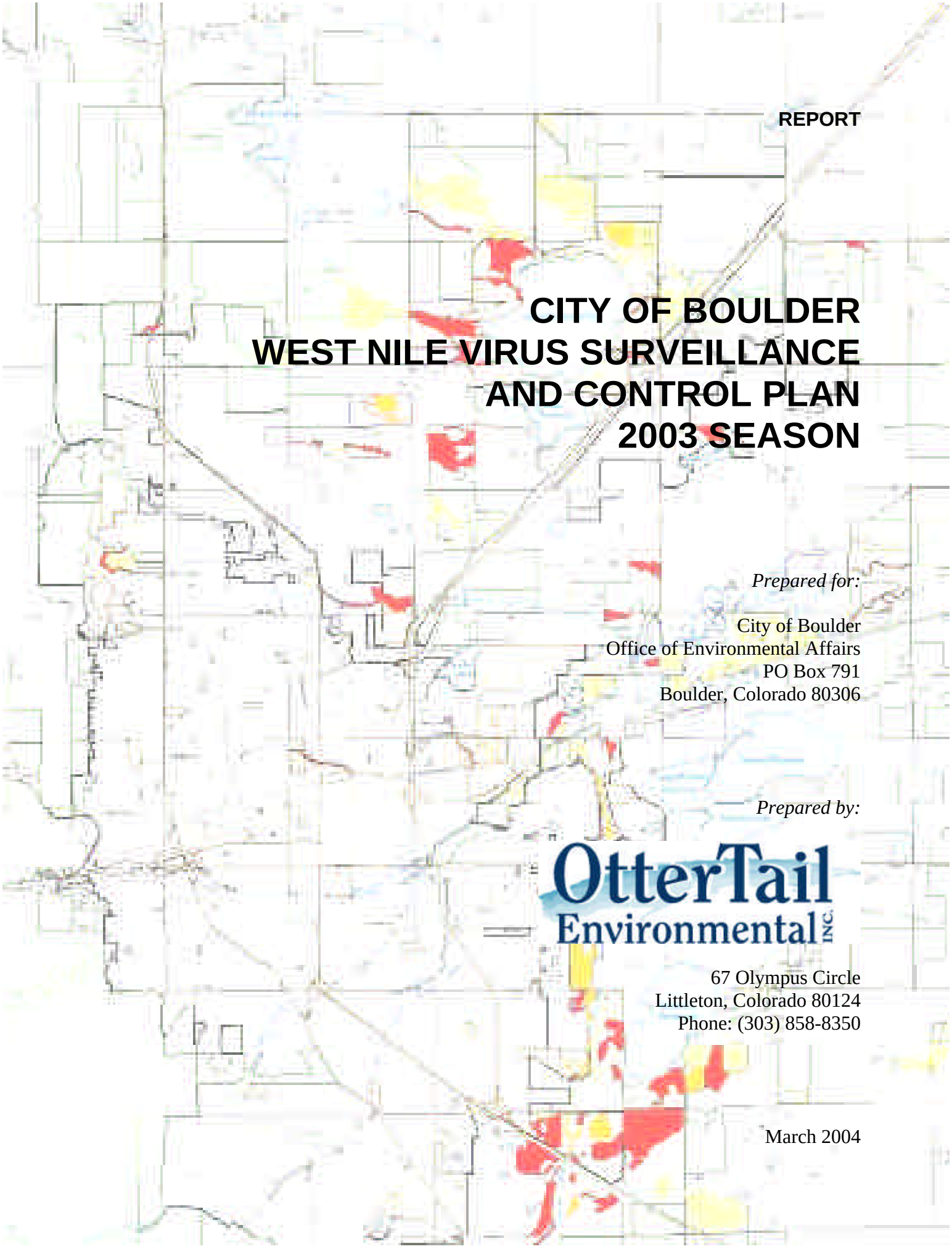

REPORT

CITY OF BOULDER WEST NILE VIRUS SURVEILLANCE AND CONTROL PLAN 2003 SEASON

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EXECUTIVE SUMMARY

For the 2003 mosquito season, OtterTail Environmental, Inc. was contracted by the City of Boulder to implement a West Nile Virus (WNV) surveillance and control program to protect public health and biological resources from the effects of WNV. This report provides the results of this project.

WNV is spread by the bite of an infected mosquito. The virus can infect people, horses, many types of birds, and some other animals. Most people (approximately 80 percent) who become infected with WNV will have either no symptoms or only mild ones. However, on rare occasions, WNV infection can result in severe and sometimes fatal illnesses. There is currently no WNV vaccine for humans, but there is for horses.

First detected within the U.S. in the state of New York in 1999, WNV has spread from East to West across the United States by mosquito hosts and carrier birds. During the 2003 mosquito season, Colorado led the nation in WNV human cases (2,945) and WNV-related deaths (55) (CDPHE 2004).

Identification and surveillance of mosquito producing areas is the primary means of identifying areas that are at high risk for transmission of WNV to humans. Accordingly, treatment of these areas with biological control methods during this stationary larval stage, before they become air borne, are the most cost-effective and efficient means to reduce mosquito populations and; therefore, to also most effectively reduce the risk of human infection of WNV. In addition, control of the larvae limited the possible future need for nonbiological control of adult mosquitoes such as ultra-low volume (ULV) pesticide spraying.

Of the approximately 54 mosquito species occurring within Colorado, species from the genus Culex have proven to be the most effective vector of WNV to humans. *Culex tarsalis* is by far the most significant contributor to transmission, due to it being the most abundant Culex species in Boulder and its high level of competency as an efficient vector. *Culex pipiens* is also a competent vector and is believed to be the second highest source of transmission in the Boulder area.

Although reasons for Colorado having the highest case numbers are not fully understood, Colorado's wet spring and hot summer of 2003, which are ideal conditions for mosquito breeding, is most likely a significant factor. These conditions created an early and abnormally large surge in *C. tarsalis* populations. This surge was about one full month earlier than is typically found in the area as documented by the 9 years of population data on the adjacent lands of the Boulder County Mosquito Control District. This allowed the virus to spread early into the bird and existing adult mosquito populations, which increased the number of adult mosquitoes infected, further increasing the risk of infection to humans.

The project area consisted of city-owned lands within city limits and selected Boulder Open Space/Mountain Parks and other city-owned parcels outside of city limits. Within the project area, potential mosquito-breeding areas (e.g., wetland areas, waterbodies, streams, ponds, ditches, and flood irrigated fields) were mapped and evaluated for larval control. Additionally, selected areas within the project area were mapped and evaluated for adult mosquito harborage areas (e.g., trees, tall grass, thick understory). The approved larval control method on city lands was *Bacillus thuringiensis israelensis* (*Bti*).

The city had specific goals for this program. First, they wanted to reduce the risk of WNV infection to human health. They also wanted to limit the effect on the environment from control materials and be as cost-effective as possible. Standard approaches to mosquito control (applying control materials to all significant mosquito populations) would not accomplish these three goals. OtterTail worked collaboratively with the City to successfully achieve these goals by devising a unique and innovative program. Mosquitoes of the genus Culex are the most effective vector of WNV to humans in the area; therefore, only areas where the target vector species were found were treated and areas with only nonvector species were bypassed. This approach saved many wetland environments from application of larval control materials. It also reduced costs by not using control materials and eliminated application time on the nonvector habitat that did not pose a threat to public health. Similarly, our adult control approach used trapping results that focused on the presence of the vector species, rather than the total population of all species. This, combined with proactive manipulation/management of high-risk adult mosquito harborage habitats, allowed us to focus our adult control efforts to very small habitat areas (around Boulder Reservoir). This resulted in use of less pesticide materials, which reduced costs and better protected the environment.

A city-specific wetland permit was obtained as part of this program that allowed us to apply the larvicide *Bti* to wetland habitats. This permit included a thorough evaluation of different alternatives to come up with the best protection of the wetlands while still accomplishing the vector species control objective. Furthermore, as part of OtterTail's field efforts, an assessment of the affect of *Bti* on nontarget aquatic organisms was conducted at one of the treatment sites on August 8-12, 2003. Quantitative triplicate macroinvertebrate Ponar samples were taken of the mosquito breeding habitat bottom substrate before, and three days after, application of *Bti*. Additionally, a qualitative sweep was conducted for supplemental information. A control site was also sampled in similar habitats without the application of *Bti*. Although not statistically reliable due to the highly heterogeneous substrates, some basic conclusions can be drawn. The data indicates no discernable affect to the aquatic community from the application of *Bti*, with many indices even slightly higher after application due to the variability of the substrate.

The results of the 2003 larval program included 278 larval/breeding sites (totaling 1948.5 acres) that were mapped and inspected at least once during the season. Of these, 104 sites were actively breeding mosquito larvae at some point during the season. Seventy-four (74) sites (294 acres) were breeding vector species during the season. Through extrapolation of the number of mosquitoes per treated site, the estimated season total of vector larvae killed was over 94 million mosquitoes. Because the project sites were focused around residential areas and that the city's mosquito infection rates were as high as 4.7 percent during its peak, it is extremely likely that the city's larval control program directly prevented human infections.

The purpose of our adult/WNV surveillance program was to provide an early warning system of the occurrence of WNV in the program area. This was accomplished primarily through 1) monitoring of the adult mosquito population and 2) determining if that population contains WNV. After submittal of adult vector specimens to the lab to determine presence/absence of WNV, the percent of the specimens with WNV is calculated; known as the infection rate (IR). The CDPHE (2004c) has determined that greater than 1 percent infection rate triggers the alarm of this early warning system. However, this trigger is only a general guideline, as total populations of the vector species must also be considered. Infection rates for the city's mosquito traps during the mosquito season ranged from a low of 0 to a high of 4.7 percent.

The adult trapping effort started the last week of May through the first week of June and ended on September 24, 2003. The last trapnight only produced a single vector mosquito total in all traps

collected. There were five traps, referred to as permanent traps, set throughout the season in harborage locations that were determined to represent the entire program area. In these permanent 5 traps, out of 27 trapnights, there was an average of 200 total adult mosquitoes per trap per night and an average of 30 percent vector species per trap per night.

Starting at the end of August, there were also rotating traps within 9 locations throughout the city's residential areas. These city traps were set as a guide to potential adult spraying operations in residential areas. The various locations for the traps were chosen based on general areas of confirmed human WNV cases, relative ease of access, and site characteristics (suitable harborage location). Although there was a fairly high percentage (20 percent average) of vector species in the traps that did have mosquitoes, very low total populations of adult mosquitoes existed (average of 16 mosquitoes per trapnight). Also, only three out of the nine traps produced mosquitoes. By deploying and collecting data for these additional traps throughout the residential areas, the city was able to make an informed decision to not spray within the residential areas during this end of August period due to the obvious natural decline in fall mosquito populations. As part of the preparation for the potential to spray residential areas, a no spray list was initiated where people could call in to choose to be bypassed if spraying were to take place. A GIS database was developed of these residents and is provided in this report.

Adult mosquito control was performed one time and at one location during the 2003 season (Boulder Reservoir). To minimize the affect on the environment, the adult control used an integrated approach consisting of both adult habitat reduction (mowing) and adulticiding of the remaining habitats. This ultra-low volume adulticiding was triggered because Boulder City and County officials determined that the level of WNV activity posed a significant threat to human health due to a specific festival to take place at the Reservoir. Therefore, the adulticiding was part of a one-time adult control effort and not part of a season-long adulticiding program. The integrated approach significantly reduced the amount of habitat and amount of adulticide materials used; 120 acres were mowed and only 9 acres were sprayed. Pre and post monitoring documented an average of an 80 percent reduction in mosquito populations using this integrated method.

Crude attack rates were calculated for all of the cities within Boulder County. Because of the inconsistencies in reporting of fever cases, the CDC recommends comparing attack rates for Encephalitis/Meningitis (M/E) only (CDC 2004a). In other words, M/E cases are more regularly and consistently reported to hospitals due to their severity. Comparison of M/E cases between each city, factoring in their population, provides one of the best means of evaluating the relative transmission of WNV. As shown on Table 2-3, the City of Boulder had the lowest attack rates of all the surrounding cities within Boulder County (11.73)—the other three cities had almost three times that of Boulder (29.7, 32.2, 32.1). This is a significant finding, given that the other three cities conducted adulticiding in their residential areas as part of their 2003 mosquito control program, and the city of Boulder did not. Because there are so many variables that could be influencing case numbers between the cities (and that there is only one year of data), clear conclusions cannot be drawn. For example differences in elevation, resident activity, educational campaigns, amounts of adult and larval habitats, and larviciding program intensities could all influence these numbers. However, it is also very possible that these results suggest that the adulticiding programs conducted within the three cities in 2003 have not reduced the number of people being infected with WNV within their boundaries. Its important to note that the adulticiding conducted within these cities in 2003 was completed after both the mosquito populations and human case numbers had peaked, making it less than ideal timing for adulticiding effectiveness.

1.0 INTRODUCTION

For the 2003 mosquito season, OtterTail Environmental, Inc. (OtterTail) was contracted by the City of Boulder to implement a West Nile Virus (WNV) surveillance and control program to protect public health and biological resources from the effects of WNV. This report provides the results of this project.

WNV is spread by the bite of an infected mosquito. The virus can infect people, horses, many types of birds, and some other animals. Most people (approximately 80 percent) who become infected with WNV will have either no symptoms or only mild ones. However, on rare occasions, WNV infection can result in severe and sometimes fatal illnesses. There is currently no WNV vaccine for humans, but there is for horses.

Identification and surveillance of mosquito producing areas is the primary means of identifying areas that are at high risk for transmission of WNV to humans. Accordingly, treatment of these areas with biological control methods during this stationary larval stage, before they become air borne, are the most cost-effective and efficient means to reduce mosquito populations and; therefore, to also most effectively reduce the risk of human infection of WNV. In addition, control of the larvae limited the possible future need for nonbiological control of adult mosquitoes such as ultra-low volume (ULV) pesticide spraying.

Of the 54 mosquito species occurring within Colorado, species from the genus Culex are the most effective vector of WNV to humans. *Culex tarsalis* is by far the most significant contributor to transmission, due to it being the most abundant Culex species in Boulder and its high level of competency as an efficient vector. *Culex pipiens* is also a competent vector and is believed to be the second highest source of transmission in the Boulder area. Other Culex species make up a relatively small percentage of the mosquito population in the area, but are included when we refer to the term vector throughout this report.

Even though it is not possible to completely eliminate mosquito populations, the goal was to effectively reduce the larval populations of the vector species throughout the project area with focus on control in areas that pose the highest risk to public health. The project was initiated on April 25th, 2003, by surveying City of Boulder lands to evaluate whether the vector species occurred. The project area included 278 larval/breeding sites totaling nearly 1,949 acres consisting of city-owned lands within the city limits (approximately 255 acres), city-owned lands outside of the city limits (approximately 55 acres) and Open and Mountain Parks lands (approximately 1,586 acres) (Figure 1-1). Measures to control mosquito larvae consisted of treating aquatic breeding habitats with the bacterium *Bacillus thuringiensis* subspecies *israelensis* (*Bti*) that attacks the digestive system of the larvae.

2.0 WEST NILE VIRUS BACKGROUND AND 2003 UPDATE

2.1 WNV PATHWAY

West Nile Virus (WNV) is carried long distances by infected birds on seasonal migration patterns and then spread locally by mosquitoes that bite the infected birds. The virus is primarily transmitted by mosquitoes of the genus Culex. In the Boulder Area, the primary species of Culex are *Culex tarsalis* and *Culex pipiens*. Of these two, *C. tarsalis* is considered the most likely to transmit the virus to mammals, such as horses and humans, which are dead-end hosts. Humans and horses are described as dead-end hosts for the virus because; once it has been contracted, they cannot transmit the disease due to inadequate amounts of viremia, or virus circulation in the bloodstream to infect other mammals or mosquitoes. Mosquito of the Culex genus overwinter and; therefore, can continue the disease the following spring.

2.2 WNV IN THE UNITED STATES

WNV was first isolated in 1937 and known to cause asymptomatic infection and fevers in humans in Africa, West Asia and the Middle East. First detected within the U.S. in the state of New York in 1999, WNV has spread from East to West across the United States by mosquito hosts and carrier birds. During 1999 and 2000, WNV encephalitis was found in U.S. residents in three northeastern states with a total of 83 human cases and 9 reported deaths. WNV spread westward in 2001 occurring in 10 states having 66 cases and 9 deaths. There is currently no WNV vaccine for humans, but there is for horses.

As of March 3, 2004 there were a total of 9,306 human WNV cases and 240 WNV related deaths in 46 states and Washington D.C. reported to the CDC for the 2003 season. Figures 2-1 through 2-4 illustrate the WNV 2002/2003 trend as it has moved westward across the US. Figure 2-5 illustrates the number of WNV cases per state in 2003. Figure 2-6 presents the age distribution of fatal Human WNV cases in the US in 2003. Table 2-1 presents the WNV Human cases in the US from 1999 to 2003, and Table 2-2 presents the WNV Human Cases by demographics and mortality in the US in 2002 and 2003.

2.3 WNV IN COLORADO

In 2002, Colorado had its first cases of humans infected by WNV with 14 documented human cases and no reported WNV-related deaths (CDPHE 2004). These 14 cases occurred despite a severe drought. Additionally, 138 bird, 3 sentinel chicken flocks, and 380 horses tested positive for WNV in 2002 (CDPHE 2004).

During the 2003 mosquito season, Colorado lead the nation in WNV human cases (2,945) and WNV-related deaths (55) (CDPHE 2004). This is likely due primarily to Colorado's wet spring and hot summer increasing mosquito breeding populations and earlier emergence as described in Section 3.1. Figure 2-7 illustrates the Colorado WNV cases by county in 2003 and Figure 2-8 presents the cases by location. Figure 2-9 presents the distribution of onset dates of human cases in Colorado in 2003 also known as the epicurve. The onset of human symptoms was first documented with less than 10 cases on June 30, 2003, by CDPHE. The number of cases from June to September followed a Bell shaped curve with the major peak in August.

Compared to 2002 data, with 4,156 positive human WNV cases nationwide, there was a 55 percent increase in 2003 with 9,306 cases. Colorado has experienced the greatest increase in WNV human cases and deaths in 2003 compared to all other states. As of February 24, 2004, WNV had been reported in 46 counties out of 64 total counties this year, which is an increase from 9 counties last year (CDPHE 2004). With the 14 human WNV cases reported in 2002, it was determined that five people were infected out of state and eight were infected in eastern Colorado (CDPHE 2002). In 2003, WNV was found to be concentrated in the residents along the north and central Front Range, and counties along the South Platte and Arkansas River Valleys. The counties in the high mountains had very low numbers or no cases reported. On the western slope, Mesa County had the most cases at 19, Delta County had 10 and Lincoln County had 8 reported cases. In Boulder County, there were a total of 430 human WNV cases in 2003. An approximate breakdown of case diagnosis is as follows: 378 (fever), 52 (meningitis/encephalitis) and a total of 5 deaths overall (CDPHE 2004). Larimer County had the most reported cases in the state with 545 cases and 8 deaths, Boulder County was second and Weld County, third with 402 cases and 6 deaths (CDPHE 2004). Denver County had 8 WNV related deaths, the same as Larimer though it only had 163 total cases. Other counties with high numbers of reported cases (exceeding 100) include Adams (231), Arapahoe (138), Jefferson (157), El Paso (114) and Pueblo (183).

Cases of WNV in Colorado ranged from the age group 0 to 4 years to people over 90 years old. The majority of positive WNV cases were in the 45 to 49 age group with a total of 387 evenly split between males (173) and females (174) (CDPHE 2004). The second largest number of WNV cases reported to CDPHE was in the 50 to 54 age group at 363. The 90 and over and 0 to 4 age groups had the fewest cases in Colorado with a total of 13. The mean age was 47.2, the maximum age was 95 and the minimum age was less than 1. The median age of infected Colorado residents was 47 for the total 2,945 people infected. The total number of females with WNV equaled 1,519 and there were a reported 1,426 total males with WNV.

In Colorado during the 2003 season, 78.9 percent of WNV cases were the viral fever syndrome, 13.2 percent meningitis, and 7.9 percent encephalitis (CDPHE 2004).

2.4 WNV IN BOULDER COUNTY

By the end of 2003 there were 430 WNV infections reported to Boulder County Public Health, 378 were diagnosed with WNV Fever and 52 with either WNV Encephalitis or Meningitis (M/E) (Table 2-3).

Based on surveillance data among human cases from 2003, it is estimated that there were approximately 7,800 individuals infected with WNV in Boulder County (only 430 reported illnesses) (Harmon 2004). When compared to a population of 277, 601 for Boulder County (est. 2002 population for Boulder County, State Demographers Office), 97.2 percent of Boulder County residents have no built up immunity against WNV infection (Harmon 2004). Figure 2-10 presents the sequence of WNV-related events that transpired during 2003 within Boulder County.

Crude attack rates were calculated for all of the cities within Boulder County. Because of the inconsistencies in reporting of fever cases, the CDC recommends comparing attack rates for Encephalitis/Meningitis (M/E) only (CDC 2004a). In other words, M/E cases are more regularly and consistently reported to hospitals due to their severity. Comparison of M/E cases between each city, factoring in their population, provides one of the best means of evaluating the relative transmission of WNV. As shown on Table 2-3, the City of Boulder had the lowest attack rates of all the surrounding cities within Boulder County (11.73)—the other three cities had almost three

times that of Boulder (29.7, 32.2, 32.1). This is a significant finding, given that the other three cities conducted adulticiding in their residential areas as part of their 2003 mosquito control program, and the city of Boulder did not. Because there are so many variables that could be influencing case numbers between the cities (and that there is only one year of data), clear conclusions cannot be drawn. For example differences in elevation, resident activity, educational campaigns, amounts of adult and larval habitats, and larviciding program intensities could all influence these numbers. However, it is also very possible that these results suggest that the adulticiding programs conducted within the three cities in 2003 have not reduced the number of people being infected with WNV within their boundaries. Its important to note that the adulticiding conducted within these cities in 2003 was completed after both the mosquito populations and human case numbers had peaked, making it less than ideal timing for adulticiding effectiveness.

3.0 CITY OF BOULDER'S PROJECT AREA/ PROGRAM OVERVIEW

The City of Boulder was concerned about the rapid increase of WNV across the country and in the State of Colorado. Prior to 2003, the City of Boulder had not controlled mosquitoes. The city's policy was that the nuisance created by mosquitoes was not significant enough to warrant pesticide application. However, the predictions were that the spread of WNV would be westward and that Colorado cases would likely be much higher than last year (as is the typical cycle found with this virus). To be proactive regarding the potential increases in WNV cases, the City of Boulder retained OtterTail's biologists to provide a plan to develop a WNV control program.

The city had specific goals for this program. First, they wanted to reduce the risk of WNV infection to human health. They also wanted to limit the effect on the environment from control materials and be as cost-effective as possible. Standard approaches to mosquito control (applying control materials to all significant mosquito populations) would not accomplish these three goals. OtterTail worked collaboratively with the city to successfully achieve these goals by devising a unique and innovative program. Mosquitoes of the genus Culex are the most effective vector of the disease to humans in the area; therefore, only areas where the target vector species were found were treated and areas with only nonvector species were bypassed. This approach saved many wetland environments from application of larval control materials. It also saved money by not using control materials and eliminating application time on the nonvector habitat that did not pose a threat to public health. Similarly, our adult control approach used trapping results that focused on the presence of the vector species, rather than the total population of all species. This, combined with proactive manipulation/management of high-risk adult mosquito harborage habitats, allowed us to focus our adult control efforts to very small habitat areas (around Boulder Reservoir). This resulted in use of less pesticide materials, which reduced costs and better protected the environment.

3.1 CLIMATOLOGICAL DATA FOR THE BOULDER AREA

The weather patterns leading into and during the mosquito breeding season are important factors that influence mosquito abundance. The following sections describe in detail the local climate in Boulder, the weather during the season, and how that affected the mosquito populations.

The City of Boulder is located in a semi-arid environment at an elevation of approximately 5,340 feet. The mosquito season for the City of Boulder program area is from April to October. Climate data has been obtained from two stations in Boulder, the NOAA NCDC Global Climate Perspectives System (GCPS) monthly station over the period 1893 to 1990, and from 1991 to 1996 at the station located at the National Center for Atmospheric Research (NCAR) Foothills Lab (NOAA 2004). The mean monthly temperature in Boulder has ranged from 48 degrees Fahrenheit (F) in April to 62 degrees F in September over the period of record. There is an approximate steady increase from April to July having a mean monthly temperature of 72 degrees F making it on average the hottest month of the year, then a steady decrease to September. Monthly mean precipitation for the same time period indicates that April and May are the two wettest months in Boulder, with mean monthly precipitation measuring 2.5 and 3.2 inches, respectively. June and July tend to receive the most precipitation second to April and May, ranging from about 1.7 to 1.8 inches. Figures 3-1 and 3-2 graphically represent the average temperature and precipitation for the period of record and for the year 2003 (NOAA 2004).

For the monitoring program in the 2003 season, from April to October, the monthly mean temperature ranged from 50.6 degrees F in April to a maximum monthly mean of 75.9 degrees F in July decreasing to 57.4 degrees F in October (NOAA 2004). The average monthly mean temperature for the season was about 62.5 degrees F with an average monthly mean precipitation of 1.9 inches. The monthly mean precipitation for the 2003 season ranged from a maximum monthly mean of 3.5 inches in August to a minimum monthly mean of 0.35 inches in September. The major storm events for the season (defined as greater than 1.0 inch of precipitation) in Boulder included the following: 1.18 inches on April 19th, 1.39 inches on May 10th; 1.32 inches on August 18th; and a maximum of 1.78 inches on August 30th (NOAA 2004).

The data presented in Figure 3-1 clearly illustrate that the precipitation was considerably higher than normal in the spring of 2003, with March being almost triple the normal amounts, and Feb and April were also above average. This, combined with the warmer than normal summer (July and August) shown in Figure 3-2, created ideal conditions for mosquito breeding. The more than double the average August rainfall also did not help, rewetting habitats that were drying up.

These data strongly suggest that Colorado's wet spring and hot summer, which are ideal conditions for mosquito breeding, was most likely a significant factor in the abundance of the vector species in 2003, especially *Culex tarsalis*.

4.0 LARVAL MOSQUITO SURVEILLANCE AND CONTROL

4.1 LARVAL SURVEILLANCE METHODOLOGY

Larval surveillance was performed using standard operating procedures outlined in the OtterTail Field Manual during the 2003 season (OtterTail 2003a). The following is a brief summary of the procedures used. To inspect a project site 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. If mosquito larvae were present, an eyedropper was used to collect a representative sample for future species identification. A representative sample consisted of mosquito larvae with all the various instars (life stages) that were present. Sites were only dipped where there was 2 inches of water or greater. On occasion, there were also sites containing less than 2 inches of water, such as depressions, which were surveyed. The majority of the sites had poor open water habitat in the center and good habitat around the perimeter of the site. At these sites, the dipping effort was completed using a linear approach (walking around the perimeter and sampling the margins). Some sites were small (1 acre or less) and had good habitat throughout the site. At these sites the dipping effort was completed using surface area guidelines where the entire site was methodically sampled.

Using the linear approach, sites 1 acre in size and less were dipped approximately every 10 to 20 feet; sites 1 to 10 acres were dipped approximately every 50 to 100 feet, and sites greater than 10 acres were dipped approximately every 200 to 500 feet. Using the surface area approach, sites 1 acre in size and less were dipped approximately every 10 to 20 square feet. Since each project site varied in size, physical characteristics, and changed as the season progressed (e.g., became drier, wetter, increased vegetation), there were field adjustments made during the season concerning appropriate number of dips. For example, at a very small site (less than 1/10 acre), the site might be dipped every 1 square foot to get good coverage. At the beginning of the season, all sites were monitored on a routine basis. During the peak season (July through August), the sites were categorized into high and low priorities due to heightened risk of WNV and professional judgment due to species present during previous visits.

4.1.1. Larval Surveillance Site Selection/Characterization Methods

The project area was separated into two regions for ease of larval surveillance fieldwork reference into North Boulder and South Boulder. The east west dividing line for the regions was Valmont Road. There were 133 sites in the North Boulder region and 145 sites in the South Boulder region. Therefore, there were a total of 278 individual sites mapped and inspected at least once within the entire project area and 23 additional sites mapped at Sawhill Ponds. The Sawhill Pond sites were not adopted into the surveillance program due to land management constraints.

In 2003, there were 40 open space parcels added to the project area starting at the end of July totaling 1,586 acres for Boulder Open Space and Mountain Parks property. The parcels were checked and existing and potential breeding habitat was mapped and monitored. Many of the parcels contained areas that were wet earlier in the season and need to be a higher priority for inspection due to potential breeding habitat in 2004.

In 2003, there were 278 total non-breeding and breeding sites mapped and inspected at least once (not including 23 Sawhill Ponds area sites) and 104 of these sites that bred mosquito larvae during the season. There were 55 breeding sites in the South Boulder region and 49 breeding sites in the North Boulder region not including the Sawhill Pond sites. As the season progressed, the sites were categorized according to larval abundance and occurrence. Low priority breeding sites consisted of sites with poor habitat, flowing water or presence of aquatic predators. High priority breeding sites bred consistently every 7 to 10 days during the peak season and contained vector larvae in the majority of hatches.

Appendix B provides a detailed physical description of each of the breeding sites where OtterTail found mosquito larvae. The site descriptions are arranged by region with South Boulder sites first followed by North Boulder sites generally moving from south to north within each region. A site-specific recommendation follows each description. Sites that are not described in detail in Appendix B were either completely dry by the time they were added to the project area or they never contained vector or nonvector mosquito larva (non-breeding) due to poor mosquito habitat features and conditions.

The natural wetland breeding sites throughout the project area were mostly wet early in the season then progressively dried up with many remaining dry for the majority of the season. Several isolated storm events during the season filled sites periodically and produced floodwater species and then vector species, but the natural wetlands followed a drying trend as the season passed and water levels naturally dropped. Several wetland sites were managed and did not follow the natural trend due to mechanical control devices or manmade diversions that maintained a fairly constant water level the last half of the season.

In farmland areas where flood-irrigation was prevalent, there were several mosquito population bursts during the season when fields were alternately flooded. Each flooding event resulted in the creation of isolated “hot spots” where large mosquito populations would originate. After the fields were flooded, a batch of floodwater species would hatch where certain localized areas held water and had become stagnant. These areas, along with numerous other naturally occurring wetland areas, consistently bred vector species throughout the season, some even reaching into early October. However, most of the flood-irrigated field sites were added to the program mid to late season, after the peak irrigation period. Therefore, the majority of the vector breeding sites encountered this season were nonflooded, irrigated wetland areas.

4.2 LARVAL CONTROL METHODOLOGY

Larval mosquito control methods employed by the City of Boulder were aimed at reducing the potential of the mosquito-borne disease, WNV. The program’s focus for larval control was to identify if Culex species (primarily *C. tarsalis* and *C. pipiens*) were present before initiating control efforts. The threshold for larval control was presence of the vector species. The method did allow for pest mosquitoes and non-biting mosquitoes to persist in the environment if vector species were not present. The approach requires significantly more surveillance and resulted in substantially less treatment of wetlands and other mosquito breeding habitats. Unlike a large-scale broad-spectrum mosquito control program, where presence of any type of mosquito larvae triggers treatment, the presence or absence of vector species had to be determined before the site-specific larval treatment could occur. Finding and documenting consistent vector breeding sites was an important component for the program because it created a pattern that can be monitored and systematically controlled. The objective of larval mosquito control is to prevent the need for adult mosquito control spraying, which is much less effective than larval control.

To accomplish the unique methodology developed by OtterTail staff, it was necessary to have a team more skilled than is typically necessary for mosquito control efforts. OtterTail maintained a strong biological staff during the season including a core team of professional aquatic biologists, wetland scientists, and mosquito control specialists, all with aquatic invertebrate identification skills. This core team was supported with a seasonal crew with natural resource and biology backgrounds and experience in mosquito larvae and adult monitoring/control. OtterTail used geographic information systems (GIS) ArcView software to compile wetland areas, mosquito habitat, adult trapping locations, etc. to aid the larval mosquito monitoring and control creating a database for quick access and queries.

4.2.1. Larvicide Application Methods

The application of *Bti* was the approved method for larval mosquito control because it serves the greatest public need. Adverse impacts to areas being treated were minimized by applying *Bti* at or below recommended concentrations, and all treatment areas were posted with bright yellow city pesticide notification signs before *Bti* was applied. A post-treatment check was conducted the following day to assure the larval population was controlled. If any larvae were found, a second application of *Bti* was applied.

The application rate for *Bti* was 5 pounds/acre or 0.2 acres treated per 1.0 pound of *Bti*. The application rate seemed to provide 100 percent control in the majority of treatments. OtterTail used appropriate personal protection equipment (PPE) when applying the *Bti* including gloves and filter masks. Proper handling of *Bti* and procedures for spill control are summarized in the 2003 City of Boulder West Nile Virus Control Plan (OtterTail 2003b).

4.3 PRE AND POST TREATMENT MACROINVERTEBRATE ASSESSMENT

An assessment of the affect of *Bti* on nontarget aquatic organisms was conducted at one of the sites on August 8-12, 2003. Quantitative triplicate macroinvertebrate Ponar samples were taken of the mosquito breeding habitat bottom substrate before, and three days after, application of *Bti*. Additionally, a qualitative sweep was conducted for supplemental information. A control site was also sampled in similar habitats without the application of *Bti*. Although not statistically reliable due to the highly heterogeneous substrates, some basic conclusions can be drawn. The data indicates no discernable affect to the aquatic community from the application of *Bti*, with many indices even slightly higher after application due to the variability of the substrate. A basic comparison of taxa and abundance for these data is presented in Table 4-1.

4.4 LARVAL SURVEILLANCE AND CONTROL RESULTS AND DISCUSSION

The 2003 larval surveillance season started on April 25 and ended on October 17, when no more mosquito larvae were observed. Monitoring for larvae throughout the project area and larvae species identification determined whether control was needed. Although adult mosquitoes reduce their taking of bloodmeals following peak season (July through August), OtterTail continued larval surveillance and control into mid October to further reduce the vector population that has the ability to overwinter and thus reduce the initial population in 2004.

In 2003, a total of 1,871 individual larval site investigations were performed within the project area. The highest number of site visits to a site was 41 times during the season due to consistent larval breeding. The average number of site visits to any one site during the season was 13.

The season's average representative larval collection resulted in a fairly even spread between Culiseta, *C. tarsalis*, and Aedes/Ochlerotatus taxa at 20 to 29 percent as shown in Figure 4-1. *Aedes vexans* and *C. pipiens* were 10 and 11 percent respectively and the remaining species were all under 2 percent for the season. To help understand patterns of presence of the larval species throughout the season, monthly species percent abundance was calculated. Figures 4-2 through 4-8 show monthly average representative populations for each larval species. *Culex tarsalis* shows an increase from May to July then an expected downward trend into the fall. *Culex pipiens* shows a similar trend except the population percent is much lower. These trends confirm that the highest populations of vector larvae are present in June, July and August, and therefore, these months should be considered the peak season in Boulder.

Detection of larval *C. tarsalis* mosquitoes in early May corresponded with other mosquito data results in other areas of Boulder County for 2003. *Culex pipiens* larvae followed the same trend (stayed relatively low compared to other nonvector species at the beginning of the season and became increasingly more abundant as the season progressed due to increasing amounts of stagnant water habitat) due to their preference for small containers and stagnant pools when the natural breeding sites became drier and more isolated. In general, *C. tarsalis* were more abundant than the *C. pipiens* throughout the project area. The other notable species, *Aedes vexans*, known as a floodwater species, was observed directly after a significant storm event (1.0 inch or greater) and during periods of flood irrigation on farmland. The pattern observed during the season was the presence of nonvector, floodwater species followed by vector species. After the farm fields were freshly irrigated or after significant rainfall, Aedes species appeared, followed by the presence of vector species 7 to 10 days later if the water was not drained or flushed and became stagnant.

Some wet areas that did not support mosquitoes included any type of moving water (rivers, streams, and creeks), deeper lakes, ornamental ponds, and duck ponds. As expected, larvae were not found within any type of moving water, not dependent on flow. The larger bodies of water such as open water ponds and lakes were unfavorable for breeding of mosquitoes because of steep banks, barren shoreline, wave turbulence, and the presence of natural aquatic predators. Some sites that appeared to have good breeding habitat characteristics did not contain many larvae, most likely because of an almost undistinguishable constant flow throughout the site or potential aquatic predators such as minnows. Breeding sites were classified according to the type of habitat using criteria developed by the U.S. Fish and Wildlife Service (Cowardin et al. 1979).

Approximately 294 acres of active vector breeding habitat were identified and treated within the entire project area in 2003. Some of the sites bred vector larvae multiple times during the season causing the treated acres at certain sites to be counted multiple times for the season total. The breeding habitat treated was approximately 15 percent of the total potential breeding site acreage (1,949 acres). Therefore, there was over 1,655 additional potential breeding acres in the project area that did not breed due to a variety of reasons. The north Boulder region had approximately 249.treated acres and south Boulder region had approximately 45 treated acres. A total of 196 individual site visits were completed to apply *Bti* larvacide during the season. Many sites were treated numerous times; therefore, the site treatment visit total was much higher than the actual number of sites in the project area.

Since this program is in its first season, the extensive data obtained by OtterTail will be a solid base for the future program and an important tool to help fight the continued threat of WNV.

5.0 ADULT MOSQUITO/WNV SURVEILLANCE AND CONTROL

5.1 ADULT SURVEILLANCE METHODOLOGY

5.1.1. Adult Surveillance Site Selection/Characterization Methods

Adult mosquitoes can dehydrate quickly during the daylight hours if they do not have a shady area to rest and escape the heat. Most mosquito species prefer to rest during the heat of the day in area known as harborage areas. A mosquito harborage area is usually a shaded, wind-protected, moist area that is most likely near a source of water. Relevant examples are groves of tall trees with a layer of shrubby undergrowth, dense bushes, tall live grasses, or under roof eaves and inside tires in residential areas. Adult mosquitoes will even collect in shorter grasses that are lush from frequent watering.

The harborage sites for the project were important for long-term monitoring; therefore, the sites were chosen carefully. The selected harborage sites were chosen for several reasons. Firstly, the project area was fairly evenly covered from north to south with three traps in the north and two traps in the south. Also, the traps were set in well-hidden areas where there was a low threat of vandalism. Finally, the traps were chosen based on their vicinity to some source of stagnant water that provided potential breeding (larval) habitat. Most of the harborage areas used in 2003 were placed near areas that had some limitations to controlling larvae (e.g., housing developments and other private property).

5.1.2. Trap Methods

There are two different types of adult mosquito traps that are typically used to monitor adult populations in the Colorado Front Range, the gravid trap and the CO2 light trap. The gravid trap collects adult female mosquitoes that have already collected a blood meal and are ready to lay their eggs. Gravid traps were not used during the season for the following reasons. Most studies have shown that light traps are the most effective at sampling our primary vector species target, *Culex tarsalis*. Furthermore, a trapping study conducted in the City of Ft. Collins in 2003, found 1) that gravid traps were essentially worthless for collecting *C. tarsalis*; and 2) that CO2 light traps sampled *C. pipiens* surprisingly well (CDC 2004c).

To capture the most representative sample of adult mosquitoes in an area adult CO2 light traps were set overnight to collect live adult mosquitoes throughout the season. The traps are based on the principle that most adult mosquitoes are attracted to light, carbon dioxide (CO2) and heat. The CO2 light trap collects adult female mosquitoes that are attracted to mammals. Therefore, the CO2 trap indicates when the vector *C. tarsalis* mosquito is no longer feeding solely on birds and has turned to mammals as their alternate bloodmeal. This is one of the first indicators that WNV is likely to be transmitted to people through the vector mosquito (assuming the trapped vector mosquitoes are found to be carrying WNV).

The trap consisted of a 1 gallon plastic insolated bucket with a battery casing hanging directly below and a finely meshed net hanging below the battery at the very bottom. The entire device was placed on a tree branch about 5 to 7 feet off the ground by a small chain letting the bucket

and net hang free. The bucket was filled with 3 to 4 pounds of commercial dry ice (CO2) and holes were punched at the base of the bucket to let a slight amount of CO2 leak out as an attractant. The battery ran a small fan and light positioned above the net. The light provided further attraction and once the mosquitoes were near the light they were sucked down into the net and trapped by the downward force of the fan. In the morning, the trap net was removed and placed into a jar with ethyl acetate that quickly killed the mosquitoes so that they could be identified.

5.1.3. Adult Trap Stations for Permanent Surveillance of the Project Area

The five permanent adult light traps (H0, H2, H4, H5, H6) were set at prime harborage locations in the project area; two traps in South Boulder and three traps in North Boulder. There was a trap (H1) set at the Bobolink Trailhead in the beginning of the season but it was moved to a permanent location (H0) approximately 3.5 miles south in mid June. The trap results are shown in Table 5-1 and Figures 5-1, 5-2, 5-3, 5-4, and 5-5. Trap H0 at Rolling Rock showed typical results for a floodwater species dominant area. The population trends rose and fell several times during the season roughly following the flood irrigation schedule in the vicinity. Trap H4 at Papini Park corresponded well with the larval control efforts. The field crew was monitoring and controlling added city-owned lands completely surrounding the trap from mid July on. There was a decrease in total population numbers at that peak season time when mosquitoes numbers should have kept increasing. Therefore, it is reasonable to assume that the larval control effort was reducing populations. However, some adults must have been hatching on private or corporate owned lands within a several mile radius of the traps and collecting in the harborage areas with the traps keeping the trap numbers from reaching zero. Trap H6 at Sawhill Ponds was a good illustration of mostly uncontrolled habitat and the resulting uncontrolled mosquito population trends. The trend seems to be steadily increasing numbers to a peak in mid July and then a general downward movement with a smaller population spike in late summer probably due to a rain event at that time. Figure 5-6 compares the vector numbers in the relatively uncontrolled H6 (Sawhill Ponds) trap area to the other adult traps in the project area to show the significantly higher populations in Sawhill Ponds.

Part of the 2003 program was an intensive adult mosquito identification effort. Species composition for the permanent traps are shown in Figures 5-7 through 5-12.

5.1.4. Adult Trap Stations for Fall Evaluation Within the City Population Centers

In addition to the permanent traps, nine adult light traps were set at rotating positions and schedules around the city. The traps were meant as a guide to potential adult spraying operations in residential areas. The various locations for the traps were chosen based on general areas of confirmed human WNV cases, relative ease of access, and site characteristics (suitable harborage location). The city trap results are shown in Table 5-1. There were fairly high percentages of vector species in the traps that did have mosquitoes; however, the data showed very low total populations of adult mosquitoes throughout the trapping period. Therefore, the data could be very misleading when presented as percent vector. Also, only three out of the nine traps produced mosquitoes.

5.2 ADULT CONTROL

5.2.1. Adult Control Background Information

There are two general strategies for mosquito adulticide applications, large-scale fogging and spot spray treatment. The first and most expensive option is to fog large areas to attempt to significantly reduce the number of adult mosquitoes in a targeted area. The effort could include many types of application methods: ground individual backpack units, foggers on ATV's, foggers on trucks, and aerial applications. To use only one method such as, fogging with a truck-mounted unit, would be far less effective than using multiple methods. However, a full-scale assault could undoubtedly reduce the adult mosquito populations but the process would use an entire season's budget in less than a month. Also, the main purpose of a large scale fogging approach is to keep the adulticide material airborne as long as possible without it evaporating. To keep the adulticide airborne a certain droplet size is needed and only expensive adulticiding equipment can do this accurately. Finally, the effectiveness of the large scale fogging effort will be compromised if the control company does not have somewhat of free range of the city area to perform control. Public concerns or the majority of residential areas on a no-spray list will make effective control almost impossible. The second option is to spray individual harborage areas, which is called spot treatment. This was the method used at the Boulder Reservoir in 2003 (see below). Treating the harborage areas where adult mosquitoes are resting in high concentrations allows more mosquitoes to come in contact with the adulticide and uses a lower, focused amount of adulticide material.

Each harborage area has unique characteristics and circumstances. Some areas can be controlled effectively with manual removal of the mosquito harborage habitat such as mowing long grass and removing vegetative understory; other areas can only be controlled effectively by spot spraying adulticides. After treatment a post check adult collection is performed to determine effectiveness. Spot treatment has many positive aspects. Firstly, it helps to identify possible overlooked mosquito breeding sites and areas where larval control should be focused. Secondly, spot treatments are publically lower profile than large-scale operations with much less adulticide material being used in the environment. Also, spot treatment potentially kills the same or more mosquitoes than fogging because the mosquitoes are concentrated in smaller areas. The only negative aspects to spot treatment are that, if initiated, they are more effective if continued all season and the public will not see an immediate reduction in adult mosquitoes.

5.2.2. Adult Control 2003 Methodology

Adult mosquito control was performed one time at one location during the 2003 season (Boulder Reservoir). To minimize the affect on the environment, the adult control used an integrated approach consisting of both adult habitat reduction (mowing) and adulticiding of the remaining habitats. This ULV (ultra-low volume) adulticiding was triggered because the City and County officials determined that the level of WNV activity posed a significant threat to human health due to a specific festival to take place at the reservoir. Therefore, the adulticiding was part of a one-time adult control effort and not part of a season-long adulticiding program.

Adult mosquito control in 2003 was performed with the adulticide known as Permanone 31-66. This specific adulticide is used for quick knockdown and control of adult mosquitoes, midges, and other biting flies. Its recommended use is by trained personnel only in mosquito control programs. The material was applied using a nonthermal ULV portable backpack sprayer unit.

The adulticide used is known as a synthetic pyrethroid. Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the oleoresin extract of dried chrysanthemum flowers.

These products cause rapid knockdown of adult mosquitoes. They exhibit low mammalian toxicity, degrade rapidly in sunlight, leave little or no residue, and do not bioaccumulate in the environment. Pyrethroids are toxic to aquatic organisms; however, recommended manufacturer dosage rates control the toxicity of these products to non-target species. Lobster, shrimp, mayfly nymphs, and zooplankton are the most susceptible non-target aquatic organisms (Mueller-Beilschmidt 1990).

Dosage rates can be low to control mosquitoes. These products are applied in small quantities per acre, referred to as ultra-low volume (ULV) application. ULV delivery techniques minimize environmental impacts at the same time they effectively manage populations of adult mosquitoes. OtterTail monitored the application of the adulticide to ensure compliance with all regulations and requirements promulgated by the EPA, State of Colorado, and City of Boulder.

In 2003, the City of Boulder held an outdoor music oriented celebration at the Boulder Reservoir. The large amount of people that would be gathered near mosquito habitat potentially raised the risk of WNV in that area. Focused control activities were deemed necessary due to these high risks to protect festival participants and employees. Fogging was not chosen as the preferred option in this situation because of the potential environment effects of the fogging material to fish-bearing waters of the Reservoir and the large area (700 acres) requiring control. Three adult light traps, referred to as the Boulder Reservoir traps, were set. The traps were set to monitor vector population numbers and determine the effectiveness of the potential adult operations to take place there. A strategy was devised that combined several adult control techniques and focused on using the least amount of adulticide material as possible but still producing effective control. First, to eliminate breeding areas, OtterTail proceeded to closely monitor and use larvacide (*Bti*) on all active larval breeding sites within a 1 to1.5 mile radius around the Reservoir for a month prior to the event. Also, manual adult habitat reduction was conducted within a mile radius of the Reservoir (a mile was determined to be sufficient for the relatively weak flying capabilities of *C. tarsalis*). The adult habitat reduction program consisted of a portion of the approximately 700-acre area around the reservoir. It consisted of mowing and weed whipping tall grass and thick weedy undergrowth from around the reservoir. After 3 days of assessment and 5 days of mowing operations, approximately 120 acres of adult harborage habitat was eliminated.

5.3 ADULT MOSQUITO/WNV SURVEILLANCE AND CONTROL RESULTS AND DISCUSSION

The adult trapping effort started the last week of May and ended on September 24, 2003. The last trapnight only produced a single vector mosquito total in all traps collected. There were five traps, referred to as permanent traps, set throughout the season in permanent harborage locations. In the permanent 5 traps, out of 27 trap nights, there was an average of 200 total adult mosquitoes per trap per night and an average of 30 percent vector species per trap per night. There were also five rotating traps, referred to as the City traps, set around the City of Boulder in residential areas of concern starting at the end of August. In the City traps, out of 10 trapnights (not every trap was set every trapnight), there was an average of only 16 total adult mosquitoes per trap per night and an average of 20 percent vector species per trap per night.

The vector species, *C. tarsalis*, adult mosquitoes were persistent throughout the survey season with population spikes in late July and early August. *Culex tarsalis* adult mosquitoes populations stayed relatively low compared to other nonvector species at the beginning of the season and became increasingly more abundant as the season progressed due to increasing amounts of stagnant water habitat.

Due to continued heightened public concern about WNV, adult control in the form of ULV spray application took place in the remaining habitat pockets surrounding Boulder Reservoir. The adult control, using Permanone 31-66, took place on the afternoon of August 28, 2003, two days after the mowing operations were completed. A total of 9 acres were treated at a rate of 2.5 ounces per acre. Table 5-2 shows results of the adulticide effort from trapping comparing pre-trap and post-trap adult mosquito numbers. The average reduction in vector species after the spraying effort was 80 percent for the three harborage-monitoring traps.

The adult mosquito data collected was used by the CDPHE and BCHD to calculate infection rates and help to determine areas of concern for public awareness and safety. The data also guided the potential adulticide effort within the city, which was a very important health and environmental issue to city residents. The city developed a contingency plan for spraying of adult mosquitoes if numbers of disease vector mosquitoes warranted it. A "no spray" list was maintained for city residents in case spraying for adult mosquitoes was initiated (Figure 5-13).

6.0 PUBLIC RELATIONS AND EDUCATION

In 2003, the City of Boulder introduced the new WNV monitoring program to the public. The goal was to promote a better understanding of the disease, provide education on ways to protect against transmission via the vector species, and the ways the public can reduce the source of mosquito breeding sites on their own property. A secondary goal was to inform the public that the program was not being implemented to control nonvector pest mosquitoes, but only to reduce the threat of WNV through vector control. Field personnel noted that the program seemed well received by the public and no negative feedback concerning the larval control portion was observed.

7.0 2003 WNV CONTROL PROGRAM SUMMARY

The program for 2003 consisted of intensive mapping and monitoring for the presence of mosquitoes that are vectors for WNV and then treatment with *Bacillus thuringiensis israelensis* (*Bti*) when disease vectors were found. The city monitored and treated on public lands and provided education and information to private landowners. The city trapped adult mosquitoes at designated locations throughout the season and cooperated with the Boulder Public Health Department and Colorado Department of Public Health and Environment (CDPHE) to test them for the presence of the WNV.

The city developed a contingency plan for spraying of adult mosquitoes if numbers of disease vector mosquitoes warranted it. A "no spray" list was maintained for city residents in case spraying for adult mosquitoes was initiated. No spraying was done within the urban areas of the city. A limited amount of spraying was done at the Boulder Reservoir in conjunction with the Rez Fest over the Labor Day weekend in early September.

Of the 1,948 acres of larval/breeding habitat monitored throughout the season, 294 acres were treated with *Bti*. Adult mosquito traps were maintained throughout the season. The mosquitoes obtained from the traps were identified and sent to the CDPHE for WNV analysis. The disease vector species *Culex tarsalis* and *Culex pipiens* comprise approximately one-third (34.37 percent) of the adult mosquitoes trapped. The distribution varies from location to location and throughout the season. The data from the adult mosquito traps will be used to determine control options for 2004.

8.0 MOSQUITO INFECTION RATES

CDC encourages surveillance programs to routinely incorporate a more informative index of relative virus activity, the virus infection rate (IR), into their mosquito-based evaluation of local virus activity patterns. At the county level or below, weekly tracking of mosquito IR can provide important predictive indicators of transmission activity levels associated with elevated human risk. The CDPHE (2004c) has determined that an IR greater than 1 percent triggers the alarm of this early warning system. However, this trigger is only a general guideline, as total populations of the vector species must also be considered.

Estimates of the IR are usually presented as the number of infected mosquitoes per 1,000 tested. The simplest estimate, the minimum infection rate (MIR), is calculated: ([number of positive pools / total specimens tested] x 1000), with the data representing a single species or species group collected over a time period and geographic area relevant to the goals of the surveillance program. The MIR uses the assumption that a positive pool contains only one infected mosquito, an assumption that may be invalid when infection rates are high, as has been observed during West Nile virus epidemics.

Dr. Brad Biggerstaff, Mathematical Statistician at CDC/DVBID, developed an easy-to-use program for calculating IR estimates from mosquito pool data using methods that do not require the assumption used in the MIR calculation. This program also includes calculation of confidence intervals, which reflect, in part, the sample sizes used in the calculations (CDC 2004c). Table 8-1 summarizes the calculated IRs for the City of Boulder for the months of June and July, 2003.

9.0 RECOMMENDATIONS FOR THE 2004 WNV PROGRAM

The WNV mosquito control program for 2004 should continue to concentrate on larval mosquito control. Improvements and/or additional activities recommended for 2004 include the following areas:

- Education to residents
- Continuation of surveillance and control of all 2003 larval breeding sites
- Outreach and technical advice to wetlands and pond owners
- Distribution of *Bti* briquettes to residents
- Habitat modification and/or source reduction
- Water management (timing, amounts, and duration modification)
- Introduction of native mosquito-eating fish
- Preventive maintenance

The City of Boulder is planning outreach to the private landowners in the community. Property owners with detention ponds and regulated wetlands will be contacted and provided information on mosquito management. A workshop on pond and wetland management is being organized for landowners to provide them with in-depth information on a range of options for management. The city will offer an audit to landowners, which would provide an on-site inspection of ponds and wetlands and provide management recommendations. Larval control products will be made available to residents for treating larval sites on their property. City staff will also be contacting irrigation companies to explore management options.

10.0 REFERENCES

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APPENDIX A¾ GIS MAPS PER SECTION WITHIN THE PROJECT AREA

These data are stored within the City of Boulder project files.

APPENDIX B ¾ BREEDING SITE DESCRIPTIONS

South Boulder

1. 1S70W Section 20 Site 01

THP Open Space, site is a temporary wet meadow area, 2.0 acres in size with mixed sedge/grass meadow on infrequently grazed open space land. Water was stagnant and the level was never more than 6 inches deep during the season*. Water originally supplied by runoff from medium-sized pond approx. ¼ mile to the northwest near highway 93. The site contained only *C. pipiens* larvae on September 18 and 25 with an average of 2.1/dip. The site stopped breeding by October 1. *Note: The site was added to project area in early August and may have been breeding prior to that date but no data was collected.

2. 1S70W Section 18 Site 05

Shannahan Ridge Water Tank, site was temporary puddles with no vegetation associated surrounding the water tank structure. The site was 0.3 acres in size and the water source was most likely overflow/leakage from the water tank. Nonvector larvae were found on September 2 and *C. tarsalis* larvae were found on September 15. The site was added to the project area following a citizen concern call in early September. The site was completely dry for the season by September 22.

3. 1S70W Section 16 Site 01

Van Vleet, south of Highway 36, east of University Property, site was a temporary backwater marsh area directly connected to South Boulder Creek. The site filled with up to 6 inches of water when the creek flooded. The site was 0.1 acres in size with dominantly cattail vegetation. The field crew found only nonvector species (Aedes/Ochlerotatus) on May 22. The remainder of the season no larvae were found. The site became dry by mid-July and is probably only a good breeding site for floodwater and other spring mosquitoes.

4. 1S70W Section 16 Site 05

Rolling Rock Ranch, site was an infrequently grazed wet meadow that was dominantly canary grass. The 5.0 acre site had numerous braided flowing ditches and the site water level fluxed with the ditch level. Larvae were found in stagnant sections of the ditches and overflow areas. Nonvectors were found throughout the season (May 22, June 23, July 31, and September 25) and vector larvae was found on June 8, 19 and September 25.

5. 1S70W Section 16 Site 06

Rolling Rock Ranch, site was a small (0.1 acre) temporary ponded area in canary grass meadow. The site was fed by irrigation ditches adjacent. During the season, only one sample of larvae was found (*C. tarsalis* and Culiseta) on June 8. The site continued to be monitored and it became permanently dry mid to late September.

6. 1S70W Section 16 Sites 08 and 09

Rolling Rock Ranch, sites were both small (0.1-0.2 acre) ponds with cattail fringes. The sites had very steep banks and appeared manmade with no apparent inlet or outlet. Nonvector larvae (Culiseta) were found at Site 08 on June 19 and vector (*C. tarsalis*) on July 10. Site 09 only bred nonvector spring species at the end of May. Both sites were completely dry by mid-September.

7. 1S70W Section 16 Site 10

East of Rolling Rock Ranch, site was a set of permanent manmade drainage ponds that had majority open water surrounded by bands of cattails and other marsh vegetation on the perimeters. The individual ponds were grouped into a single site of 10.0 acres because of their proximity to one another and similar site characteristics. The ponds have mostly steep banks and abundant waterfowl. The breeding habitat was located at the southern edges of several of the ponds in very orange, stagnant water in tall cattails. Nonvector larvae were found in May, June,

and July. Only one sample of vector (*C. tarsalis*) was found during the season on July 31. The site edges became dry by mid September leaving only poor open water habitat.

8. 1S70W Section 16 Site 11

East of Rolling Rock Ranch, site was a temporary manmade drainage pond that contained some open water but mostly cattails and other grass/sedges. The site has slightly less steep sides compared to site 10 and was 4.0 acres in size. Along with Culiseta larvae, *C. tarsalis* was found at the site on May 22 and not again until June 24. The site became dry by mid July and did not become wet again.

9. 1S70W Section 16 Site 12

East of Rolling Rock Ranch, site had similar characteristics to Site 11 (above) and was 3.0 acres in size. Only found *Culiseta* larvae on May 22 and one unidentifiable Culex genus larva on July 10 (larva was physically damaged). The site was dry by mid-July and filled briefly at the end of September. Only nonvector larvae were found after the refill period.

10. 1S70W Section 16 Site 14

Fancher Open Space, site was a temporary wet meadow with majority canary grasses and sedges. There was a small, fish-bearing pond in the center and the northern end near South Boulder Creek contained stands of cottonwood trees. The site was 10.0 acres in size and most of the site was dry from late May on. Only one sample of *C. pipiens* was found on July 10 in the pools at the north end of the site under the cottonwoods. Water was mostly likely left from flooding on South Boulder Creek. No more larvae were found.

11. 1S70W Section 16 Site 15

Fancher Open Space, site was a steep elongated manmade ditch with cattails around borders. Site was 0.3 acres in size and had raised berms as site boundaries. Water was stagnant and never more than 12 inches deep during the season. Vector larvae were found one time during the season on July 31. All other larvae collected were non vector *Culex territans* and Aedes/Ochlerotatus genus.

12. 1S70W Section 16 Site 16

Van Vleet, south of Highway 36, east of South Boulder Creek, site is a temporary shallow marsh, 6.0 acres in size with majority cattails and some tall native grasses. The site extends from north to south adjacent to South Boulder Creek and is slightly sloped towards the creek. Water was stagnant and the level was never more than 6 inches deep during the season. The water at the site was probably left from creek overflow periods. Only one sample containing *C. tarsalis* was found at the site on June 20. The site continued to be monitored approx. every 10 days and was dry by the end of July.

13. 1S70W Section 16 Site 17

Van Vleet, south of Highway 36 along South Boulder Creek, site encompassed a section of South Boulder Creek and the adjacent canary grass wet meadow to the east. The site was 7.0 acres in size and larvae were only found in the canary grass meadow bordering the creek. Only one sample of vector larvae (*C. tarsalis* and *C. pipens*) were found on July 31. Only floodwater species found the remainder of the season.

14. 1S70W Section 10 Site 01

Van Vleet, north of Highway 36, southwest of Boulder Open Space Administration buildings, site was small (0.2 acres) wet meadow area in grazed field. Larvae were not found until August 8. Vector (*C. tarsalis* and *C. pipens*) were found and treated on August 8 and 13. The site was dry by August 25.

15. 1S70W Section 10 Site 2

Van Vleet, north of Highway 36, southwest of Boulder Open Space Administration buildings, site was small (0.4 acres) wet meadow/woodland area. The water in the site was from South Boulder Creek diversion ditch to feed irrigation ditches to the east. Nonvector larvae were found when site flooded in July and the only vector (one *C. tarsalis* larva) ever found was in the

drainage culvert in a temporary stagnant area on August 1. The site was dry except for the flowing ditch by end of August.

16. 1S70W Section 10 Site 03

Van Vleet, Junction of South Boulder Creek and Highway 36, site was a backwater cattail marsh (0.2 acres) adjacent to South Boulder Creek. The diversion structures in the area created the marsh. Only one sample was found at the site for the entire season, nonvector Culiseta at 0.1/dip on August 8. The site did not breed because of a very slow flow movement through the site.

17. 1S70W Section 10 Site 04

Van Vleet, South Boulder Creek between Highway 36 and South Boulder Road, this section of South Boulder Creek was flowing heavily during the season. The site was 3.0 acres in size and the only larvae found were nonvector Aedes/Ochlerotatus species at an average of 2.0/dip west of the barn in the center of the site in a small backwater pool. The larvae were found July 3, 13 and August 13.

18. 1S70W Section 10 Site 05

Van Vleet, north of Highway 36, south of South Boulder Road, northwest of Boulder Open Space Administration buildings, site was temporary grazed wet meadow hay field that was 2.0 acres in size with majority grass and one area of cattails at the southern end. The water in the site was from flood irrigation and never more than 3-4 inches deep. The only larvae ever found were nonvector (Culiseta), at less than 0.1/dip, in the cattail area, on August 13. The site was periodically flushed with fresh irrigation water, most likely keeping the vectors from breeding.

19. Van Vleet, north of Highway 36, south of South Boulder Road, northwest of Boulder Open Space Administration buildings, site was similar to Site 05 (see above) and separated from Site 05 by a low earthen berm. The site was entirely flood irrigated wet meadow. Vector and nonvector species were found as early as May 22 but, the flushing effect of the flood irrigation stopped any further breeding until August 1. The vector larvae were treated on a weekly basis from August 1-13. The flood irrigation ended and the site was dry by the end of August.

20. 1S70W Section 10 Site 07

Van Vleet, north of Highway 36, west of Cherryvale Road, south of South Boulder Road, west of Boulder Open Space Administration buildings, site was a temporary grazed flood irrigated wet meadow of 20 acres. The site could also be described as the Boulder Open Space Administration's "front yard". The water level was never more than 2-3 inches deep and the majority of the season the field had scattered soggy areas. Vector and nonvector species were found as early as May 22 but, the flushing effect of the flood irrigation stopped any further breeding until the end of July. Floodwater species were found at the end of July and beginning of September. Vector larvae (*C. tarsalis*) were found only once on August 13 at less than 0.1/dip. The site was dry by mid-September.

21. 1S70W Section 10 Site 09

Van Vleet, north of Highway 36, west of Cherryvale Road, south of South Boulder Road, west of Boulder Open Space Administration buildings, site is permanent drainage ditch along Cherryvale Road of 0.2 acres in size. Site contained majority cattails and in flooded conditions, it is connected to Site 07 (see above). The site contained only floodwater species until August 1 when a single *C. tarsalis* larva was found in a sample of other nonvectors. The site was dry by mid September.

22. 1S70W Section 10 Site 11

Van Vleet, south of Highway 36, west of Cherryvale Road, site was a temporary wet ungrazed meadow of 3.0 acres in size. The site is majority canary grass and sedges with a few cattails. Water in the site came from overflow from irrigation ditch that extended across the northern portion of the site. Nonvector larvae were found in May and vector larvae were found on June 14. No larvae were found for the remainder of the season. The site was dry by August 1.

23. 1S70W Section 10 Site 12

Van Vleet, south of Highway 36, west of Cherryvale Road, site is a temporary cattail dominant marsh area that extends into a fairly wide irrigation ditch. The site is 5.0 acres in size with water coming from ditch overflow. Nonvector were found at the end of May and vector found on June 14 and August 1. The site and ditch were dry by the end of August.

24. 1S70W Section 10 Site 13

Van Vleet, south of Highway 36, west of Cherryvale Road, site was a dominantly cattail marsh area along the main irrigation ditch of 0.3 acres in size. The site bred nonvector floodwater species in mid May, mid June and early August. The site only bred vector species once during the season on June 14. The site was dry by the end of August.

25. 1S70W Section 10 Site 15

Van Vleet, south of Highway 36, west of Cherryvale Road, site was a temporary wet meadow that was 5.0 acres in size. The site was filled with ditch overflow water in the spring and had a slow flow at that time. The site dried up by July 1 and filled again briefly in early August and then dried for the season by the end of August.

Only nonvector larvae were found at the end of May (Culiseta) and early August (floodwater).

26. 1S70W Section 10 Site 16

Van Vleet, south of Highway 36, west of Cherryvale Road, site was temporary wet meadow area that was 1.2 acres in size. The site was a low area in the field that ponded a small amount of water during the season. The site was dry until mid August when a sample containing *C. tarsalis* was found. The site was completely dry again by the end of August.

27. 1S70W Section 10 Site 20

Van Vleet, north of Highway 36, east of Cherryvale Road, site was a huge flood irrigated agriculture field of approximately 100 acres with several small low spots containing cattails (less than 1 percent of the total acreage). The water in the site was scattered and followed a loose conformity to the braided irrigation ditches throughout the site. The water in the site was never more than 3 to 4 inches deep. The site contained nonvector larvae in May, June and July. The only vector larvae were found on August 13 in the scattered cattail areas in very low abundance.

28. 1S70W Section 10 Site 21

Van Vleet, north of Highway 36, east of Cherryvale Road, site was a finger of flood irrigated agriculture field south of site 20 (see above) across a large flowing irrigation canal. The site had similar characteristics to site 20 and but was only 5.0 acres in size. The site bred nonvector larvae in May and August and vector (*C. tarsalis* and *C. pipens*) on August 1 and 25. The site was dry by mid September.

29. 1S70W Section 09 Site 01

Van Vleet, south of Highway 36, directly east of University property, site was a 12 acre flood irrigated pasture field that extended north to south along South Boulder Creek. The site had several irrigation ditches and a line of large cottonwood trees that contained woodland pools underneath. The pools seemed independent of irrigation schedules and the water had dried up by late July. Nonvector (Aedes/Ochlerotatus) larvae were found in late April, mid May, mid June, late June, and mid July. Vector larvae (*C. tarsalis*) were found starting on June 12 and then again on June 24 and July 10. All of the larvae during the season were located under the cottonwood trees in the extremely stagnant shallow pools and cattle hoof prints.

30. 1S70W Section 09 Site 02

Van Vleet, south of Highway 36, directly east of University property, site was a large (30 acre) flood irrigated pasture with three main irrigation ditches extending through the site north to south. The ditches all stayed flowing or completely dry throughout the season and the only problem areas were adjacent to Highway 36 along the north edge of the site. Water tended to collect and pool there creating a cattail dominant strip along the Highway. Nonvector floodwater species were found early in the season in scattered wet areas in the western half of the site but by

June the north edge had started breeding vector larvae in the cattails. *Culex tarsalis* were only found twice during the season on June 12 and 24. The site was dry by July 24.

31. 1S70W Section 09 Site 03

Van Vleet, south of Highway 36, directly east of University property, site was a temporary elongated cattail dominant marsh along the west side of South Boulder Creek. The site was 0.4 acres in size and contained runoff water from nearby flood irrigation. The site bred nonvector larvae in late April to late June. The vector species both *C. tarsalis* and *C. pipens*, appeared on June 12 and 24. The site went dry by the end of July.

32. 1S70W Section 09 Site 05

Van Vleet, north of Highway 36, south of Table Mesa Road at 55th Street, site was a large flood irrigated agricultural field of 20 acres. There were scattered low pocket areas with cattails throughout the site. The site bred nonvector larvae in late May, mid June, and mid July. *Culex tarsalis* appeared on June 25 and July 10 both in low abundance. The field was dry and cultivated by late July.

33. 1S70W Section 09 Site 07

Van Vleet, south of Highway 36, directly east of University property, site was a manmade holding pond with steep sides and barren gravel bottom. The site was 0.3 acres and contained numerous frogs and minnows. The site seemed to have no inlet but the outlet flowed into site 02 periodically when flooding occurred. Rainwater possibly filled the site during the season. Nonvector Aedes/Ochlerotatus larvae were found in late May and no other larvae were found the remainder of the season.

34. 1S70W Section 08 Site 01

Southern shore of Viele Lake, site was composed of scattered inlet pockets of cattail areas bordering southern shoreline of Viele Lake. The samples taken from the site contained nonvector and vector species with each collection. Vectors were found in shoreline pools and inlet areas June 10, 19, July 7, 21 and September 2. In early September there was construction in the site area that involved installation of drainage pipes. The construction left deeply incised tire ruts and open ditches in several areas in the south and southeast sections of the site that filled with rainwater and became stagnant. *Culex pipens* began breeding in these areas in early September and continued through end of September.

35. 1S70W Section 03 Site 01

Burke I Open Space, south of Baseline Road west of South Boulder Creek, site was temporary grassy wet meadow that contained a permanent pond in the southeast corner of the site. The site was 5.0 acres in size and the majority of the water was located along Baseline road in a low ditch area that was filled with cattails. The site bred nonvector in the spring (late April-mid June) and then dried up in mid July until early September. It bred one batch of floodwater larvae in mid September before drying up again for the season. The site only bred vector (*C. tarsalis*) on June 17.

36. 1S70W Section 03 Site 02

South Boulder Recreation Center, site was the southernmost in a set of manmade drainage ponds along 55th Street near the South Boulder Recreation Center. The site was the only one that contained open water with cattails around the perimeter. The water level was 1 to 3 feet and contained numerous frogs. The site bred nonvector in late April and both nonvector and vector on August 15. The vector were treated and there were no larvae found for the remainder of the season.

37. 1S70W Section 03 Site 04

South Boulder Recreation Center, site was northernmost manmade drainage pond near South Boulder Recreation Center. Site was majority cattail marsh and 6.0 acres in size. Site only bred floodwater larvae in early September.

38. 1S70W Section 03 Site 09

Burke I Open Space, corner of Baseline Road and Cherryvale Road, site was a small (0.3 acre) ditch area near the side of the road. Water level was never more then 1-3 inches deep. The vegetation was mostly willows with scattered cattails. Site only bred nonvector (Aedes/Ochlerotatus) larvae in late June. No larvae were found the remainder of the season.

39. 1S70W Section 03 Site 10

Burke I Open Space, northwest corner, south of Baseline Road, site was temporary grassy wet meadow that contained majority canary grass in the north and extended along the western boundary of the Burke Open Space in more a wooded area. The field was flood irrigated and the water tended to collect in the northern end of the site along Baseline Road. Nonvector larvae were found in early June, mid August and early September (floodwater species). The site was dry by mid September. No vector larvae found.

40. 1S70W Section 03 Site 12

Burke I Open Space (north) and Gebhard Open Space (south), west of Cherryvale Road, (Bobolink Trailhead fire access road), site was a flood irrigated wet meadow that curved through the southern half of Burke I and the northern ¼ of Gebhard. The site was 9.0 acres in size and majority cattail vegetation with some grasses and sedges. The water level varied throughout the site from 1 to 12 inches. There was also some flow movement in certain areas in the site. *Culex tarsalis* was found with nearly every sample taken from the site and they were always found near the center of the site. *Culex tarsalis* (along with other nonvector species) were found June 16, 27, July 11, and August 4. The site was mostly dry by end of August.

41. 1S70W Section 03 Site 15

Gebhard Open Space, along Cherryvale Road, site was a temporary cattail marsh that was created by overflow from nearby irrigation canal. The site was 0.6 acres in size and contained an average of 4 to 6 inches if water during the season. Nonvectors were found in spring with *C. tarsalis* appearing on June 27 and July 11. The breeding habitat in the site was excellent with nearby shaded harborage areas. There were numerous adults around the site, which were probable due to several ponds to the north that were on private property that were most likely breeding. The site did not dry up until mid August.

42. 1S70W Section 03 Site 16

Gebhard Open Space, along Cherryvale Road, site had very similar characteristics to site 15 (see above) except it was 1.0 acres in size. Nonvectors were found in early-mid June, mid July and early August. *Culex tarsalis* was found on June 16 and July 11 in very low numbers (less than 0.1/dip). The site was dry by mid August.

43. 1S70W Section 03 Site 19

Suitts Open Space, north of South Boulder Road, site was a small (0.2 acre) wet meadow area that had mixed cattails and grasses. Water on the site came from flood irrigation north of the site. The site was grazed by cattle and contained an above ground well structure. Larvae were only found twice during the season, August 4 and 13, and the samples contained both nonvector and vector (*C. tarsalis* and *C. pipens*). The larvae were found in the cattail area. The site was dry by mid September.

44. 1S70W Section 03 Site 20

Suitts Open Space, north of South Boulder Road, site was a small (0.2 acre) temporary marshy pond area that held up to 12 inches of water in the spring. The vegetation was dominated by cattails with numerous aquatic predators. The site bred nonvectors in late April and did not breed again until it had dried up leaving water filled hoof print habitat in early August. *Culex tarsalis* was found August 8 and never again for the remainder of the season. The site went dry by mid September.

45. 1S70W Section 03 Site 21

Suitts Open Space, north of South Boulder Road, site was a small (0.2 acre) temporary marshy ponded area that contained mainly grasses and was dry by mid June. The site only bred nonvector in the early spring.

46. 1S70W Section 03 Site 22

Suitts Open Space, north of South Boulder Road, site was a large (22.0 acre) flood irrigated hay field/pasture. The site held no more than 1-3 inches of water throughout the season when wet. Larvae (both nonvector and vector) were found twice during season on June 26 and August 14. The areas the larvae were found in were small and isolated patches in the site. The site was dry by mid September.

47. 1S70W Section 03 Site 24

Gebhard Open Space, directly north of South Boulder Road, site consisted of two temporary marsh ponds. The site was 0.5 acres in size, no more than 12 inches of water, and the vegetation was dominantly cattails. The water on the site came from a drainage pipe that emptied the flood irrigated field to the east. The site never bred vector species but consistently bred nonvectors such as, Aedes/Ochlerotatus and Culiseta larvae. The site was dry for the season by mid September following the ending of flood irrigation in the area.

48. 1S70W Section 03 Site 25

Burke I Open Space, directly south of Baseline Road along South Boulder Creek, site was temporary woodland cattail marsh of 0.3 acres that extended along the South Boulder Creek Trail near the Bobolink Trailhead. The site held stagnant water no more than 1 to 2 feet deep at the wettest time. The site dried completely during July and August and filled again at the end of the season. The site bred nonvector species consistently when wet but, vector (*C. tarsalis*) were found only on June 30.

49. 1S70W Section 02 Site 01

St. Walburga Open Space, west parcel, site was a 20 acre flood irrigated pasture/hayfield. The site was not observed to have been directly irrigated during the season but, received water from fields surrounding it. The site was dry until August when one sample of floodwater species were found at very low abundance. No other larvae were found the remainder of the season.

50. 1S70W Section 02 Site 07

Abbey Easement, southeast corner, site was a large flood irrigated agricultural field of 30 acres. The larvae were found only in a semi permanent ditch that extended along the southeast boundary of the Abbey and the Catholic School. The ditch site was dominantly cattails and held no more than 12 to 18 inches of water, the majority of the time only several inches. The site was added to the project area in August and bred nonvector consistently from August to the end of September. Vector (*C. tarsalis*) were only found on September 4.

51. 1N70W Section 33 Site 01

Hatch Open Space, site was an elongated drainage area/holding pond created in summer of 2003. The site had no outlet and several inlets. The site was added to the project area after public concern about standing water. The south half of the site stayed dry for the monitoring period (July 30 through October 7) but the northern section held standing water the entire period creating excellent vector breeding habitat. The site held an average of 6-8 inches of water, newly revegetated grasses, cattails and sapling trees, level shoreline, sandy substrate, and steep berms surrounding the site to protect from wind movement. The site bred mainly *C. tarsalis* and some *C. pipens* consistently from end of July to end of September. The vector larvae were completing their life cycle in 4 to 5 days in August and 5 to 6 days in September. The site stopped breeding by September 30.

52. 1N70W Section 34 Site 01

Burke II Open Space, site was a large flood irrigated pasture/hay field grazed by cattle with several active ditches flowing throughout. The site contained a permanent cattail marsh area in the northwest corner that was wet the entire season. The marsh contained excellent habitat but

was periodically flushed with fresh water from flowing canal to the north. The only larvae found were nonvector, Aedes/Ochlerotatus species in very low abundance (less than 0.5/dip average). The field was mostly dry by the end of July.

53. 1N70W Section 34 Site 02

Flatirons Golf Course, site was a naturally occurring temporary marshy area adjacent to a golf tee. The site held no more than 4 to 5 inches of stagnant water in the early summer but dried completely by mid June. The site bred nonvector floodwater species in late April and unidentifiable Culex species in late May.

54. 1N70W Section 34 Site 03

Flatirons Golf Course, site was a ditch along fairway #3. The site contained flowing water and several stagnant areas. Nonvector larvae were found once during the season at the end of July in the stagnant areas.

55. 1N70W Section 34 Site 17
1N70W Section 35 Site 01

Sombrero Marsh, western end (17 acres), eastern end (22 acres) combined 34 acre restored wetland area north of Baseline Reservoir. The combined sites consisted of near perfect mosquito breeding habitat and consistently bred many different species of mosquito during the season. The site was a shallow (12 to 14 inches at the deepest), level wetland with broad bands of cattail shallows encircling the perimeter. The site has no outlet and two inlets controlled by gates. The sites were inspected starting May 18 and vector species were found at every monitoring event weekly throughout the season. The last site inspection was October 15 with no larvae found.

56. 1N70W Section 29 Site 01

Goose Creek Greenway, west end, site was the western half of an approximately one mile long stretch of slow moving creek from Folsom Street in the west to Foothills Highway in the east. The site was 1.0 in size and contained newly revegetated banks and rockwork in the stream channel. The larvae were found in the main pond area at the western end and in small stagnant pools below the main pond. Vector larvae of both species were found with each sample in August and end of September. The site had stopped breeding by the end of September. The remainder of Goose Creek section did not breed mosquitoes.

57. 1N70W Section 28 Site 04

Valmont City Park Greenway, site was a permanent stream channel flowing southeast through Valmont City Park from Valmont Road to Pearl Street. The Park area was under construction/restoration in the beginning of the summer and the site was dry until August. The site contained revegetated native grasses and wildflowers and scattered cattails. The stream was very slow moving and overgrown with algae and other aquatic plants. The only larvae samples found contained *C. tarsalis* and other nonvector species. The vectors were always found in tiny stagnant areas near bridges of in pond areas along the stream in very low abundance (less than 0.1/dip). The site stopped breeding by end of September

58. 1N70W Section 27 Sites 02 and 03

Colorado Open Lands, south of KOA Lake, sites were low profile stream channels, north of bike path bridge, with grassy wetland borders created by overflow that drained KOA Lake to the south. The site were 3.0 acres in size each and held no more than 12 inches of water during the season. In the spring, the flooded sites did not breed but, as the season progressed and the site dried small stagnant pools were created. The habitat seemed good but, site 02 only bred nonvector species in late August.

59. 1N70W Section 27 Sites 04 and 05

Colorado Open Lands, south of KOA Lake, sites were low profile stream channels, south of the bike path bridge, with grassy wetland borders created by overflow. The sites were 13 acres total in size and contained scattered patches of cattails near the south ends. Both sites bred nonvectors consistently throughout the season in selected pools in the northern and southern ends

away from the flowing water. The only vector larvae found were *C. pipens* on August 11 in small pools at the southern end of site 05.

60. 1N70W Section 27 Site 07

Stazio Ballfields, site was a semi-permanent cattail marsh north of the playing field. The site contained a small flowing ditch through the center of the site that dissipated to the north. The site contained no more than 6 to 8 inches of water during the season and progressively dried as the season went on. The site bred both vector and a variety of nonvector species consistently during the season with *C. tarsalis* May through September and *C. pipens* starting in August. The site was mostly dry by the end of September.

North Boulder

1. 2N70W Section 33 Site 02

Axelson Open Space, site was a chain of open water ponds and associated wetlands connected by a culvert system. The site vegetation was dominantly cattails with some flood irrigated pasture grasses. The site was 4.5 acres in size and the majority of the site had a slow eastern flow. The site was added to the project area in mid August and only monitored from August 15 to October 1. The site bred *C. tarsalis* and other nonvector larvae species on August 15 and 28 in backwater nonflowing areas of the site.

2. 2N70W Section 34 Site 02

Coot Lake, site consisted of a permanent 23 acre wetland area directly to the west of the actual open water lake. Due to Coot Lake's barren shores and open water that creates wave motion, there was no breeding habitat within the lake. The larval monitoring was focused on the wetland area to the west. The site contained numerous small connected trout ponds and bordering cattail marshes. The only larvae found were nonvector and only in isolated areas away from the fish bearing waters. The site seemed to be excellent mosquito breeding habitat but, it was observed that the site had a slow flow throughout most of the area circulating fresh water.

3. 1N71W Section 01 Site 01

Mann Open Space, site was an isolated 2.0 acre temporary cattail marsh near a culvert under Highway 7. The majority of the site was dry for the season except in and near the culvert. The water level in the site was never over 1 to 3 inches. In the spring only nonvector (Culiseta and floodwater) species were found. There was a brief flood event in early September that caused more floodwater breeding. *Culex tarsalis* was found on July 8 and *C. pipiens* was found on September 23 several weeks after the brief flooding event.

4. 1N70W Section 01 Site 02

Canino Open Space, site was a temporary cattail drainage area in a pasture field near Mineral Road. Most of the site was on a sloped hillside. The water in the site came from a stream drainage to the southeast. The water level in the site was very low during the season, never more than several inches. The problem area in the site was numerous deep hoof prints left in the mud from grazing cattle. The prints filled with water and became stagnant creating perfect breeding habitat for mosquitoes. The site has a strong flow in the spring and did not breed until early June. Nonvectors and vector species (*C. tarsalis* and *C. pipens*) were found on June 18 and after the September rains on the 9, 17, and 24. The site continued to breed only nonvector Culiseta into October.

5. 1N70W Section 03 Site 05

Boulder Reservoir, east side, site was a 2.0 acre flowing drainage/ditch system that extended almost from Coot Lake south for ½ mile. The site vegetation was dominantly cattail. Nonvector Culiseta larvae were found three times in August and September in a tiny area near a culvert at the trail. Vector (*C. tarsalis*) was found only once during the season on August 29.

6. 1N70W Section 03 Site 06

Boulder Reservoir, 63rd and Foothills, site was a permanent cattail drainage extending from Foothills Highway west towards the Boulder Reservoir directly south of the Boulder Water Lab facility. The site had one main channel with numerous sink holes and side ditches. The main channel had a slow flow and some larvae were found in the shallow cattail sides but the main persistent problem area was an area of grassy wet sink holes to the south of the main channel. The holes breed extremely high numbers of mosquito larvae (15 to 20+/dip), most of which were *C. tarsalis*. The site breed only nonvector Aedes/Ochlerotatus species in early spring but *C. tarsalis* larvae were found on a consistent basis starting June 15 until the end of September. The site finally stopped breeding vectors in early October.

7. 1N70W Section 04 Site 01

Boulder Reservoir, Model Airplane Airport, site was a large cattail floodplain area 80 acres in size. The majority of the water in the site came in from the north through one main drainage (see Axelson Open Space above). The majority of the site stayed dry during the season except an area bordering the model airplane airstrip. The problem area consisted of approximately 0.5 acres of tall grasses with old tire ruts. The ruts held water that was several inches deep and bred mosquito larva throughout the season. Nonvector and both vector species were found on July 19, 31, August 19, September 8, 24, and October 1.

8. 1N70W Section 04 Site 03

Boulder Reservoir, west side, directly north of main entrance, site was a cattail wetland of 20 acres in size. The site had a small flowing ditch through the center that spilled overflow water 1 to 4 inches deep throughout the site. The site consistently bred both nonvector and both vector species throughout the season starting in mid June and ending in late September. The problem areas in the site were along the shoreline of the Reservoir in the shallow sedge/cattail marshy areas and in the cattails near the culvert at the road. The upland area surrounding the site was mowed in late August to reduce adult harborage locations.

9. 1N70W Section 04 Site 06

Boulder Reservoir, west side, directly north of site 03, site was a cattail wetland with similar physical characteristics to site 03. Nonvector species were found on July 18 and 31 and the site only bred vector (*C. tarsalis*) one time during the season on July 18 along the shoreline sedge/cattails. The site was mostly dry the latter half of the season with water only in flowing ditch through the center of the site.

10. 1N70W Section 11 Site 01

Twin Lakes, north, site was a 18 acre flood irrigated field. The site vegetation was majority grasses but, there was a cattail ditch that extended from west to east along the northern boundary of the site. A variety of nonvector species and vector (*C. tarsalis*) larvae were found as early as May 15. The site continued to breed vector until it became dry in mid July. The tire ruts at the western end became wet for a brief period at the end of August but, only bred nonvector floodwater and other Aedes/Ochlerotatus species.

11. 1N70W Section 11 Site 02

Twin Lakes, east, site was a cattail ditch extending south to north along the eastern side of Twin Lakes 3.0 acres in size. The water in the site was deep in some areas (up to 3 feet) in the north. The site was added to the project area in mid June and only nonvector (Culiseta, Aedes/Ochlerotatus) larvae were found in June and July. The larvae were always found at the north end due to flowing water in the south end of the site.

12. 1N70W Section 08 Site 01

Papini City Park (North Central Boulder), site consisted of a flood irrigated field with a chain of small ponds through the center. The site was partly wooded and was 11 acres total. The site bred nonvector floodwater species in the spring and an isolated breeding of Aedes/Ochlerotatus species at the end of August. The site has a majority of poor habitat due to flowing water and open water ponds with fish and other aquatic predators.

The site has good adult harborage and one of the permanent light traps was place at the site. The trap found vector species throughout the season but, no vector larvae were found in a ¼ mile radius of the trap. The conclusion that was made was that the adult were traveling from over a ¼ mile away to use the shaded harborage areas around the trap.

13. 1N70W Section 08 Site 02

Papini City Park (North Central Boulder), site was a long winding mostly flowing irrigation drainage ditch connected to site 01 (see above). The site was 0.5 acres total and only bred nonvector Aedes/Ochlerotatus species.

14. 1N70W Section 08 Site 03

Hendrickson Open Space, site was a cattail wetland drainage area that extended west to east through the east side of the parcel. The site was added to the project area in mid July because of WNV positive found in light trap in Papini City Park directly to the north. The site contained stagnant water 3 to 6 inches deep. Nonvector larvae were found on July 16, August 15, and September 25. The only vector larvae found during the season were *C. tarsalis* found on August 15.

15. 1N70W Section 08 Site 06

Blip II Open Space, site was an open water fish-bearing pond with cattail fringes in a chain a ponds along a wide drainage valley. The site was added to the project area in mid July because of WNV positive found in light trap in Papini City Park to the northeast. Nonvector and vector (*C. tarsalis*) species were found on July 16 in the cattails at the western end. No larvae were found again until September 26 (nonvector).

16. 1N70W Section 09 Site 03

Gallegher Open Space, site was a small cattail dominant pond and grassy overflow area below. The site was 1.5 acres and added to the project area in mid July because of WNV positive found in light trap in Papini City Park directly to the west. The site was created by an leakage of water from a nearby heavily flowing irrigation ditch. Vector larvae were found immediately and consistently each week from July 17 until September 23 when the site dried up.

17. 1N70W Section 09 Site 04

Gallegher Open Space, site was a large (80 acre) flood irrigated agriculture field. The water moved from west to east through the site and it was added to the project area in mid July because of WNV positive found in light trap in Papini City Park directly to the west. The only larvae found on the site was a sample containing 20+/dip of nonvector and vector (*C. tarsalis*) on July 17. The site went completely dry several days later and never became wet again.

18. 1N70W Section 13 Sites 07, 08, and 09

Kolb Open Space, sites all had similar physical/vegetative characteristics. All sites were slowly flowing shallow irrigation ditches overgrown with tall canary grass and other grasses. Site 07 had shorter grasses because of active livestock grazing. The sites totaled 20 acres in size and were added to the project area in early August due to increasing concern from the wastewater treatment plant to the west. Site 07 consistently bred both nonvector and vector larvae weekly from August 8 to September 22. Site 08 bred nonvector and vector only in August and site 09 never bred vector larvae species only floodwater species.

19. 1N70W Section 15 Site 02

Andrus Open Space, north, site was a 15 acre flood irrigated field with grasses, thistle and scattered cattails. The site was situated in a V-shaped plain and the site water came from a drainage flowing through the center. The site had very little water with most coming from rainfall when it occurred. The site was added to the project area at the end of July due to increasing WNV concern. The site only bred vector (*C. tarsalis*) once during the season on August 21. It bred only nonvector species at the beginning and end of September before it became dry for the season.

20. 1N70W Section 16 Site 03

Louisberg Open Space, site was an open water stock pond with tall cattail fringes and thick duckweed surface coverage. The site contained numerous frogs and other aquatic predators. The site was 1.2 acres in size and was added to the project area in early August due to WNV concern. Larvae were only found once during the season on August 25, both nonvector and vector (*C. tarsalis and C. pipens*).

21. 1N70W Section 17 Site 02

Palo Park, site was an elongated area of 30 acres that has several different habitat types. The western end was Wonderland Creek with sandy deposits, grassy side banks and scattered cattails. The western end tended to created flooded areas adjacent to the creek in the spring. The center was the actual playground and turf fields. The area was only wet in spring flood periods. The northeastern end contained woodland pools and slowing flowing creek. There were several ponds that did not breed due to fish. The southeast end is lowland marsh that was fed from creek overflow and seepage. The site bred nonvector mostly on the eastern end and one occurrence of vector (*C. pipens*) on August 13 in the backwater of the creek at the eastern end of the site.

22. 1N70W Section 19 Site 03

Parkside Park, site was a cattail drainage area bisecting the park. The site was 0.2 acres in size and added to the project area in early August due to increasing residential WNV concerns. The site consistently bred both nonvector and vector (*C. pipens*) larvae weekly. Most likely the site would have been much wetter throughout the drainage channel earlier in the season but, by August all the water had pooled near the culvert under Juniper Road. The site had stopped breeding by early October.

23. 1N70W Section 13 Site 02

Wonderland Lake, site consisted of the west, south and north lake margins totaling 5.0 acres in size. The vegetation was mostly cattails. Larvae were only found twice during the season, in June and July. Vector (*C. tarsalis*) larvae was found on June 16 in the northern shore cattails. By mid August the lake level had dropped leaving barren exposed shoreline, which is extremely poor breeding habitat for mosquitoes.

24. 1N70W Section 13 Site 03

Wonderland Lake, site was the west to east deeply incised drainage from Wonderland Lake. The site was mostly flowing during the season but slowed at the eastern end into a small pond area. A sample of nonvector and vector (*C. pipens*) larvae were found near the pond area in cattails on August 22. No other larvae were found the remainder of the season.

25. 1N70W Section 20 Site 03

Elmer's Two Mile Park, site was a section of the Goose Creek Greenway extending north to south through the Park. The site was 2.0 acres in size and was mostly flowing during the season. The vegetation associated was water cress, some cattails and other aquatic plants. The problem area was at the north end near the culvert under Iris Street. The site flow slowed and created stagnant side pools where larvae could breed. The site breed both nonvector and vector fairly consistently from mid July to late August. At the end of August the Park facility mowed the edges of the creek and removed the aquatic growth in the stream channel allowing unrestricted flow of the water. The action along with site drying ceased the mosquito breeding.

26. 1N70W Section 21 Site 01

Christenson Park, site was a small (0.5 acre) cattail drainage in Christenson Park. The site was flowing for the first part of the season until mid July when it dried and created stagnant pools near the culvert at the north end. Larvae were found twice in the culvert pool both nonvector and vector (*C. pipens*) each time.

27. 1N70W Section 22 Site 02

Colorado Open Lands, site was a cattail marsh, 4.0 acres in size. The site water was extremely stagnant and averaged 6 to 8 inches in the wettest part of the season. The site bred only nonvector larvae in the spring and started breeding vector on June 6. The site continued to breed

both nonvector and vector species throughout the season until the end of July when only nonvector bred again.

28. 1N70W Section 22 Site 04

Cottonwood Farms, site was a northern lake margin of unnamed pond. The vegetation was dominantly cattails and the site was 1.0 acres in size. The site did not breed in the spring and was mostly dry in July and August. The site did breed both nonvector and vector (*C. pipens*) in late August and early September at very low numbers.

29. 1N70W Section 22 Site 06

Cottonwood Farms, site was a tiny (0.1 acres) lowland cattail marsh area. The site only bred once (nonvector) during the season in early May.

30. 1N70W Section 22 Site 10

Short-Milne Open Space, site was a broad cattail marsh island between two branches of the creek. The site was 4.0 acres in size and the field crew was not able to access the site until early July when the water level in the creek dropped. The only larvae ever found at the site were floodwater species in the northeast corner on September 11 after a rain event. The site was dry by September 17.

31. 1N70W Section 22 Site 12

Andrus Open Space, south, site was part cattail wetland and part flood irrigated field of 10.0 acres in size. The site was added to the project area in late July due to increased concern over WNV. The only larvae (both nonvector and vector, *C. tarsalis*) found during the season were near the eastern end of the site in the flooded grasses near the road. The water in the site came from the west and flowed out into the field pooling and becoming stagnant in the east near the road.

APPENDIX C¾ FIGURES AND TABLES

(Appendix C is included as a separate document.)