Stapleton MCA
Integrated Mosquito Management
2016 Annual Report

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SUMMARY

For the 2016 season, Stapleton MCA (Stapleton) contracted OtterTail Environmental, Inc. (OtterTail) to operate an Integrated Mosquito Management (IMM) program to protect public health from the transmission of West Nile Virus (WNV) and other vector-borne diseases, and to suppress local populations of nuisance mosquitoes. On June 26, 2016 OtterTail Environmental's mosquito control assets and operations were acquired by Vector Disease Control International, LLC (VDCI). As such, the operation of the 2016 Stapleton IMM program prior to June 26 was conducted by OtterTail, while operations conducted after June 26 were conducted by VDCI, with considerable cooperation between both entities throughout the transition.

Through surveillance of potential mosquito breeding sites (larval sites); areas that produced mosquito larvae were identified and treated with control materials known as larvicides. The primary larval control materials used were the microbial larvicide *Bacillus thuringiensis israelensis* (*Bti*) and BVA-2 larvicide oil. During the 2016 season, VDCI/OtterTail performed 300 individual larval site inspections and conducted 111 site treatments covering approximately 11.7 acres of active mosquito breeding habitat.

Surveillance of adult mosquito populations and WNV activity were also conducted during the season by using five adult mosquito traps placed within the contract area. Weekly trap collections enabled VDCI/OtterTail to monitor nuisance levels and provide any needed mosquito pools for WNV testing throughout the season.

The State of Colorado experienced a third consecutive year of relatively low WNV activity in 2016. The climate patterns and temperatures that occurred during the 2016 season caused mosquito populations to remain at average levels throughout the majority of the season; consequently, there was relatively low WNV activity within the region. Stapleton MCA's IMM program coupled with education and personal protection measures, also likely continued to help reduce mosquito populations and WNV activity in the Stapleton area during 2016.

1.0 INTRODUCTION

In 2016, Stapleton MCA (Stapleton) partnered with both OtterTail Environmental, Inc. (OtterTail) and Vector Disease Control International, LLC (VDCI) to operate their Integrated Mosquito Management (IMM) program. As explained in the Summary section, VDCI took over the program on June 27, 2016. As in previous years, the Stapleton's primary mission was to protect local residents from the effects of West Nile Virus (WNV) and to suppress the local populations of nuisance mosquitoes.

Specific objectives for the program included (1) the identification, monitoring, and treatment of habitats with a high potential for mosquito breeding; (2) monitoring adult mosquito populations by speciation and population counts; (3) use the adult surveillance data as an early warning system for the occurrence and severity of WNV activity in the program area; and (4) to limit any adverse effects on the environment from control materials in a cost-effective manner.

This report explains the methods used in the IMM program and provides a detailed summary of the results of this year's effort.

2.0 WEST NILE VIRUS (AND OTHER MOSQUITO-BORNE DISEASE) UPDATE

As of November 8, 2016, there were 1,521 WNV human cases and 71 WNV related deaths in 45 states and the District of Columbia reported for the 2016 season (**Table 1**). Colorado ranked fifth in the national case count with 137 human WNV cases and 7 WNV related deaths reported as of October 28, 2016. Most WNV cases occurred in Colorado within the populous regions of the Front Range **(Table 2)**. The Colorado Department of Public Health and Environment (CDPHE) did not report any mosquito pools, horses, birds or humans as positive for St. Louis Encephalitis or Western Equine Encephalitis during the 2016 season**.** The slightly elevated WNV activity and number of human infections in Colorado may be attributed to the temperature and precipitation patterns observed during the 2016 mosquito season and the affect they had on mosquito populations, as discussed further in **Section 3.0**.

Table 1 WNV Incidence, 2002 - 2016

Total WNV Human	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Cases in the United States[1]	4,156	9,862	2,539	3,000	4,269	3,630	1,356	720	1,021	712	5,674	2,469	2,205	2,175	1,521
Deaths in the United States[1]	284	264	100	119	177	124	44	32	57	43	286	119	97	146	71
Highest State Count in United States[1]	884	2,947	779	880	996	576	445	115	167	158	1,868	379	801	783	242
Cases in Colorado[2]	14	2,947	291	106	345	576	71	103	81	7	131	322	118	108	137
Deaths in Colorado[2]	0	63	4	2	7	7	1	3	4	0	5	7	5	3	7
Cases in Denver County[2]	1	162	3	5	5	27	3	1	1	1	16	12	5	12	10
Deaths in Denver County[2]	0	9	0	0	0	2	0	0	0	0	0	1	2	1	*
Total WNV Positive	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
Mosquito Pools in Denver County[2]	0	28	1	3	5	10	0	0	0	0	0	0	0	9	7
Birds in Denver County[2]	0	60	0	0	4	2	0	0	0	0	0	0	0	0	1
Horses in Denver County[2]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1. Reported by the Center for Disease and Control (CDC); 2016 data reported as of November 8, 2016.
2. Reported by the Colorado Department of Public Health and Environment (CDPHE); 2016 data reported as of October 28, 2016.

* = data was not available from CDPHE at the time of report

As of October 28, 2016 there were 10 WNV related illnesses and no WNV related deaths in the City and County of Denver reported by CDPHE **(Table 1)**. The number of City and County of Denver human cases and WNV positive mosquito pools comprised approximately 7.3 percent and 3.3 percent of the state totals, respectively (**Table 2**). The slightly lower number of human WNV cases and WNV positive mosquito pools suggests that the viral activity in the City and County of Denver was lower in 2016 than last year. It is likely that the continued mosquito control efforts to reduce mosquito populations, coupled with public education and personal protection measures, helped to reduce exposure and disease transmission within Stapleton and its surrounding areas.

Table 2 Colorado WNV Cases and WNV Positive Mosquito Pools, 2016

County	Human Cases[1]		Positive Mosquito Pools[2]	
	Number	% of State	Number	% of State
Adams	12	8.8%	12	5.6%
Alamosa	0	0.0%	0	0.0%
Arapahoe	8	5.8%	3	1.4%
Bent	2	1.5%	0	0.0%
Boulder	19	13.9%	21	9.8%
Broomfield	5	3.6%	0	0.0%
Costilla	1	0.7%	0	0.0%
Crowley	0	0.0%	0	0.0%
Delta	0	0.0%	2	0.9%
Denver	10	7.3%	7	3.3%
Douglas	1	0.7%	0	0.0%
El Paso	1	0.7%	0	0.0%
Gunnison	1	0.7%	0	0.0%
Freemont	0	0.0%	0	0.0%
Huerfano	1	0.7%	0	0.0%
Jefferson	3	2.2%	0	0.0%
La Plata	1	0.7%	3	1.4%
Larimer	32	23.4%	115	53.7%
Logan	3	2.2%	0	0.0%
Mesa	0	0.0%	1	0.5%
Morgan	1	0.7%	0	0.0%
Otero	3	2.2%	0	0.0%
Pueblo	1	0.7%	0	0.0%
Rio Grande	0	0.0%	0	0.0%
Rio Blanco	2	1.5%	0	0.0%
Sedgewick	3	2.2%	0	0.0%
Washington	0	0.0%	0	0.0%
Weld	27	19.7%	50	23.4%
Colorado Totals	**137**		**214**	

1. Reported by CDPHE as of October 28, 2016
2. Reported by CDPHE as of September 30, 2016

3.0 REGIONAL 2016 CLIMATOLOGICAL DATA AND MOSQUITO ACTIVITY OVERVIEW

The weather patterns leading into and during the mosquito season (April – September) are important factors that influence mosquito abundance and WNV activity. The following section describes the regional climate, weather during the season, and how that may have affected the mosquito populations.

Stapleton is located in a semi-arid environment with an elevation of approximately 5,200 feet above sea level (CDATA). The typical mosquito season for Stapleton MCA's program area is from May to September. Current and historical climate data from the High Plains Regional Climate Center's (HPRCC) Denver International Airport weather station was used for regional temperature and precipitation patterns.

Historical records for the mean monthly temperature at the station suggest that temperatures usually have a steady increase from April to July, making July, on average, the hottest month of the year. Typically there is then a steady temperature decrease into September. In 2016, every month of the mosquito season except May and August had temperatures above normal. The month of May and June experienced the highest variations from normal during the season. May had a monthly mean temperature that was approximately 2 degrees below normal, while June's monthly mean temperature was approximately 3 degrees above normal (**Figure 1**).

The historical averages for the monthly mean precipitation indicate that May, June and July are usually the wettest months of the year (**Figure 2**). During 2016, the accumulated precipitation from January through September was lower than the historical average for the same period. During this time period in 2016, there was an accumulation of 10.29 inches. This is approximately 19.4 percent less than the normal amount of accumulation when compared to the historical average, which is 12.77 inches. Five of the nine months received precipitation amounts slightly higher than their normal averages. The most significant variations during the mosquito season were the months of April and August. April received approximately 37 percent more precipitation than average, making it the wettest month of 2016, while August received approximately 13 percent of its normal precipitation, making it the driest month of 2016 (NOAA 2016).

Temperatures and precipitation amounts varied throughout the 2016 mosquito season. Wetter and near normal temperatures for the months of April and May were followed by a drier and warmer than average June. The month of July was then much drier and warmer than average. These warmer temperatures during the first half of the 2016 mosquito season likely caused mosquito larvae to develop at a much faster rate. August and September were then much hotter and drier than average, causing many of the sites to quickly dry up during the second half of the season. These climate patterns were the likely cause of the slightly elevated abundance of nuisance and *Culex species* mosquitoes experienced throughout much of the 2016 mosquito season.

Figure 1 2016 Monthly Mean Air Temperature and Historical Averages*

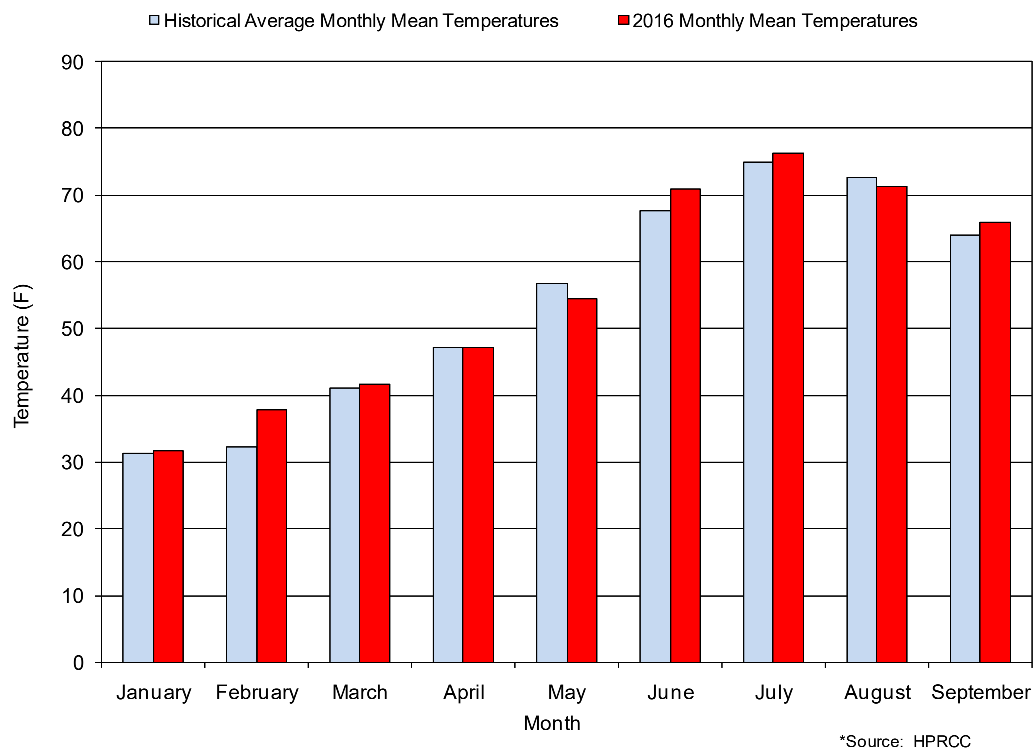
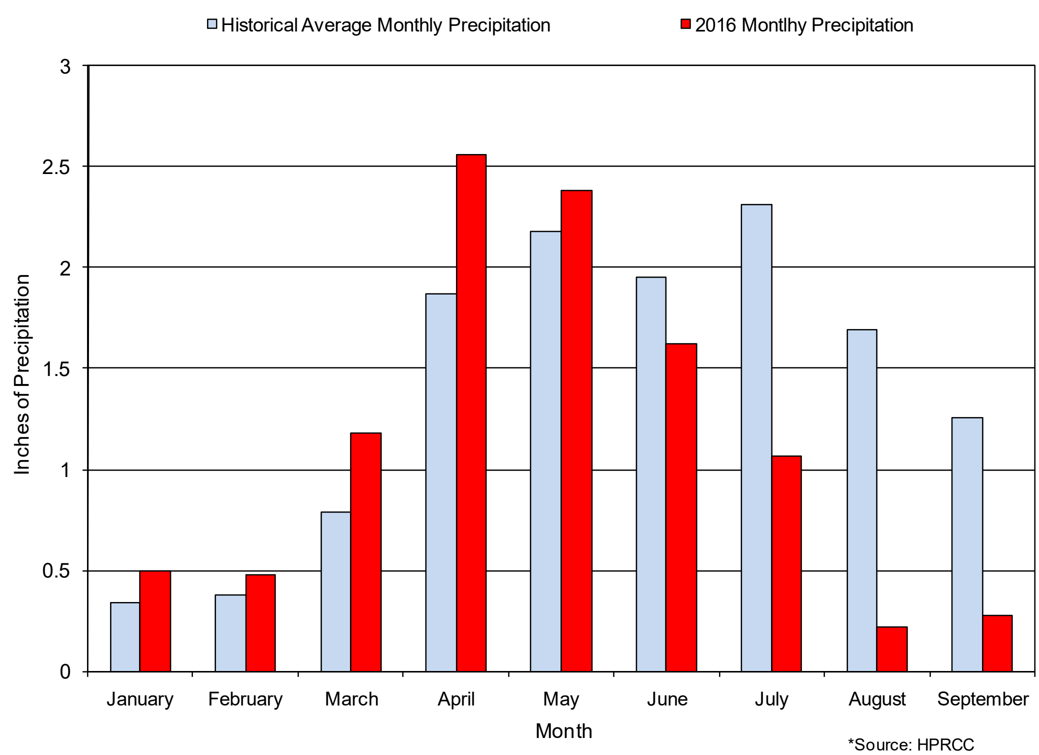


Figure 2 2016 Monthly Total Precipitation Data and Historical Averages*



4.0 LARVAL MOSQUITO SURVEILLANCE AND CONTROL

LARVAL SURVEILLANCE METHODOLOGY

OtterTail staff began the season by identifying and inspecting the larval habitat sites within the Stapleton MCA project area. Many of the habitats were those with stagnant water high in nutrients and organic matter including: cattail marshes, small stagnant ponds, temporary pools, and drainage structures. Sites were generally inspected once a week. Habitat sites were added and refined throughout the field season as needed. A detailed explanation of the larval surveillance methodology used during the 2016 season can be found in **Appendix A** and a map of the larval surveillance area can be found in **Appendix C**.

LARVAL CONTROL METHODOLOGY AND APPLICATION METHODS

The primary focus for VDCI/OtterTail's IMM program is to control mosquitoes while in the larval stage. Larval mosquito control methods employed by VDCI/OtterTail were aimed at preventing adult mosquito emergence, which reduces the potential of the mosquito-borne disease, WNV, and minimizes the annoyance level of mosquitoes to local residents. To achieve a high level of effectiveness and efficiency of larval control efforts, VDCI/OtterTail identified and inspected mosquito larval habitats on a regular basis. The threshold for larval control was presence of any mosquito larvae. Finding and documenting consistent mosquito producing sites was an important component of the program because it created a pattern that is monitored and systematically controlled to help understand mosquito populations and WNV trends.

The application of *Bacillus thuringiensis israelensis* (*Bti*), *Bacillus sphaericus (Bs),* and BVA-2 mosquito larvicide oil (BVA-2) are VDCI/OtterTail's primary methods used for larval mosquito control. Control materials were applied within the labeled rates, thereby minimizing any potential adverse impacts to areas being treated. Routine post-treatment checks were conducted to ensure the larval control was effective. If any larvae were found during the post-check, a second application was conducted.

In balancing environmental resources, cost effectiveness, and public health needs, *Bti* was selected as the primary treatment product. *Bti* is a naturally occurring protein that is toxic to mosquito larvae upon its ingestion. It provides a residual treatment that lasts for approximately two days. Since new mosquito larvae may hatch after the product dissipates, the sites must be inspected for mosquito larvae every one to two weeks. The presence of mosquito larvae between monitoring periods has the added benefit of allowing these larvae to continue to be part of the aquatic food web. However, larvae are eliminated before they can emerge as adults. This helps protect the public from potential WNV transmission, while still providing a food source for many aquatic animals.

Bacillus sphaericus is a larvicide very similar to *Bti* but has a longer residual time. The protein in *Bs* products is able to provide continuous treatment of mosquito larvae for up to four weeks and was typically used on sites that were found to be continuously producing mosquitoes. Although the longer residual time of this larvicide allows for fewer site checks and cost savings in labor and travel, it is only practical in certain situations because it costs substantially more than *Bti*.

It should be noted that *Bti* was the primary control material used, but this product is ineffective if pupae are found at a site. Mosquitoes do not feed during their pupal stage; therefore, the use of *Bti* and *Bs* is ineffective against mosquito pupae since they must ingest the proteins. In these instances of pupae occurrence, BVA-2 is used. BVA-2 is a highly refined mineral oil that creates a thin film on the water surface. The film interrupts the air and water interface during the mosquito's larval and pupal development stages, causing them to drown.

LARVAL SURVEILLANCE AND CONTROL RESULTS AND DISCUSSION

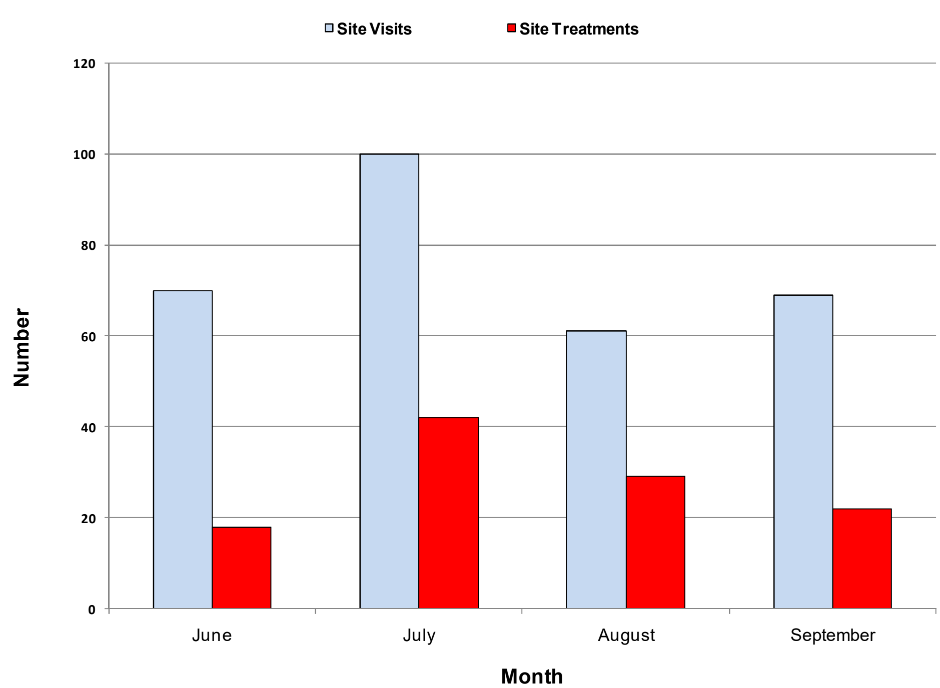
The 2016 larval surveillance season started in June and lasted through September. During the season, a total of 300 individual larval site visits were performed within the program area. Approximately 90 lbs. of *Bti*, and 0.3 ounces of BVA-2 were used to treat 11.7 acres of active habitat during 111 treatments (**Table 3**).

Table 3 Larval Surveillance Summary, 2016

Habitat Site Surveillance	2016 Totals
# Site Visits	300
# Site Treatments	111
Amount of Treated Acreage	11.7

Many of the habitat sites produced mosquito larvae multiple times during the season causing the treated acres at certain sites to be counted multiple times for the season total. As the season progressed, the sites were categorized according to larval abundance and occurrence. Low priority mosquito sites which were not producing mosquitoes had poor habitat or had the presence of aquatic predators. High priority mosquito sites typically had larvae when sampled and consistently produced mosquitoes every seven to ten days during the peak season. **Figure 3** shows the number of site visits and treatments performed each month during 2016. It is likely that Stapleton MCA's larval control program helped reduce the adult mosquito population levels in the local and surrounding areas during 2016.

Figure 3 Numbers of Site Visits and Treatments per Month, 2016



5.0 ADULT MOSQUITO SURVEILLANCE AND CONTROL

ADULT SURVEILLANCE METHODOLOGY

Adult mosquito population surveillance is a crucial component of any successful IMM program. Adult surveillance provides information on what types of mosquito species are in an area as well as information on their abundance. Mosquitoes collected from the mosquito traps can be tested for a variety of mosquito-borne diseases and are critical for monitoring and forecasting vector threats, particularly WNV.

Most mosquito species prefer to rest during the heat of the day in areas known as harborage areas. A mosquito harborage area is usually a shaded, wind protected and moist area because adult mosquitoes can dehydrate quickly during the daylight hours if they do not have a shady area to rest and escape the heat. Relevant examples are groves of tall trees with a layer of shrubby undergrowth, tree-lined waterways and water bodies, dense bushes, tall live grasses, or in residential areas under roof eaves and inside tires. Adult mosquito trapping efforts target these harborage areas to monitor adult mosquito populations.

VDCI/OtterTail used the CDC style carbon dioxide (CO_2) light trap to monitor the adult mosquito populations within the Stapleton area. The CO_2 light trap is based on the principle that most adult mosquitoes are attracted to light, CO_2 (via respiration), and heat. The CO_2 light trap collects adult female mosquitoes that are seeking a blood meal, so that she may produce eggs. This type of trap is set overnight and on the following morning the nets are collected and returned to VDCI/OtterTail's lab to be identified and counted. Once identified, the mosquitoes were then sorted and counted by species. A detailed explanation of the CO_2 light trap used during the 2016 season can be found in **Appendix B**.

Beginning in June, five light traps were set to capture adult mosquitoes on a weekly basis until mid-September. The traps were set in adult mosquito harborage locations throughout the Stapleton area (**Appendix C**). VDCI/OtterTail and Stapleton MCA used the adult mosquito data collected to help determine local areas of concern for public awareness and safety as well as to monitor the local nuisance mosquito populations.

ADULT SURVEILLANCE RESULTS AND DISCUSSION

Over the season, from the five traps within the Stapleton MCA project area, there was an average of 29 total adult mosquitoes per trap per night and an average of 11 adult vector mosquitoes per trap per night. The total adult mosquitoes collected during the season from all 5 traps resulted in *Aedes/Ochlerotatus species* (60.7 percent) being the most abundant, followed by *Culex* (vector) *species* (38.9 percent), and *Culiseta species* (0.4 percent as shown in **Table 4**. This results in approximately 61 percent non-vector vs. 39 percent vector adults being collected over the entire season.

Culex pipiens accounted for approximately 55 percent and *Culex tarsalis* accounted for approximately 45 percent of the vector mosquitoes captured from all 5 traps during the season. This species of mosquito is often found breeding in containers and a likely source for the high populations were man- habitats (e.g., storm retention areas, pools, tires, buckets, eves, troughs, bird baths, and other similar containers) in the neighborhoods surrounding the traps.

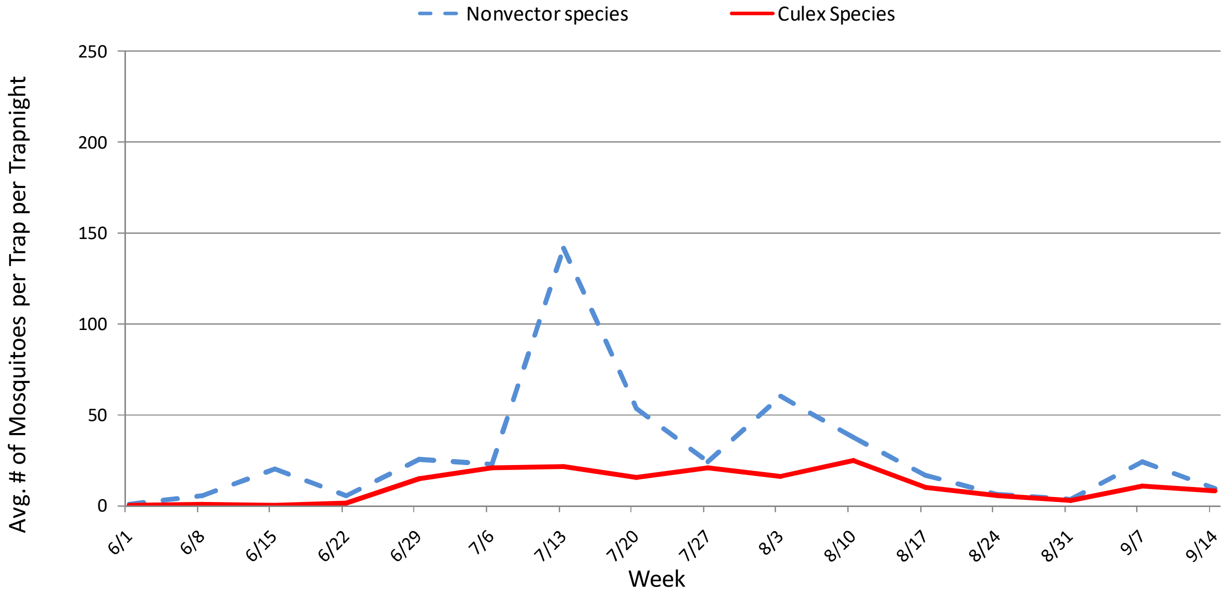
Table 4 Total Number of Adult Mosquitoes per Trap for the 2016 Season[1]

Trap Name	ST-01	ST-02	ST-03	ST-04	ST-05	Total
Trap Locations	Maverick Pool	Jet Stream Pool	Aviator Pool	F15 Pool	Puddle Jumper Pool	
Trap Type	Light Trap	Light Trap	Light Trap	Light Trap	Light Trap	
Species						
Culex pipiens	58	9	103	178	122	470
Culex tarsalis	85	37	51	124	85	382
Total Culex	**148**	**46**	**154**	**302**	**207**	**857**
% RA Culex	**81.8%**	**66.7%**	**16.5%**	**82.5%**	**31.5%**	**38.8%**
Aedes vexans	24	19	759	37	422	1261
Oc. dorsalis	2	1	0	2	3	8
Oc. increpitus	1	0	10	14	8	33
Oc. melanimon	2	0	0	1	2	5
Oc. nigromaculis	4	1	0	3	0	8
Oc. trivittatus	0	2	7	1	15	25
Total Ae./Oc.	**33**	**23**	**776**	**58**	**450**	**1,340**
% RA Ae./Oc.	**18.2%**	**33.3%**	**83.3%**	**15.8%**	**68.4%**	**60.7%**
Anopheles spp.	0	0	0	0	0	0
Total Anopheles	**0**	**0**	**0**	**0**	**0**	**0**
% RA Anopheles	**0.0%**	**0.0%**	**0.0%**	**0.0%**	**0.0%**	**0.0%**
Coquillettidia perturbans	0	0	0	0	0	0
Total Coquillettidia	**0**	**0**	**0**	**0**	**0**	**0**
% RA Coquillettidia	**0.0%**	**0.0%**	**0.0%**	**0.0%**	**0.0%**	**0.0%**
Culiseta inornata	0	0	2	6	1	9
Total Culiseta	**0**	**0**	**2**	**6**	**1**	**9**
% RA Culiseta	**0.0%**	**0.0%**	**0.2%**	**1.6%**	**0.2%**	**0.4%**
Trap Total	**181**	**69**	**932**	**366**	**658**	**2,206**

Notes: 1. Adult Surveillance season was June 1 to September 14, 2016 (76 trapnights). RA= Percent Relative bundance

VECTOR DISEASE CONTROL OtterTail Environmental INC

Figure 4 Season-Wide Weekly Adult Trap Counts of All Trap Locations, 2016



6.0 PUBLIC OUTREACH AND EDUCATION

Public education is an important component to any mosquito control program and is vital in combating West Nile Virus. VDCI/OtterTail and Stapleton MCA provided valuable educational materials to residents and the general public through local media outlets, educational materials and their internet websites. The educational materials stressed the importance of actions that residents could take to aid in the effort to combat WNV; topics included personal protection, property maintenance for source reduction, and general information related to mosquito biology and the WNV disease cycle.

Educating residents on the need for property maintenance, source reduction and the use of personal protection measures was crucial in the fight against WNV in 2016. The resulting actions taken by the public likely helped reduce the mosquito populations and the WNV activity levels and cases in the area during 2016.

7.0 REFERENCES

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APPENDIX A - DETAILED LARVAL SURVIELLANCE AND SITE SELECTION METHODOLOGY

Larval Surveillance Methodology

The following is a summary of the procedures used by VDCI/OtterTail during larval surveillance. To inspect a mosquito source, a plastic dipper cup with a 3-foot wooden handle was used to collect water from the site. Each sample (dip) was closely examined for mosquito larvae presence. Many of the sites inspected had mosquito-sustaining habitat around the perimeter of the site, but the middle remained mosquito free due to water circulation and/or natural predators. At these sites, the dipping effort was completed using a *linear approach* (walking around the perimeter and sampling the margins).

In sites with widespread mosquito habitat, the entire site was methodically sampled using the *surface area approach*. With this approach, sites were dipped approximately every 10 to 20 square feet. Since each site's characteristics could vary as the season progressed (e.g., become drier, wetter, increased vegetation), there were changes made during the field season to adjust the appropriate number of dips.

Larval Surveillance Site Selection/Characterization Methods

VDCI/OtterTail used a series of maps, generated with Geographic Information Systems (GIS) technology, for identifying and monitoring the larval habitat areas within the project area that could support mosquito larvae. Mosquito larvae require stagnant water and will thrive in areas where the water is high in nutrients, organic matter, or other organic pollutants. Common habitats include: wetlands, riparian groundwater sinks, non-flowing irrigation ditches, flood irrigated fields, floodwater retention ponds, lake and river shores, and a wide array of man-made habitats including pools, tires, pots, buckets, eves troughs, bird baths and other similar containers. Since habitat sites can change over time, the sites were re-evaluated and classified, based upon their breeding potential, at the beginning of the season.

APPENDIX B - ADULT CDC-STYLE MOSQUITO TRAP DESCRIPTION

For the season, carbon dioxide (CO_2) baited Centers for Disease Control (CDC) Light Traps were incorporated into Stapleton MCA's adult mosquito surveillance system. The following is a detailed description of the CO_2 light trap.

CO_2 Light Trap

To capture the most representative sample of adult mosquitoes in an area, CDC Light Traps are baited with CO_2 in the form of dry ice and set overnight in adult mosquito harborage areas throughout the mosquito season. The traps are designed with the knowledge that the female mosquito species we target are attracted to light, CO_2, and heat. The number and types of mosquitoes captured in these traps can provide local officials with a valuable early indication of the threat of WNV.

The traps consist of a plastic insulated thermos filled with enough dry ice (CO_2) to last throughout the trapping cycle. Units consist of a light, fan unit, and fine mesh net which hang below the thermos. The device is placed on a tree branch with the thermos approximately five to seven feet off the ground and is suspended by a small chain or rope to allow the thermos and net to hang free. Holes at the base of the thermos allow dissipating CO_2 to be emitted as an attractant around the trap. Batteries run the small fan and light positioned above the net. The light provides further attraction and once the mosquitoes are near the light, they are pulled down into the net by the downward force of the fan.

In the morning, the mosquitoes are removed and frozen to prepare for identification. During the identification process, the mosquitoes are sorted by species and sex. Female vector mosquitoes are routinely submitted to the Colorado Department of Public Health and Environment (CDPHE) lab for WNV testing as needed.

APPENDIX C – PROJECT AREA MAP