 2009. The average amount of rainfall (mm) received during the sampling periods (May to September) is shown in blue line. Numbers inside each bar indicates the percentage of larvae identified. Note: \* indicates non-vector species; \*\* indicates vector and non-vector species contributing <1% of identified larvae are not shown in the pie chart since their proportions were very small to graphically display. These species include *An. earlei*, *An. walkeri*, *Cs. inornata*, *Cx. salinarius*, *Cx. tarsalis*, *Oc. mercurator*, *Oc. punctor*, *Oc. trivittatus* and *Ps. ferox*



The primary vectors, namely the *Cx. pipiens* and *Cx. restuans*, accounted for about 20% of the larvae identified from the wetlands (2005 to 2009), stressing that although the key vectors did occur in the wetlands, their overall abundance was less than 1/3 of the non-vector species found in the wetlands.

Yearly data summaries from previous reports indicated that the vector species *Anopheles punctipennis* was the most commonly found species in the wetlands next to *Cx. territans* (TRCA, 2006b; 2007; 2008; 2010), although their abundance is less. However this species is not a competent vector when compared to the *Culex* species as *Anopheles punctipennis* prefers to bite mammals (Ontario Ministry of Health and Long-term care, 2003) and WNV transmission to mammals requires mosquitoes that bite both bird and mammals. Some of the less common species such as *Psorophora ferox* and *Culiseta inornata* were also found in wetlands, suggesting that although the wetlands had more mosquito larvae breeding, they support diverse species, which perhaps play a role in the food chain supporting other wetland fauna.

### 3.1.2 Stormwater Management Ponds

A total of 5568 larvae were identified from the SWMPs during the 2005 to 2009 sampling seasons, of which 675 (12%) larvae had died due to the same reason mentioned in the previous section. Among the identified larvae, 395 (8%) were non-vectors and 4498 (92%) were vector larvae. Sampling data from 2005 to 2009 showed a reverse trend from that seen in the wetlands in terms of the percentage of vector vs. non-vectors identified. In the SWMPs (**Figure 3a**) the vector percentages were consistently higher than the non-vector larvae. The percentage of vector larvae ranged from 67% (2008) to 99% (2007), while the percentage of non-vector collected was between 1% and 33% for the same years.

Eight species of mosquito larvae were identified over the years, of which *Culex territans* and *Anopheles earlei* were the only non-vectors collected from the SWMPs. The vector species included *Culex pipiens*, *Culex restuans*, *Anopheles punctipennis*, *Anopheles quadrimaculatus*, *Aedes vexans*, and *Culex salinarius* (in order of decreasing abundance). The majority of the larvae identified were represented by the key vector *Culex pipiens* (85%), (**Figure 3b**). Species such as *Culex territans* and *Anopheles punctipennis* which were common in the wetlands represented only 8% and 3% of the total species composition respectively in the SWMPs. Another important point worth noting was that *Culex restuans*, the species most widely implicated in WNV along with *Culex pipiens*, only occurred in small proportion (3%) in wetlands as well as the SWMPs (**Figures 2b** and **3b**).

When compared to the wetlands, the overall species diversity and abundance of larvae were low in the SWMPs (**Figure 2b** and **3b**). The reasons for the reduced diversity and abundance may be that the SWMPs typically contain high concentrations of organic pollutants and have low thermal stability (fluctuating temperatures due to constant mixing of runoff water), that species such as *Culex pipiens* perhaps can tolerate, while species like *Culex territans* and *Anopheles punctipennis* may require less pollution and constant temperature, which is more likely to exist in wetlands.

Figure 3a

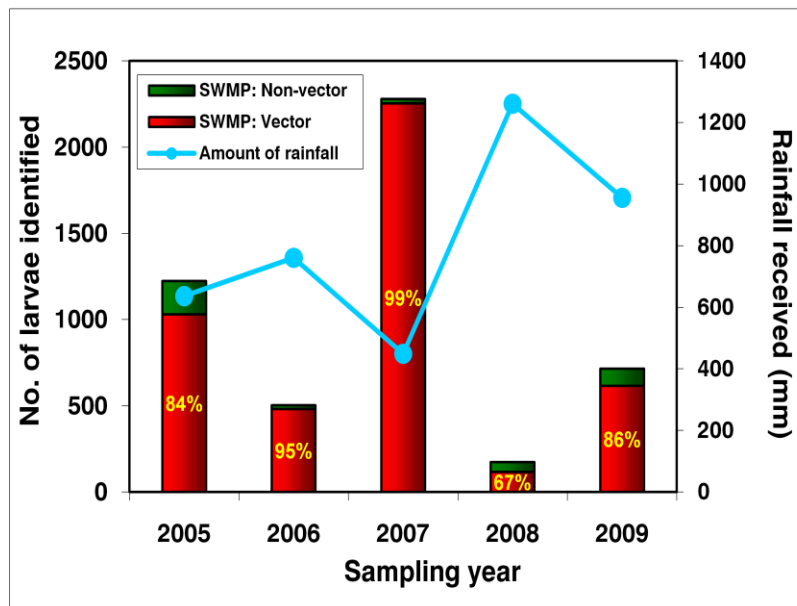
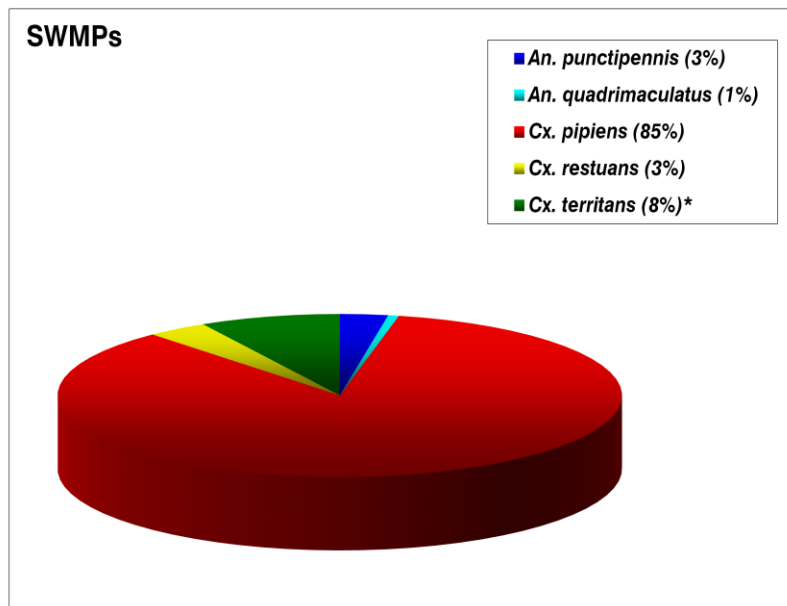


Figure 3b



**Figure 3.** Abundance and species composition of vector and non-vector mosquito larvae in stormwater management ponds during 2005 – 2009. The average amount of rainfall (mm) received during the sampling periods (May to September) is shown in blue line. Numbers inside each bar indicates the percentage of larvae identified. Note: \* indicates non-vector species; The vector and non-vector species contributing <1% of identified larvae are not shown in the pie chart since their proportions were very small to graphically display and they include *Cx. salinarius* and *An. earlei*

### 3.1.3 Total Vector Abundance: Wetlands vs. SWMPs

When the data for vectors from 864 samples were analyzed, the number of vectors was shown to be significantly greater in SWMPs compared to wetlands (**Table 3**;  $p < 0.02$ ). The average number of vector mosquitoes sampled was nearly three times higher in SWMPs compared to wetlands.

**Table 3.** One-way ANOVA results for total WNV vector counts by type 2005-2009 for 58 ponds (N=864). \* Significant at  $\alpha = 0.05$

Type	Number	Mean	Std Error	F	p
SWMP	181	6.228	1.489	5.753	<b>0.017*</b>
Wetland	683	2.215	0.766		

#### 3.1.3.1 By Species: Wetlands vs. SWMPs

When the data for all mosquito species occurring in wetlands and SWMPs were subjected to one-way ANOVA, only three species of mosquitoes were found to be significantly different between SWMPs and wetlands (**Table 4**). *Anopheles punctipennis*, a vector species, were found in common in wetlands compared to SWMPs but were found in very low numbers in both water body types. *Culex pipiens*, the key vector species, were found in high numbers in SWMPs; almost six times the numbers found in wetlands. *Culex territans*, a non-vector species, was found significantly in higher numbers in wetlands compared to SWMPs.

**Table 4.** One-way ANOVA results by species and water body type 2005-2009 (N=864). Results are shown for only those species that are occurring both in wetlands and SWMPs. \* Significant at  $\alpha = 0.05$

Species	Type	Number	Mean	Std Error	F	p
<i>Ae. vexans</i> (Vector)	SWMP	181	0.010	0.369	0.997	0.318
	Wetland	683	0.422	0.190		
<i>An. earlei</i> (Non-vector)	SWMP	181	0.003	0.003	0.033	0.856
	Wetland	683	0.003	0.001		
<i>An. punctipennis</i> (Vector)	SWMP	181	0.192	0.143	4.318	<b>0.038*</b>
	Wetland	683	0.526	0.073		
<i>An. quadrimaculatus</i> (Vector)	SWMP	181	0.041	0.032	2.588	0.108
	Wetland	683	0.099	0.016		
<i>Cx. pipiens</i> (Vector)	SWMP	181	5.728	1.363	9.607	<b>0.002*</b>
	Wetland	683	0.978	0.701		
<i>Cx. restuans</i> (Vector)	SWMP	181	0.236	0.147	0.106	0.745
	Wetland	683	0.182	0.076		
<i>Cx. salinarius</i> (Vector)	SWMP	181	0.023	0.009	3.007	0.083
	Wetland	683	0.005	0.005		
<i>Cx. territans</i> (Non-vector)	SWMP	181	0.551	0.681	12.191	<b>0.001*</b>
	Wetland	683	3.224	0.350		

The differences in vector abundance between wetlands and SWMPs were still evident when the data from the 30 ponds that were continuously monitored for five years were compared. These results showed that the stormwater ponds had 3.5 times more vector larvae than the wetlands. The one-way ANOVA again indicated that SWMPs had significantly higher numbers of vector species compared to wetlands (**Table 5:  $p=0.03$** ) confirming the larval summaries discussed above.

**Table 5.** One-way ANOVA results for differences in total WNV vector abundance by water body type for 30 ponds monitored continuously from 2005-2009 (N=150). \* Significant at  $\alpha = 0.05$

Type	Number	Mean	Std Error	F	p
SWMP	40	7.018	1.989	4.778	0.030*
Wetlands	110	1.941	1.199		

### 3.2 Effect of Rainfall on Larval Abundance

The rainfall data analysis suggests that the differences in the abundance and diversity of mosquito larvae between the wetlands and SWMPs can be attributed, at least in part to the amount of rainfall. For example, 2008 and 2009 were wet years; the total amount of rainfall received was 1261 and 956 mm and the number of mosquito larvae found in wetlands was 2589 and 4448 respectively. Conversely in 2007 the rainfall received was 449 mm (**Figure 2a** and **3a**) and the number of larvae found in the wetlands was 676. The exception to this pattern was the 2005 data: where more larvae (5669) were collected than any other year, but the amount of rainfall received was the second lowest (636 mm) recorded in the five year period. The reason for the high number in 2005 was that the majority of the larvae were collected from a few sites (Carruther's Creek swamp, Duckweed pond and Topham pond) that had stagnant water in isolated pockets. These sites have the tendency to get flooded during storm events and hold water in small pools providing ideal breeding habitat for the mosquito larvae. So it appears that in the wetlands, dry summers reduce the number of larvae collected while moderate amounts of rainfall seemed to result in an increased number of larvae sampled, and excessive rainfall appears to have a negative effect on the larval abundance (e.g., 2008).

It can be speculated that the increase in larval numbers under moderate rainfall can be related to wetland characteristics. Unlike the SWMPs, the water does not drain quickly from wetlands, giving more time for the larval development and the larvae can find refuge among the vegetation such as cattails during rain events. Excessive rainfall however might prevent larval development; reduce abundance by washing larvae away, altering the thermal regime due to frequent runoff and making the habitat less suitable for the development of certain species.

In contrast, the amount of rainfall received during the summer months had the opposite effect on the larval numbers in SWMPs. There was an overall reduction in the number of larvae sampled from the storm ponds even under a moderate amount of rainfall. The number of larvae collected was highest

(2279) during 2007, which corresponded to the dry year where the total amount of rainfall received was the lowest (449mm) and this was followed by larval samples from 2005 where the number of larvae collected were second highest (1223 larvae) and the amount of rainfall received was 531 mm (**Figure 2a** and **3a**). The wettest year, 2008 had the lowest number of larvae (173) identified from the SWMPs.

The reasons for the increased number of larvae in SWMPs during dry summers can be attributed to stormwater management pond characteristics; although the SWMPs are designed to hold water for a short period of time, many ponds do hold water for longer than the prescribed 72 hours and during dry summers when the rain events are not frequent, the chances of water being stored for longer is increased after the initial run off. During dry spells the disturbance due rain fall and the movement of water would be minimal, creating a container like environment which is likely to increase the chances for more mosquitoes to complete their development. In addition when the rain events are less the water held in isolated pockets and among armouring rocks will not drain quickly and this may also present an ideal breeding ground for the mosquitoes.

The effect of rainfall on the number of mosquito larvae collected has been reported in previous studies. For example, Bolling *et al.* (2005) have reported that the abundance of *Culex salinarius* and *Psorophora columbiae* were positively related to two-week accumulation precipitation. Similarly, Rey *et al.* (2006) noted that total rainfall for five days prior to sampling had a significant negative effect on the total number of mosquitoes collected and on the number of *Culex nigripalpus* and *Culex quinquefasciatus* from two Florida cities. Also, the amount of rainfall received during winter months (February – March) reduced the population density of *Cx. quinquefasciatus* in the summer months in California (Su *et al.*, 2003). Kwan *et al.* (2008) speculated that precipitation might cause frequent runoff into storm water structures that disturb the habitat, potentially impairing or eliminating the ability of mosquito to hatch and develop and may wash existing larvae from the storm ponds. In addition, continuously disturbed water may also deter egg laying behaviours of the gravid females. Support for these theories was found in our data, which suggested that the short-term heavy summer rain fall did not contribute to the increased presence of mosquito larvae from both wetlands (e.g. 2008 data) and the storm ponds while moderate rainfalls favoured the mosquito proliferation in the wetlands.

### 3.3 Water Quality in Wetlands and SWMPs

The results of comparisons of water quality between SWMPs and wetlands from the one-way ANOVA are presented in **Table 6**. When the data from all 743 sampling events were analyzed, all five water quality parameters showed significant differences between SWMPs and wetlands. SWMPs had higher conductivity, TDS, dissolved oxygen, pH and temperature compared to wetlands. Although these results are statistically significant, they may not be biologically significant. For example, a pH difference of 7.5 compared to 7.8 may be statistically different but may not affect the biological community.

**Table 6.** One-way ANOVA results for in-situ water quality samples by waterbody type 2005-2009 (N=743). \* Significant at  $\alpha = 0.05$

Water Quality Parameter	Type	Number	Mean	Std Error	F	p
Conductivity	SWMP	172	988.4	46.4	5.574	<b>0.0185*</b>
	Wetland	571	863.4	25.4		
TDS	SWMP	172	0.649	0.030	6.304	<b>0.012*</b>
	Wetland	571	0.563	0.165		
Dissolved Oxygen	SWMP	172	8.61	0.36	32.13	< <b>0.001*</b>
	Wetland	571	6.31	0.19		
pH	SWMP	172	7.75	0.042	31.44	< <b>0.001*</b>
	Wetland	571	7.48	0.023		
Temperature	SWMP	172	21.51	0.27	9.11	<b>0.003*</b>
	Wetland	571	20.57	0.15		

When the data from the 30 ponds that were continuously monitored for five consecutive years was examined, the results of the ANOVA indicated that SWMPs had slightly lower mean conductivity and TDS than wetlands, but higher dissolved oxygen, pH and the temperature relative to wetlands (**Table 7**). However, only the dissolved oxygen showed statistically significant ( $p = 0.014$ ) difference between the SWMPs and wetlands.

**Table 7.** One-way ANOVA results testing the difference in in-situ water quality (2005-2009) between water body types for 30 ponds monitored continuously for five years (N=150). \* Significant at  $\alpha = 0.05$

Water Quality Parameter	Type	Number	Mean	Std Error	F	p
Conductivity	SWMP	40	962.78	94.19	0.021	0.088
	Wetland	110	978.84	56.80		
TDS	SWMP	40	0.634	0.0610	0.007	0.935
	Wetland	110	0.640	0.040		
Dissolved Oxygen	SWMP	40	8.46	0.57	6.19	<b>0.014*</b>
	Wetland	110	6.81	0.34		
pH	SWMP	40	7.72	0.066	2.96	0.088
	Wetland	110	7.58	0.041		
Temperature	SWMP	40	21.53	0.41	1.23	0.269
	Wetland	110	21.00	0.25		

Conductivity is influenced by the amount of total dissolved solids (TDS); in turn, the quantity of TDS in the storm ponds is influenced by the run-off and storm events. If the samples were consistently taken

after the rain events then we would expect their values to be higher than samples taken before the rain events. Since our samples were taken once in three weeks on a rotation and not necessarily coinciding before or after the rain events, we should interpret the results with caution. Similarly, we would expect to see a higher amount of DO since there is a constant mixing of run-off and water in the SWMPs, while in the wetlands the mixing due to run-off will be limited. The same reasoning can hold for the higher pH and water temperature. In addition, differences in the sample sizes between the water body types will also affect the outcome of the analysis.

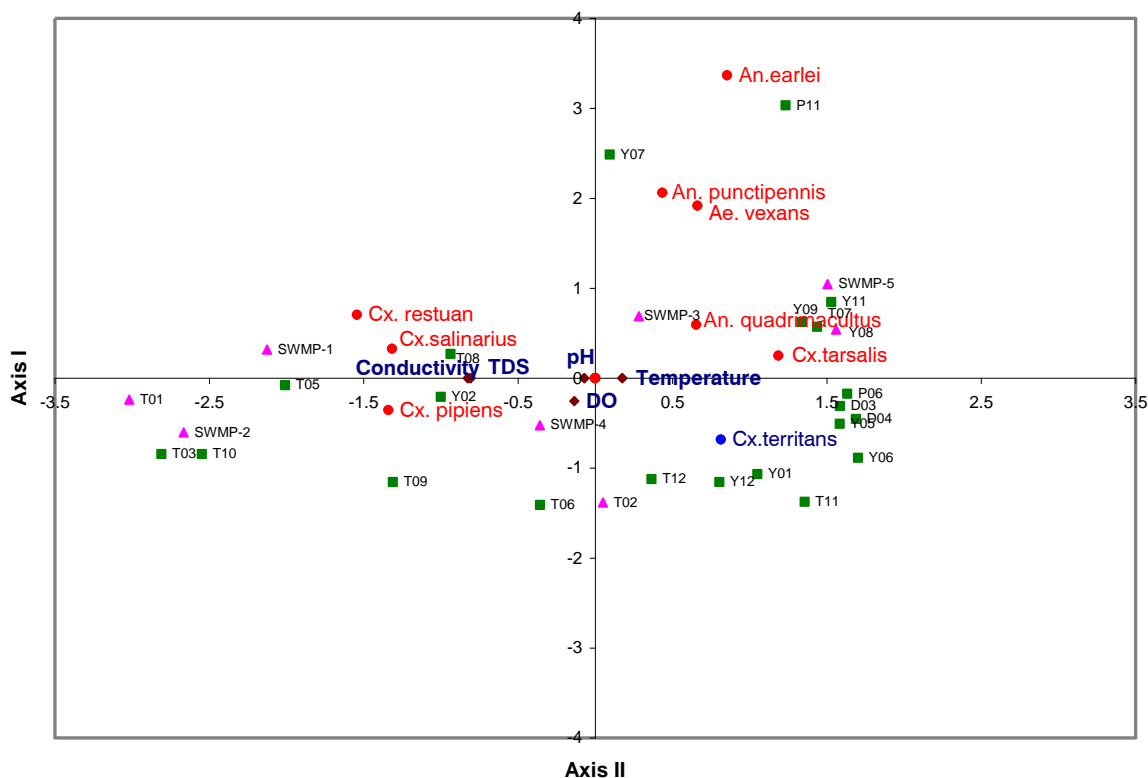
### 3.4 Effect of Water Quality on Mosquito Species Distribution

The outcomes of Canonical Correspondence analysis investigating the associations between larval data and in-situ water quality are presented in **Table 8**. The first two axes of the CCA explained over 92% of the variation caused by the water quality variables. The CCA associations of the 2005 to 2009 mosquito species data along with the in-situ water quality are graphically displayed in **Figure 4**. Axis I of the CCA can be described as a gradient of conductivity or TDS. The CCA revealed a clear association of the *Culex* species with conductivity/TDS along Axis I. The three main *Culex* vector species were associated with higher conductivity of water. The other three water quality parameters used in the CCA did not have a strong influence on the mosquito community although the DO differed significantly between the wetlands and the SWMPs in the ANOVA (see **Table 7**).

**Table 8.** Eigen values from CCA of the mosquito community and water quality data

CCA Axes	Eigenvalues	Cumulative % of Eigenvalues
Axis I	0.2108	0.6136
Axis II	0.1047	0.9184
Sum of Eigen values	0.3436	

Both TDS and conductivity were good candidates for further investigation due to their strong associations with the abundance of vector species. Since these two water quality variables are highly correlated and contained similar information, our analysis used one of these variables for further analysis. TDS was chosen to further explore the influence on the *Culex* sp. because it has been shown to influence the abundance of mosquito species in monitoring reports by TRCA (TRCA, 2006a; 2008). These previous analyses indicated a positive association with *Culex pipiens* and TDS (TRCA, 2006a) and a negative association with *Culex salinarius* and *Culex restuans* (TRCA, 2008). Even though the current CCA suggested that the abundance of some vector species was related to concentrations of TDS, the average TDS concentration at each location was not significantly correlated with increased total abundance of all vector species at each location (**Table 9**,  $p = 0.4865$ ).



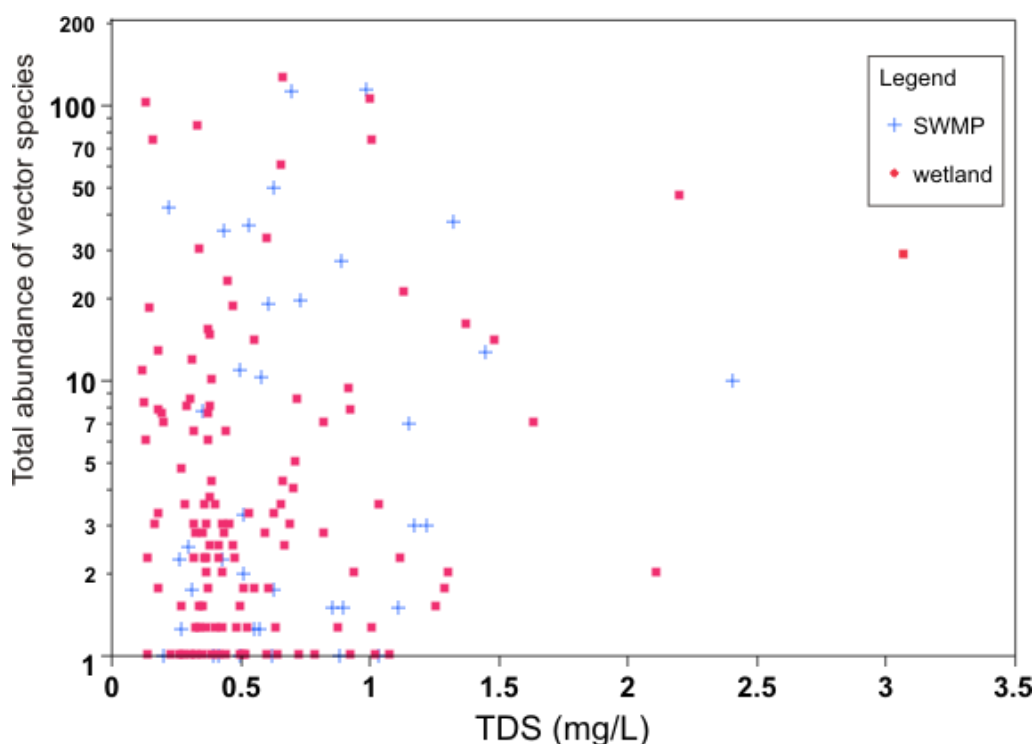
**Figure 4.** Triplot of a Canonical Correspondence Analysis (CCA) showing associations between different species of mosquito larvae and in-situ water quality variables from SWMPs (pink triangles ▲) and wetland locations (green squares ■). WNV vector species are highlighted in red font. Relationships between variables or similarity of samples are indicated by close clusters of points

**Table 9.** Spearman rank correlation between TDS and total vector abundance (N= 836)

Variable	By variable	Spearman's $\rho$	$p$
TDS	total vector	0.0398	0.4865

A scatter plot of total abundance of all vector species and concurrent measurements of TDS indicated there were no underlying differences between SWMP and wetlands (**Figure 5**). Considered together, these results suggest that differences in the total abundance of vector species between SWMPs and wetlands were not related to differences in TDS between the two habitat types.





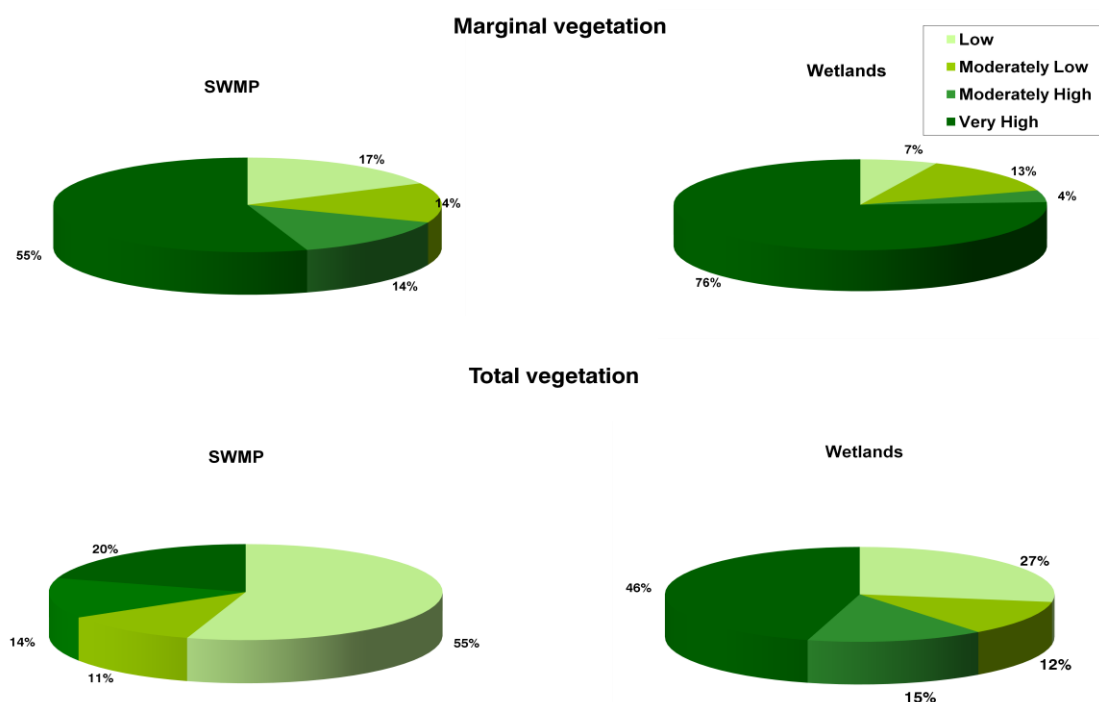
**Figure 5.** Relationship between total abundance of vector species and TDS concentrations in SWMPs and Wetlands

Although concentrations of TDS do not appear to be strongly linked to the total abundance of all vector species combined in the present study, the results of previous studies indicate that individual species may be favoured or hindered by variations in TDS (TRCA, 2006a; TRCA, 2008). As noted in the previous section, water quality parameters can fluctuate over the course of a day and with the environment (e.g. precipitation) and our sampling (which was conducted only 4 times a year) may not accurately reflect the dynamic conditions in each water body. Consequently, our analysis may not have been able to detect true relationships between environmental variables and the mosquito community. Conversely, a relationship between an environmental variable and the abundance of mosquito larvae shown by statistical analysis may not necessarily have been caused by a direct biological effect of the environmental variable on the mosquito community. Where significant differences in environmental factors such as water quality are found, we have considered if (and how) they are likely to have a biological impact.

### 3.5 Effect of Aquatic Vegetation on Mosquito Abundance

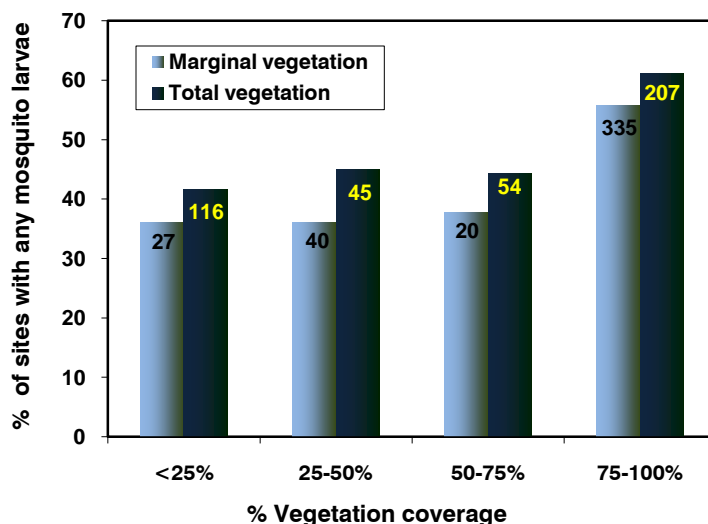
Analyses of the aquatic vegetation data collected from the wetlands and the storm water management ponds showed that the majority of sites where larvae were sampled had very high vegetative coverage in marginal areas but low coverage when the total area was considered (**Figure 6**). In general, wetlands had higher vegetation coverage in both marginal and total areas, relative to SWMPs. Ordinal logistic

regression to test for differences in the frequency of vegetation cover between SWMPs and wetlands indicated that both Marginal and Total vegetation coverage *were* significantly different between SWMPs and wetland sites (both  $n = 839$ ,  $p < 0.0001$ ). This is expected since physical characteristics such as bottom features and depth of wetlands may be more conducive to support more emergent vegetation in wetlands than SWMPs. In addition, SWMP vegetation control measures may also keep the Marginal and Total vegetation under control.

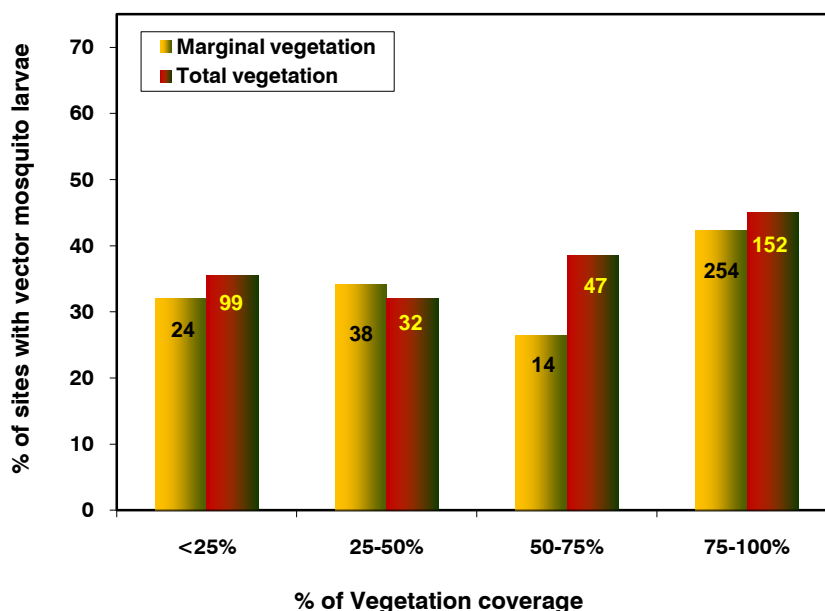


**Figure 6.** Differences in Marginal (top panel) and Total (bottom panel) vegetation coverage during sampling events at SWMPs and Wetland sites between 2005 and 2009. The numbers indicate the proportion of samples from SWMPs or wetlands with each level of vegetation coverage

When all sampling events between 2005 and 2009 were considered, the data analysis showed that increased vegetation cover offered favourable habitat to mosquito larvae in general. The increase, however, was not consistent over the range of coverage, but rather increased sharply above 75% coverage. When there was a very high degree of vegetation (75 – 100% coverage), larvae were most likely to occur (**Figure 7**); this pattern was the same for Total and Marginal vegetation in both SWMPs and wetlands. When larvae of vector species alone were considered, the effect of the vegetation on the occurrence of larvae followed a similar pattern (**Figure 8**).



**Figure 7.** The proportion of sampling events in which mosquito larvae of any species (vector or non-vector) were found, grouped by percentage of vegetation coverage. Actual numbers of events in which larvae were found are indicated within each bar (N=422). Marginal vegetation coverage (light blue bars) or Total vegetation (dark blue bars) was assessed during each of 839 sampling events between 2005 and 2009



**Figure 8.** The proportion of sampling events in which mosquito larvae of vector species were found, grouped by percentage of vegetation coverage. Actual numbers of events in which larvae were

found are indicated within each bar (N=330). Marginal vegetation (Yellow bars) or Total vegetation (Red bars) coverage was assessed during each of 839 sampling events between 2005 and 2009.

When logistic regression was used to test the effect of vegetation coverage on the presence or absence of larvae, there was a significant effect of both Marginal and Total vegetation on the likelihood that larvae of any species of mosquito were found (**Table 10**, models 1, 2). Multiple contrasts of the models to test for significant differences between levels of vegetation showed that contrasts in models 1 and 2 (assessing presence of any species of mosquito) indicated that there was significantly higher probability that mosquito larvae were present during sampling events where Marginal or Total vegetation coverage was 75% or greater ( $p < 0.0001$ , for contrasts of groups above and below 75% vegetation in both models).

**Table 10.** Results of logistic regressions testing the effect of vegetation coverage (Marginal or Total) on the presence of mosquito larvae during 839 sampling events in SWMP and Wetlands between 2005 and 2009. Separate models tested whether the effect was significant when only vector species were considered. \* Significant at  $\alpha = 0.05$

Model	Species considered	Effect tested (Vegetation Coverage)	$\chi^2$	df	$P > \chi^2$
1	All	Marginal	25.874	3	<0.0001*
2	All	Total	8.435	3	0.0378*
3	Vector	Marginal	8.795	3	0.0321*
4	Vector	Total	8.435	3	0.0378*

Similar results were found when the analysis was restricted to the effect of vegetation on vector species (**Table 9**; models 3, 4). There was a significant effect of both Marginal and Total emergent vegetation on the likelihood that a vector species was found. Multiple contrasts of vegetation levels within each model indicated that there was a similar threshold at 75% coverage: the presence of vector species increased significantly at sites with greater than 75% coverage in Marginal ( $p = 0.003$ , model 3) and Total areas ( $p = 0.007$ , model 4).

The Effect of vegetation on the presence of vector larvae was reported previously by the Durham Regional Health Department (2003) when Cattails (*Typha* spp.) and other emergent vegetation were found to significantly increase the chances of finding *Culex* larvae in SWMPs. Similarly, Gingrich *et al.* (2006) reported a strong association between *Culex pipiens* and emergent vegetation such as loosestrife (*Lythrum* spp.), water primrose (*Ludwigia* spp.) and grasses (family: Poaceae) during late summer. Similarly *Culex salinarius* was found to be strongly associated with Common reed (*Phragmites* spp.) during early summer.

Our results have also indicated that vegetation does have an effect on the ability of an aquatic environment to support mosquito larvae and that in this context, the SWMPs and wetlands sampled as

part of the TRCA monitoring offered different habitat to mosquito larvae. Since wetlands have greater vegetation coverage, we would expect to find that mosquito larvae occur more frequently at wetlands than at SWMPs. The next step in the analysis was to determine if there were differences between SWMPs and wetlands in the presence or absence of mosquito larvae which might be driven by underlying differences in vegetation coverage.

The frequency of detection of mosquito larvae at all events from 2005 to 2009 indicated that larvae (of any species) were found during approximately half of the sampling events (**Table 11**). Mosquito larvae (of any species) were found slightly more frequently at wetlands than at SWMPs; larvae were found at 52% of the sampling events from wetlands, but were only detected at 46% of the events from SWMPs. However, the difference in mosquito occurrence between SWMPs and wetlands was not statistically significant ( $n=839$ ,  $p = 0.0875$ ; Fisher's exact test), suggesting that although SWMPs and wetlands have different vegetation cover (which we know to effect the potential for larvae to be found), there are likely other differences between the two environments which alter their suitability as habitat for mosquito larvae.

**Table 11.** Presence and absence of mosquito larvae (vector and non-vector) in wetlands and SWMPs . Sampling events without information about vegetation coverage are not included

Larvae of (any species)		Vector species	SWMPs	Wetlands
Present			81 (46%)	341 (52%)
Absent			97 (54%)	320 (48%)
<b>Total number of events (839)</b>			<b>178</b>	<b>661</b>
Present	Absent		6 (7%)	86 (25%)
Present	Present		75 (93%)	255 (75%)
<b>Total number of events (422)</b>			<b>81</b>	<b>341</b>

Since this study is primarily focused on the occurrence of species which are vectors for the WNV, additional analysis was done which was restricted to sampling events where larvae of vector species were found. The purpose of this criterion was to focus on conditions favouring vector species, rather than mosquito larvae in general. We examined differences in the occurrence of vector species between SWMPs and wetland sites during sampling events where larvae of at least one mosquito species (vector or non-vector) were found.

Of the 422 sampling events during which larvae were present (i.e., where conditions were thought to be favourable for mosquito larvae), a high proportion of them had samples which included at least one vector species (**Table 11**); the proportion of larval samples which included vector larvae was higher in SWMPs (93%) than in wetlands (75%). When Fisher's exact test was used to test for the difference in the frequency of vector species occurrence in samples where mosquito larvae (of any type) were found, the results indicated that wherever larvae were found, the probability that they included vector species was significantly higher at SWMPs than at wetland sites ( $n = 422$ ,  $p > 0.001$ ; Fisher's exact test). This pattern is the reverse of what would be expected based on our findings of vegetation distribution and effects

(i.e., that wetlands, which generally have higher vegetative cover, are better able to support populations of mosquito larvae). Taken together, the results of this study indicate that vegetation is not the primary factor controlling the differences in the occurrence of vector larvae between SWMPs and wetlands. This again emphasizes the results obtained earlier that although wetlands have higher percentage of vegetation and mosquito larvae, they do not necessarily support the vector breeding.

## 4. Conclusions

The detailed analyses of data collected over the past five years suggest that wetlands continue to produce higher overall numbers of mosquito larvae when compared to the SWMPs. However, wetlands support a diverse species composition and higher percentage of non-vector species. Our study also indicated that *Culex territans* a non-vector species continues to dominate the wetland ecosystems. In contrast, the SWMPs had lesser overall numbers of larvae and fewer species; the majority of which were the vector species implicated in West Nile Virus transmission. *Culex pipiens* was the dominant vector species found in most SWMPs.

When the effect of an abiotic factor like rainfall on the larval abundance and on the occurrence of vector species was examined, we found that heavy rainfall (wet summers) negatively affected the larval abundance in wetlands, while moderate rainfall increased the number of larvae collected. It should be stressed that although the number of mosquito larvae collected were highest, the majority of them were non-vector species. In SWMPs, any amount of rainfall had a negative impact: the higher the amount of rainfall received, the lower the number of mosquito larvae collected. Larvae collected in SWMPs were highest during dry summers (e.g. 2007).

When the effect of water quality on larval composition was examined, the general analysis showed that all five water quality parameters tested (TDS, pH, DO, Temperature and Conductivity) had significantly different and higher values for SWMPs than the wetlands. Contrary to the expectation, when the analysis was restricted to data from ponds that were constantly monitored for five years, our results indicated that the SWMPs had slightly lower mean conductivity and TDS than wetlands. We found higher dissolved oxygen content, pH and the temperature relative to wetlands, however, only the dissolved oxygen showed a statistically significant difference between the two waterbody types. Since there is usually a frequent influx of TDS due to snow melts and runoffs from rain events, the TDS content would be expected to be higher in stormwater ponds, but our final results showed the opposite. We believe that the number of samples collected (frequency) and the time of sample collection (before or after the rain events) influenced the outcome of our analysis of water quality parameters. The results of Canonical Correspondence Analysis support this conclusion by way of showing a closer association between the TDS and the vector species in the stormwater ponds. Further correlation between the total vector species and TDS did not prove to be statistically significant, reinforcing the need to collect more frequent data and maintain consistency in the time of sample collection (before or after rain events).

The influence of biotic factors such as the Total and Marginal Emergent vegetation was found to be significantly different between the wetlands and SWMPs. Wetlands had significantly more Marginal and Total vegetation as expected. Higher vegetation appears to be good habitat for larvae: where vegetation coverage (in total or marginal areas) was greater than 75%, there was a significantly higher probability that mosquito larvae (vector or otherwise) were present. However, vector larvae did not occur more frequently at wetland sites (as predicted by the amount of vegetation); the proportion of larvae in samples which included vector larvae was higher in SWMPs than in wetlands. Fisher's exact test showed that where ever mosquito larvae were found, the probability that they included vector species was significantly higher at SWMPs than at wetland sites and this suggests that factors other than vegetation are controlling the differences in the occurrence of vector larvae at SWMPs and wetlands.

Based on the outcomes of our five year data analyses, we recommend the following for the mosquito and WNV vector management in the wetlands and the SWMPs:

- Vegetation control and proper maintenance should be undertaken in SWMPs to reduce the presence of mosquito larvae since denser vegetation coverage seems to promote higher number of mosquito larvae,
- Conductivity and total dissolved solids need to be monitored on a regular basis especially in the SWMPs since they favour the vector breeding compared to the wetlands,
- Continuous monitoring of wetlands and SWMPs is necessary to detect and prevent the occasional vector hotspots, and
- Additional sampling of water quality and abiotic (physical) factors in SWMPs and wetlands be undertaken in order to further determine their influence on vector breeding.

## 5. References

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## Appendices

**Appendix 1.** List of wetlands and stormwater management ponds (SWMPs) with sampling information on water quality. \* Incomplete data set (i.e. data from some sampling events are not available due to pond being dry, too shallow water levels for collecting data, site inaccessible etc.). Site names in bold italic fonts indicate that the data from these ponds were used in the detailed analysis of water quality and larval abundance

Site name	Habitat type	Data availability
Frenchman's Bay West Shore	Wetland	2007*
Frenchman's Bay Promenade	Wetland	2007, 2008, 2009
Carruthers Swamp Complex	Wetland	2005*, 2006*, 2007*, 2008*, 2009
<b>Claremont Wetland-1</b>	Wetland	2005, 2006, 2007, 2008, 2009
<b>Claremont Wetland-2</b>	Wetland	2005, 2006, 2007*, 2008, 2009
Altona Forest	Wetland	2008*, 2009*
Bruce's Mill	Wetland	2007, 2008, 2009
Frenchman's Bay Promenade	Wetland	2007, 2008, 2009
Greenwood Marsh	Wetland	2007*, 2008, 2009
Greenwood Pond	Wetland	2007*, 2008, 2009*
Heart Lake Main Lake	Wetland	2005*, 2006*, 2007*
Heart Lake Duckweed Pond	Wetland	2005*, 2006*, 2007*, 2008
Heart Lake New Site	Wetland	2007*, 2008, 2009
Albion Hills Pond-1	Wetland	2006*, 2007*, 2008, 2009
Albion Hills Pond-2	Wetland	2006*, 2007*, 2008*, 2009*
Albion Hills Pond-3	Wetland	2005*
<b>Albion Hills Pond-4</b>	Wetland	2005*, 2006*, 2007*, 2008, 2009
Claireville Wetland-1	Wetland	2005*, 2006*, 2007*, 2008, 2009
Claireville Wetland-2	Wetland	2005*, 2006*
Claireville Wetland-3	Wetland	2006*
Glen Haffy Flooded Forest	Wetland	2005*
<b>Glen Haffy Trout Pond-1</b>	Wetland	2005*, 2006*, 2007*, 2008, 2009
<b>Glen Haffy Trout Pond-2</b>	Wetland	2005*, 2006*, 2007, 2008, 2009
Marie Curtis Park	Wetland	2007, 2008, 2009
<b>Pond-88.2</b>	SWMP	2005, 2006, 2007, 2008, 2009
<b>Pond-139</b>	SWMP	2005, 2006, 2007, 2008, 2009
<b>Pond-253</b>	SWMP	2005*, 2006*, 2007, 2008, 2009
<b>Pond-262</b>	SWMP	2005*, 2006, 2007, 2008*, 2009
<b>Pond-279.1</b>	SWMP	2005*, 2006, 2007, 2008, 2009
Pond-303	SWMP	2005, 2006, 2007*
Pond-174	SWMP	2005, 2006, 2007
<b>L'Amoreaux North Pond</b>	SWMP	2005, 2006, 2007*, 2008, 2009
<b>L'Amoreaux South Pond</b>	SWMP	2005, 2006, 2007*, 2008, 2009
<b>Milne Hollow</b>	Wetland	2005*, 2006, 2007*, 2008, 2009

Stevenson Swamp	Wetland	2005*, 2006*
<b>Topham Pond</b>	Wetland	2005*, 2006*, 2007*, 2008, 2009
<b>Col. Samuel Smith Mini Pond</b>	Wetland	2005*, 2006*, 2007, 2008, 2009
<b>Col. Samuel Smith Main Pond</b>	Wetland	2005*, 2006*, 2007, 2008, 2009
<b>High Park Grenadier Pond</b>	Wetland	2005*, 2006*, 2007, 2008, 2009
<b>Woodland Pond</b>	Wetland	2005*, 2006*, 2007, 2008, 2009
<b>Mimico Amphibian Pond</b>	Wetland	2005*, 2006*, 2007, 2008, 2009
<b>TTP Triangle Pond</b>	Wetland	2005*, 2006*, 2007*, 2008, 2009
<b>TTP Goldfish Pond</b>	Wetland	2005*, 2006*, 2007*, 2008, 2009
TTP Flooded Grassland	Wetland	2005*
Rouge Marsh	Wetland	2005*, 2006
<b>Cold Creek Wetland</b>	Wetland	2005*, 2006, 2007, 2008, 2009
<b>Cold Creek Pond</b>	Wetland	2005*, 2006*, 2007*, 2008, 2009
Lake St. George Main Lake	Wetland	2005*, 2006
Lake St. George Wetland	Wetland	2005*, 2006*
<b>Leman's Reservoir</b>	Wetland	2005*, 2006, 2007, 2008, 2009
<b>Stouffville Reservoir</b>	Wetland	2005*, 2006, 2007, 2008, 2009
<b>Toogood Pond</b>	Wetland	2005*, 2006, 2007, 2008, 2009
<b>Killian Lamar</b>	SWMP	2005, 2006, 2007, 2008, 2009
<b>Kortright Centre Marsh</b>	Wetland	2005*, 2006*, 2007, 2008, 2009
Granger Wetland North	Wetland	2005, 2006*
<b>Granger Wetland South</b>	Wetland	2005, 2006*, 2007, 2008, 2009
<b>Keffer Marsh</b>	Wetland	2005*, 2006, 2007, 2008, 2009
Boyd Conservation Area	Wetland	2007, 2008, 2009
Major McKenzie Drive and Hwy. 27, Kleinberg	Wetland	2009*

**Appendix 2.** List of TRCA's rain gauge locations that are closure to the West Nile Virus monitoring sites

Rain Gauge Locations	Region
Albion Hills CA	Peel
Claireville Dam	Peel
Glen Haffy CA	Peel
Heart Lake CA	Peel
Bruce's Mill CA	York
Boyd Field Centre	York
Dufferin Reservoir	York
King Creek at Mill Road	York
Milne Dam	York
Stouffville Dam	York
Ajax Works Yard	Durham
Claremont CA	Durham
Petticoat Creek CA	Durham
Etobicoke near Queen Elizabeth Way	Toronto
Danforth and Coxwell TTC Bus Depot	Toronto
Kennedy Pump Station	Toronto