MOSQUITO POPULATIONS IN THE POWDER RIVER BASIN, WYOMING: A COMPARISON OF NATURAL, AGRICULTURAL AND EFFLUENT COAL BED NATURAL GAS AQUATIC HABITATS

By

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ABSTRACT

Coal bed natural gas development in northeastern Wyoming has increased surface water in ranching and agricultural areas over undeveloped land. This increase of water increases larval habitat for mosquitoes, potentially increasing adult populations of West Nile virus vector mosquitoes. I compared adult and larval mosquito populations in four different habitat types in the Powder River basin including agricultural, natural, CBNG and upland sagebrush steppe.

Adult mosquitoes were sampled weekly (2004) or bi-weekly (2005) using CDC miniature black-light traps baited with dry ice. A fixed-effect mixed model indicated that in a normal rainfall year (2005) mature CBNG ponds had the highest adult mosquito populations of all sites sampled, and the highest population of the WNV vector *Culex tarsalis*. In a drought year (2004) where total rainfall from May – August was 59% of the seasonal average, agricultural areas had the highest mosquito abundance, likely due to increased irrigation. Adult *Culex tarsalis* tested positive for WNV across the PRB in 2004 and 2005, with highest minimum infection rates in those areas with large *Culex tarsalis* populations.

Larval mosquitoes were sampled bi-weekly from 13 May - 24 August 2005, using a 350 ml dipper in a 20 point vegetated transect along the pond perimeter. Pond vegetation characteristics were recorded between 3 and 17 August including vegetation density, type and class. Larval *Culex tarsalis* were the most abundant mosquito in the region, representing 47.7% of the total sampled population. A fixed-effects mixed model found *Culex tarsalis* produced at similar rates in natural, new, old and outlet CBNG sources; irrigated agriculture produced significantly less ($P \leq 0.02$) *Culex tarsalis* in 2005. New and old CBNG ponds and outlets also produced *Culex tarsalis* over a longer period of time than natural or irrigated agricultural sites.

This study indicates that CBNG ponds are significantly increasing the overall population of vector mosquitoes in the PRB, as well as adding to the duration of larval habitats that would normally be ephemeral. Thus CBNG ponds and associated habitats enhance mosquito abundance and may serve to increase pathogen transmission in an otherwise arid ecosystem.

CHAPTER 1

REVIEW OF RELAVENT LITERATURE

Introduction

The Powder River basin (PRB) includes the Powder River and its tributaries in northeast Wyoming and southeastern Montana. This area reaches east from Gillette, Wyoming, west to the Bighorn Mountains, and north to Miles City, Montana (Environmental Protection Agency 2006) (Figure 1). The PRB is in a semi-arid climate dominated by sagebrush grassland which is primarily used for grazing and wildlife habitat. The dominant shrubs in this system are Wyoming big sagebrush, *Artemisia tridentata* ssp. *wyomingensis* Beetle and Young, and silver sagebrush, *A. cana* Pursh. Smaller patches of native short grass prairie, conifer forest, greasewoods, riparian woodlands and non-native grasses are common throughout the region (Hemstrom et al. 2002, Walker et al. 2004).

Historically, the major industries in the Powder River basin include cattle ranching and coal mining. These industries have now expanded to include coal bed natural gas (CBNG) production (formerly termed coal bed methane) which extracts natural gas from sub-surface coal seams. Fifteen surface coal mines are located around Gillette, Wyoming, and several large sub-surface coal seams extend west from Gillette toward the Bighorn Mountains (Vicklund 2000). These coal seams contain large amounts of natural gas (61 Tcf: trillion cubic feet; a volume measurement of natural gas),

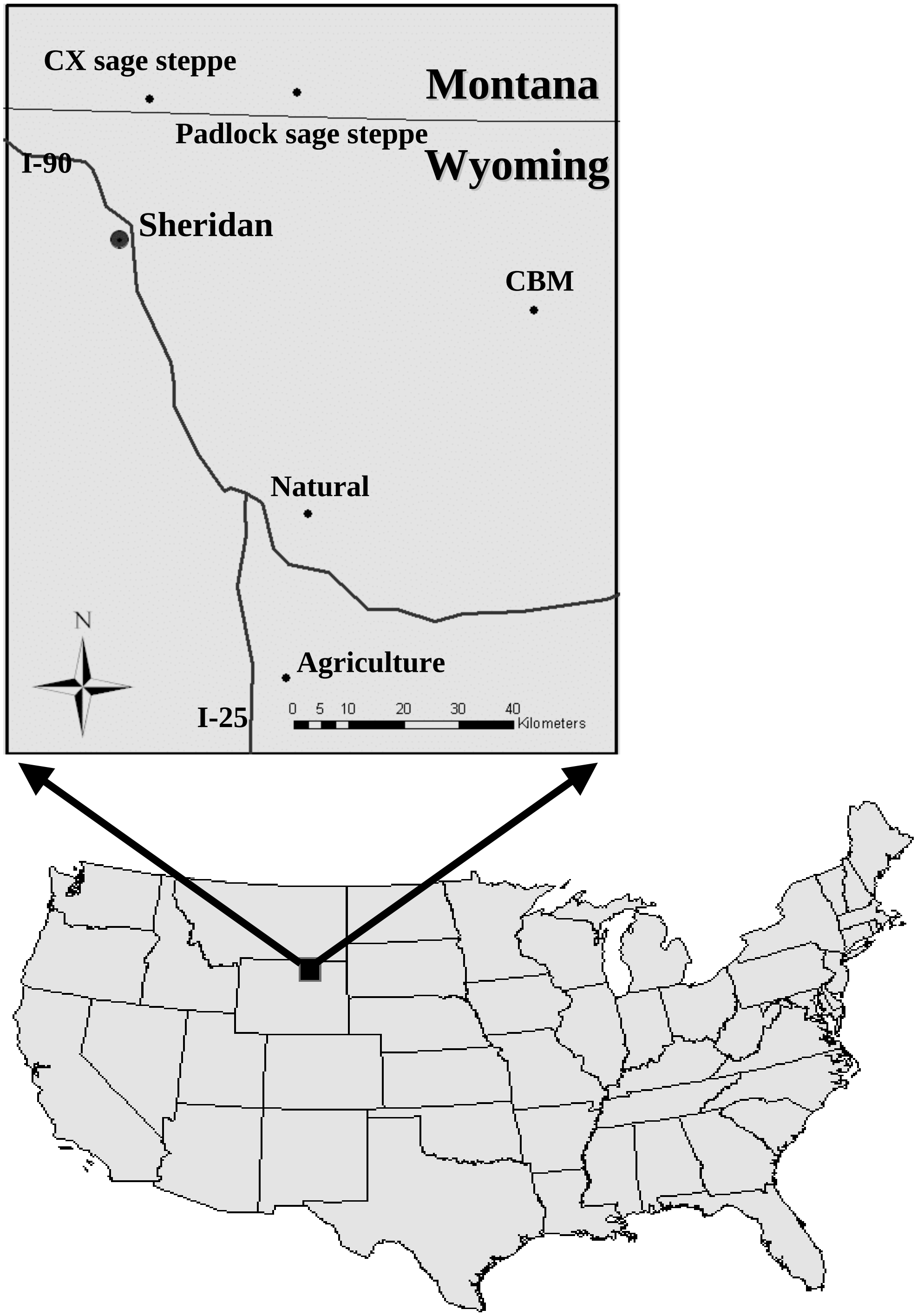

Figure 1. Study site locations for adult mosquito trapping in 2004 and 2005 within the Powder River basin of Wyoming and Montana

which is currently being extracted for commercial use by the natural gas industry at the rate of 23,304,764 m^3 per day (DOE 2002). Methane extraction includes the removal of groundwater from a coal seam to allow confined natural gas to flow in sub-surface voids. This effluent water is discharged into existing stock ponds, newly constructed ponds, or surface drainages that do not continue in to larger water bodies (Clark et al. 2001). Since 1999, an estimated 19,000 CBNG well heads have been constructed in the PRB, with 20,000 more projected in the next ten years, each of which will produce discharge water that will potentially be held in additional CBNG ponds (DOE 2002). A recent GIS study on mosquito habitat in the PRB indicates that CBNG development has increased potential mosquito larval habitat by 75.2% from 1999 to 2004 (Zou et al. 2006). This corresponds with a recent land-use change study in the same region that indicates a 9-fold increase in surface water in ranching areas, and a 2-fold increase in surface water in agricultural zones (Naugle et al. unpublished data).

Concerns have been expressed by the public as well as local natural resource agencies regarding the environmental impacts of CBNG, including surface disturbances from roads, wells, power lines and ponds; dewatering of local aquifers, and methane discharge water quality (Regele and Stark 2000). While these ponds do provide water to native wildlife and habitat for migratory duck species, CBNG ponds have the potential to produce mosquitoes that could transmit pathogens such as West Nile virus (WNV). An increase in mosquitoes and pathogen transmission in the PRB could pose a health risk to humans and livestock in the region, as well as native wildlife populations. This research project was initiated to monitor WNV in 2003 after the first sage-grouse was detected with WNV in Northeastern Wyoming. My objectives were to assess adult and larval mosquito population trends as well as the impacts of CBNG development on mosquito production in areas of sage-grouse use in the PRB.

West Nile Virus

Historical Distribution.

West Nile virus is an encephalitic virus and a member of the Japanese encephalitis group in the genus *Flavivirus*, family *Flaviviridae*. This pathogen is closely related to both eastern equine encephalitis (EEE) and Saint Louis encephalitis (SLE), which are endemic to North America. West Nile virus was first isolated from a febrile woman near the Nile River in Uganda in 1937, and has since caused large human epidemics in Africa, Europe and Asia (Smithburn et al. 1940, Baqar et al. 1993). Human outbreaks of WNV have been documented in southern France in 1962, southern Russia in 1963, Belarus in 1977, the Ukraine in 1985, Romania in 1996, Czechland in 1997 and again in Russia in 1999 (Hubalek and Halouzka 1999). These outbreaks have been geographically associated with wetlands and flooding from heavy rains and are more likely to occur in the summers of warm, wet years.

Eurasian and African outbreaks of WNV have been closely associated with bird-feeding mosquitoes. The virus has been isolated from 43 Old World species of mosquitoes in the genera *Culex* and *Aedes* including the trans-Atlantic species *Cx. pipiens* L. and *Ae. aegypti* L. (Hubalek and Halouzka 1999). The primary Old World vectors of WNV include *Cx. univittatus* Theobald in Africa, *Cx. modestus* Kamimura and Wada and *Cx. pipiens* in Europe, and *Cx. quinquefasciatus* Say in Asia (Hayes 2005). Further research has shown that WNV is enzootic in wild birds migrating between Africa and Asia, and these animals are considered the primary vertebrate hosts for this disease in the Old World (Hayes 1989). Mammals, reptiles and amphibians do not play a large role in maintaining Old World transmission cycles; although, horses, lemurs and frogs have been shown to obtain transmissible infection rates in the laboratory (Rodhain et al. 1985).

North American Distribution.

West Nile virus was first detected in the Western Hemisphere in New York City in the summer of 1999. That year, there were 62 human infections around the New York City area, and 7 deaths. Since its introduction to North America, WNV has spread westward across the United States, as well as into Canada, Mexico and parts of the Caribbean (Rochrig et al. 2002).

It is unknown how WNV was introduced into the U.S in the initial 1999 outbreak of WNV in New York City. Speculation regarding WNV transmission to NYC include movement of infected mosquitoes via air transportation, illegal importation of exotic birds, lost avian migrants and possible terrorist acts. Biologists confirmed mortalities due to WNV infections in 18 species of native and non-native birds in 1999 including more than 3,000 American crows (*Corvus brachyrhynchos* Brehn). It was anticipated that the corvids would be the most vulnerable family of birds to the virus as they were highly susceptible to WNV infection via mosquito bite, and had mortality rates >40% (Hayes 2005, Steele et al. 2000). Since 1999 WNV has spread at a rate of approximately 67 km per month throughout the spring and summer, and now has been found in 284 species of North American birds (Rappole and Hubalek 2003, CDC 2006). West Nile virus's rapid rate of spreading and annual reoccurrence in native biota indicates that this pathogen likely will remain an enzootic disease in our environment for the foreseeable future.

As of the September 2006, there have been 21,340 human cases of WNV in the United States, with 837 fatalities (CDC 2006). Of the reported human WNV cases in the U.S., 71% were neuroinvasive, 28% were uncomplicated West Nile fever, and 6.8% were fatal (n = 4,146). The median age for fatal cases in the U.S. is 77.5 years, with the fatality to case ratio increasing significantly with age. The risk of WNV is also significantly higher in males among middle aged (>40 years) and elderly individuals, with the fatality to case ratio 1.3 times higher for men > 70 years old (O'Leary et al. 2004).

There are several methods used for detecting WNV in the environment by monitoring agencies in the United States. These methods include pooling collected adult mosquitoes for virus detection, collecting dead birds, drawing and testing of sentinel chicken blood for antibodies indicating exposure to WNV, and testing non-human mammal serum for WNV antibodies (primarily equine) (Morris et al. 1994). In 2002, 72% of primary detections were from virus-infected dead birds, 18% were from non-human mammals, 6% were from infected mosquitoes, and 2% were from sentinel birds (O'Leary et al. 2004). While it seems that dead bird surveillance is the most effective monitoring technique for WNV surveillance, this technique is more effective in densely populated areas where dead birds are noticed and reported to the proper authorities. In rural areas, methods such as mosquito monitoring and sentinel chickens are the most effective methods for disease monitoring. Dead bird surveillance may become a less effective form of virus monitoring in the future if native bird species acquire immunity to WNV through repeated exposures.

Northeastern Wyoming Distribution.

West Nile virus was first detected in Wyoming three years after it was found in NYC. The first documented case of WNV in Wyoming was on 18 August 2002 in a horse in Goshen County. This case, along with two humans and 95 other horse cases, were documented in the fall of 2002 (Wyoming Department of Health 2006). In 2003 a major outbreak of WNV occurred throughout the western United States including Wyoming, Montana and Colorado. That season Wyoming had a total of 393 human and 230 horse cases, with 10 human fatalities (Table 1).

On 24 July 2003 WNV was detected in a radio-collared greater sage-grouse, *Centrocercus urophasianus urophasianus* Aldrich, hen on the Montana/ Wyoming border. That summer a total of 18 sage-grouse died from WNV between four radio-marked populations in the western US and southern Canada, creating a 25% average decline in survival for this time period (Naugle et al. 2004). Late-summer survival of sage-grouse in the northern Powder River Basin was markedly lower at 1 site with confirmed WNV mortalities (20% survival) than at 2 sites without (76% survival) (Walker et al. 2004). Moreover, declines in male and female lek attendance at the WNV site in spring 2004 indicated that outbreaks have threatened local populations with extirpation (Walker et al. 2004). In 2004 WNV spread to sage grouse populations in Colorado and California, and female survival in late summer was 10% lower at 4 sites with confirmed WNV mortalities (86% survival) than at 8 sites without (96%). West Nile virus mortality decreased to 2% during the cool summer of 2005 (x = 67°F), increased again in 2006 when hot temperatures (x = 71ºF) returned in 2006 (D. Naugle, University of Montana, unpublished data).

Wildlife Susceptibility to West Nile virus

Historically, the impact of emerging diseases on wildlife populations has not taken much notice: however, attention has been elevated around WNV outbreaks in wildlife populations because of its potential threat to human health. While we do not know how WNV broke into the Western Hemisphere, we know that wildlife disease emergences historically are amplified by changes in host pathogens, ecology or the environment (Daszak et al. 2000). Often times these changes introduce diseases to naive hosts who have no natural resistance to the said virus. In the case of WNV, almost all of our North American wildlife fauna was naïve to infection, and it is unknow which species will acquire resistance through immune response (ie. antibody production), which will become amplifying hosts to the pathogen, and which will remain susceptible.

Clinical Symptoms in Wildlife.

West Nile virus is an encephalitic pathogen that affects the brain and neural tissues, causing bleeding, fever and cell death in infected animals. In general, birds are more susceptible to this virus. Symptoms of this disease in birds include weight loss, head tremors, blindness, ataxia,weakness in the legs and seizures. Birds that survive a WNV infection may have neural damage as well as damage to the pancreas, kidney and heart (Steele et al. 2000). Detection of WNV in avian carcasses can be done through necropsies of organ tissues and oral and cloacal swabs followed by PCR to detect WNV (Komar et al. 2002). WNV has also been found in ovarian and testicular tissues in birds, suggesting that infected adults may be able to vertically pass an infection to their offspring (Komar et al. 2003).

Avian Susceptibility.

While many different species of birds have been found to be infected with WNV, only those that have high viremias can be considered amplifying hosts for the virus. Birds are the only amplifying host for this pathogen in the Western Hemisphere. In order for a feeding mosquito to become infected, a bird must have a viremic titer of at least $10^{7.1}$ PFU/ml (Komar et al. 2003). Birds that have been challenged with WNV in the laboratory, and have reached sufficient titers to serve as an amplifying host include Passeriformes (perching birds), Charadriiformes (wading shore birds), Strigiformes (owls) and Falconiformes (diurnal birds of prey) (Molaei et al. 2006). Birds able to sustain high viremic levels have a high susceptibility to the disease. A high mean infectiousness was ranked for reservoir competence by Komar et al. (2003). They found that the blue jay (*Cyanocitta cristata* L.), common grackle (*Quiscalus quiscula* L.), house finch (*Carpodacus mexicanus* Muller) and American crow were the top four species of 25 tested as competent reservoirs for WNV in southern California. Of these birds, the blue jay and American crow were found to transmit virus between infected animals and non-exposed cage mates through fecal and salivary secretions with a cage transmission rate of 1.0 (on a 0 – 1 scale) for both species. This may have contributed to the high infection rate and mortality seen in the field since both of these species of birds have social or semi-social behaviors. Young altricial birds may also be more exposed to mosquito feeding due to incomplete feather covering and immobility. Colonial species, such as the American white pelican *Pelecanus erythrorhynchos* Linnaeus, may occupy habitats near mosquito production areas, which increases exposure to juvenile birds, and may concentrate the mosquito-avian amplification cycle in some areas (Rocke et al. 2005).

Sage-grouse infected with WNV show symptoms similar to other avian groups. Radio-marked grouse rarely move more than a few meters 2 days before death and have a weak flight when flushed (Walker et al. 2004). Intact sage-grouse that died from WNV were often found facedown in good condition with no external signs of trauma. Infected grouse may also be at elevated risks of predation, potentially contributing in 2004 and 2005 to a reduced survival rate. A total of 363 sera samples were taken from wild grouse across Wyoming, Montana and Alberta in 2004 and none tested positive for WNV antibodies, indicating that these birds at that time had not developed an immune response to this pathogen (Naugle et al. 2005).

Mammal Susceptibility.

Equines, as well as several other domestic animals have exhibited WNV symptoms. These symptoms include symmetrical or asymmetrical ataxia, staggering, stumbling, toe dragging, leaning and wide-based stance (McLean et al. 2002). The strain of WNV that occurs in North America is particularly virulent in horses, causing a clinical infection rate of 42% in seropositive animals and a death rate of 36% in those animals with clinical symptoms (Bunning et al. 2002). A vaccine is available to protect equines from WNV, and its use has greatly reduced the WNV morbidity and mortality. Other mammals that have been experimentally tested for WNV infections include dogs, cats, cattle, sheep, chickens, turkeys, domestic geese, pigs and goats. None of these animals, including horses, has been found to carry a virus titer high enough for them to serve as amplifying hosts to the New York strain of WNV (Bunning et al. 2002, Austgen et al. 2004, McLean et al. 2002). Many of these animals, including house pets such as dogs and cats, have been found to develop antibodies to this disease, and occasionally mild symptoms such as lethargy and a loss of appetite occur. These symptoms are not debilitating and may go unnoticed by the caregiver (Austgen et al. 2004).

Most wild mammals in the New World appear to be resistant to WNV. Some species including several lagomorphs carry high viremias without showing clinical symptoms, indicating they may serve as reservoir hosts within their range. The majority ff those mammals that have been challenged with WNV in the laboratory do not get viremias higher than $10^{7.1}$ PFU/ml, which is the level required for transmitting virus to a feeding mosquito (Bunning et al. 2002, Austgen et al. 2004). An exception to this is the cottontail rabbit (*Sylvilagus floridanus* Linneaus), which has been shown to carry WNV titers of $\geq 10^{4.3}$ PFU/ml for approximately 2.2 days (Tiawsirisup et al. 2005). These animals do not show clinical signs of infection and are able to infect *Cx. pipiens* and *Cx. salinarius* with minimum estimated infection rates of 11.5/ 1000 ± 5.5 and 20.5/ 1000 ± 6.4% respectively. While little research has been done on their role in WNV amplification in the field, cottontail rabbits, as well as other lagomorphs, are widespread across the Western Hemisphere south of Canada, and may play a role in virus amplification or virus overwintering in some systems.

West Nile Virus Implication for Wildlife.

The effects of WNV on wildlife populations are virtually unknown for any species in the Western Hemisphere, however work is being done to try and predict which species will undergo the greatest consequences from this disease (Marra et al. 2004). Birds, such as the sage-grouse that are already under population stresses due to habitat changes from CBNG development and are susceptible to WNV, may need additional conservation management regulation in areas recently affected by epizootics to sustain current population levels. There is also some indication that scavenger and predatory species may contract WNV from consuming infected prey, and their populations may be at risk in outbreak years (McLean et al. 2002). Domestic cats presented with up to three infected mice contracted WNV from consuming infected carcasses in the laboratory (Austgen et al. 2004), and there have been several incidental cases of predatory birds such as Cooper's hawks (*Accipiter cooperii* Bonaparte) and great horned owls (*Bubo virginianus* Gmelin) succumbing to WNV after consuming infected prey in the wild (McLean et al. 2002). As more research is done on WNV epidemiology in natural systems, we will be able to build better models to assess risk factors to wildlife populations, and be more equipped to make informed decisions in wildlife management.

West Nile Virus Vector Biology

Since its appearance in the western United States in 2002, WNV has been one of the most important vector-borne diseases in the region. The competency of the local mosquito vector *Cx. tarsalis*, public and equine health risks, and threat to native wildlife populations has generated many research programs to investigate the biology and ecology of mosquitoes and epidemiology of this disease. We now have a basic knowledge of regional vectors and mosquito infection rates in North America, and are continuing to learn about the over-wintering mechanism of this pathogen and reservoir hosts utilized in each ecosystem.

The primary mode of transmission for WNV in North America is by the bite of an infected mosquito. In the United States, WNV has been isolated from 60 mosquito species; however many of these species are not bridge vectors for this pathogen (Turell et al. 2001, Molaei et al. 2006). Mosquitoes that are bridge vectors must feed on both avian and mammalian hosts forming a link between the amplifying and susceptible hosts (Riesen and Reeves 1990). These are the mosquitoes of greatest concern for human health.

The isolation of WNV from a mosquito does not necessarily mean that a mosquito species is capable of virus transmission. Primary vectors are insects that are (1) physiologically competent to acquire virus from an infected host and transmit to a susceptible host, (2) are frequently infected with a virus in nature, and (3) naturally occur in areas that are foci for virus transmission (Molaei et al 2006). These insects must feed on both avian and mammalian hosts, and disseminate virus through the midgut in order to transmit virus through the salivary gland. Vector mosquitoes spread WNV between amplifying hosts, thus amplifying the virus in the ecosystem.

In North America, there are less than 10 species of mosquitoes that are considered bridge vectors for WNV (Turell et al. 2001). *Culex pipiens* is considered a moderately efficient vector of WNV, and is the primary vector of WNV in the northeast and midwest along with *Cx. restuans* and *Cx. salinarius* Coquillett (Nasci et al. 2001, Molaei et al. 2006). This species has the highest percentage of reported positive pools in the United States in 2001 and 2002, with 57% and 47% respectively. Outbreaks of Saint Louis encephalitis have been reported in humans with minimum infection rates of 3/1000, indicating that this species of mosquito has the ability to spread encephalitic viruses at low infection rates (Nasci et al. 2001). After 2002, infection rates have dropped yet this species remains in the top three for percentage of total positive pools in the U. S. (Hayes 2005).

In the southeastern United States, the southern house mosquito, *Cx. quinquefasciatus*, is a bridge vector of WNV with 51.4% of total positive mosquito pools from the U.S. in 2004 (Hayes 2005). While this species of mosquito was considered a low to moderate vector of WNV in a laboratory study, its abundance and preference to feed on both birds and mammals make it a competent vector for WNV in the south (Turell 2005). *Culex quinquefasciatus* has also been found to undergo non-viremic transmission between infected and non-infected mosquitoes feeding simultaneously on naïve mice, with infection rates as high as 5.8% (Higgs et al. 2005). No detectable viremia was found in the host mice post feeding, and transmission was thought to be through high virus titers secreted in mosquito saliva while feeding at high densities. This phenomenon has not been described in the field or in other vector species of mosquitoes in North America. Non-viremic transmission may however explain high WNV infection rates within the *Cx. quinquefasciatus* geographical range, as the mosquito infection rate could increase much faster if mosquitoes are able to obtain WNV infections by feeding adjacent to an infected mosquito rather than having to obtain an infected bloodmeal from a viremic host.

Other species of mosquitoes that may be important vectors of WNV in the United States include *Cx. restuans* Theobald, Cx. *nigripalpus* Theobald and *Cx. salinarius* Coquillett (Turell 2005). These species are all found in the eastern United States, and have been found to be competent WNV vectors under laboratory conditions.

The most common mosquitoes in the Powder River basin of Wyoming and Montana include the floodwater mosquitoes *Aedes vexans* Meigen, *Aedes melanimon* Dyar, and *Aedes dorsalis* Meigan, and the freshwater mosquito *Cx. tarsalis*. Each of these species has unique life histories as both immature and adults which allow them to survive in this region. I will first discuss basic mosquito biology, and then describe species-specific characteristics.

Larval Distribution.

Immature mosquitoes pass through four larval stages in aquatic habitats before pupating and emerging as adult mosquitoes. Each species of mosquito has different habitat requirements for optimal development ranging from flooded grasses to stagnant wastewater treatment plants. Within a given water body, microhabitats may exist that support different species of mosquitoes over both space and time. An Iowa study found that temporary pools supported *Cx. tarsalis*, *Cx. pipiens* and *Ae. vexans*, while intermittently flooded vegetation areas around the perimeter of their study site included species such as *Anopheles punctipennis* Say, *Culiseta inornata* Williston and *Cx. pipiens* (Mercer et al. 2005). Of the total larval mosquito population within their study areas, 65.7% was found in temporary pools with intermittently flooded and permanently flooded areas providing habitat for the remaining 34.3%. Open-water habitats contained no mosquito larvae in this study, and generally provide habitat for very few mosquitoes in wetland areas (Thullen et al. 2002). Factors such as vegetation density, dissolved nitrogen content, organic matter and phosphate availability contribute to the productivity of a wetland for mosquito development, and the availability of these resources in any given microhabitat may be the determining factor on the species that will utilize that habitat (Lawler and Dritz 2005, Jiannino and Walton 2004).

Laboratory results show that mortality among larvae at densities greater than 500 per pan was increased by 60% in *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata* (Buth et al. 1990). A shorter development time due to warmer water temperatures reduced mortality under laboratory conditions, but was not seen in the field, likely due to fluctuating ambient temperatures. *Culex tarsalis* and *Cs. inornata* occurring concurrently under natural conditions can have higher densities than single species populations, indicating that these two species may fill different niches within the same aquatic environment (Fanara and Mulla 1974).

Adult Dispersal Patterns.

Distribution of adult mosquitoes after eclosion vary both by species and environmental conditions. Mosquito flights have been classified as migratory, appetential and consummatory, and commence for one of five reasons: (1) resting sites, (2) carbohydrate sources, (3) blood meals, (4) ovipositional sites or (5) mates (Bidlingmayer 1985, Service 1997). Migratory flights have been observed in *Cx. tarsalis* in southern California in pre-diapausal insects including unidirectional flights of up to 17.7 km (Bailey et al. 1965). This type of dispersal may be common in the Powder River basin where overwintering habitat is sparse. Appetential flights are upwind or downwind searching flights for olfactory host clues, mates or carbohydrate sources (Bidlingmayer 1985). Once a food source or mate is detected, consummatory flight begins in which a food source is sought after and consumed. In cases where food sources are sparse, adult mosquitoes may fly several kilometers in the appentential flight mode, often times moving long distances from their original larval habitat. Cases have been observed where high larval densities have also increased dispersal distances by newly emerged adults spiraling several meters into the atmosphere in an attempt to catch wind currents (Bailey et al. 1965). In any case, once a mate and or blood meal is found, appentential flight mode begins again in search of a suitable oviposition site based on a species individual needs.

One of the main reasons that *Cx. tarsalis* is such an efficient vector of WNV in the western United States is because it feeds on both birds and mammals. A study conducted in central California indicates 97.2% of all blood-fed mosquitoes in the spring fed on avian hosts, whereas between May and October, 58.5% of blood meals were from avian hosts, and 41.4% were from mammals (Tempelis and Washino 1967). This shift in feeding habits is most likely due to avoidance behavior by avian host species or the relatively high availability of mammalian hosts over avian hosts in late summer when altricial nestling birds have fledged (Kilpatrick et al. 2006). A shift in feeding hosts may contribute to the spread of WNV among mammals (Kilpatrick et al. 2006).

After a female mosquito takes an infected blood meal, a specific amount of time is required before that insect is capable of transmitting the virus. This is called the extrinsic incubation period (EIP). The EIP is dependent on the species of vector mosquito, virus replication rate and ambient weather temperatures. The movement of adult mosquitoes to cool, shaded resting places during the day, and subsequent host seeking behaviors at night allows them to maintain a composite thermal environment with less temperature variation than in the surrounding habitat (Meyer et al. 1990). This may reduce the EIP in insects that occupy environments with a wide range of maximum and minimum temperatures. *Culex tarsalis* requires a relatively long extrinsic incubation period at normal temperatures in Montana and Wyoming, requiring adult females to survive three gonotrophic cycles, or 109 degree-days, before they are able to transmit WNV (Riesen et al. 2006). Reisen indicates that virus activity in the western United States were closely linked to above-average temperatures in 2004 and 2005, which reduced EIP's in *Cx. tarsalis* to a point where transmission could occur after two gonotrophic cycles indicating that EIP’s can be reduced by high temperatures.

Information regarding EIP and temperature relationships has been used to create a predictive model for WNV outbreaks based on degree-day accumulations over time. In a hot year (2003), this model predicted the WNV cases in Wyoming with a 91.3% total accuracy, and was 65.2% accurate in 2004, which was relatively cool and dry (Zou et al. In Press). Predictive modeling such as the proceeding degree-day model may be useful in the future to forecast WNV outbreak in high risk areas along with proper surveillance.

Mosquitoes have several different survival strategies for overwintering in cool climates. Some species over-winter as adults under diapause, others lay eggs that remain viable over the winter, and several species survive the winter as larvae (Clements 1992). Mosquitoes that over-winter as adults have a higher rate of survival if they enter diapause directly, rather than taking a blood meal first. Female mosquitoes are stimulated to enter diapause by short day lengths and low water temperatures as early instar larvae (Tauber and Tauber 1976). As these mosquitoes prepare for dormancy the development of the primary ovarian follicles stops and production of trypsin and chymotrypsin-like proteases that are used for digesting bloodmeals are reduced (Tauber and Tauber 1976, Robich and Denlinger 2005). These females swich from blood meals to sugar gluttony shortly before entering diapause as a way to increase hypertrophy of the fat bodies before winter (Robich and Denlinger 2005). The only exception to this is when females take a blood meal and develop it as a fat body rather than eggs, a process called gonotrophic disassociation. This is the only known way that an adult mosquito can over-winter WNV without undergoing vertical transmission of the disease (Turell et al. 2002).

Species Specific Biology.

Culex tarsalis. *Culex tarsalis* is a widely distributed mosquito species preferring rural areas west of the Mississippi river from Canada into Mexico. This species is a highly efficient vector of WNV, and it has remained one of the top four species of mosquitoes in the United States for total positive pools since WNV spread west of the Mississippi River in 2002 (Hayes 2005, Turell 2005). This species of mosquito has been widely studied throughout its range because of its ability to transmit pathogens such as WNV, St. Louis encephalitis and western equine encephalitis between birds and mammals. *Culex tarsalis* was the only species of mosquito collected in abundance in the PRB that regularly takes both avian and mammalian blood meals, and thus it has the most veterinary and medical importance.

Culex tarsalis populations have been reported to have high numbers of host-seeking females in August and September in northern climates, as their populations build though the summer from over-wintered females (Knight et al. 2003). In the Powder River basin we saw *Cx. tarsalis* populations build through the middle of August, with a decrease in late August and September, possibly due to shorter day lengths in northeastern Wyoming. *Culex tarsalis* emerges from diapause in the spring, seeks a bloodmeal and completes a gonotrophic cycle. Adults mate in large swarms at dusk, with males copulating each evening, and most females mating 1-2 days post emergence (Riesen et al. 2002). Females lay eggs on the surface of freshwater pools in rafts of 100 eggs or more, seeking out suitable ovpositional habitats by using non-volatile chemical cues (Isoe et al. 1995). Some of the ovipositional cues that female *Cx. tarsalis* use include flooded grass, cattle manure and bacterial composition of the water. *Culex tarsalis* larvae have been observed at highest densities in vegetation cover dominated by *Typhia* spp. root masses and high stem density (Walton et al. 1990). The eggs that are laid are not drought resistant and depending on environmental conditions will hatch several days after being deposited. (Clements 1992)

Larvae of *Cx. tarsalis* are found in newly flooded habitats, and are often the first species of mosquito to colonize a water source (Fanara and Mulla 1974). Flooded areas with high percentages of plant cover (example saltgrass) have the highest larval populations of *Cx. tarsalis* in California, and this affinity for colonizing freshly flooded grasslands most likely is true for this species throughout its range (De Szalay and Resh 2000). The two factors that were found to be most significant in predicting larval abundance of this mosquito in California include maximum water temperature and pond age with newly flooded habitats as the most productive. In this system, duck ponds are flooded annually to provide waterfowl with winter habitat, and gravid *Cx. tarsalis* females are the first mosquito species to utilize this resource. This behavior may be initiated to avoid predators who take 3-4 weeks to reach abundance levels that have a significant effect on larval mosquito populations (Walton et al. 1990). The range of temperatures that are optimal for larval *Cx. tarsalis* development in the laboratory is between 10°C and 37°C, with a mean of 32°C (Fanara and Mulla 1974). The development time for *Cx. tarsalis* larvae under natural conditions ranges from 19.8 to 25.3 days in Southern Manitoba, and may be shorter in warmer climates (Buth et al. 1990).

Adult females are opportunistic feeders, taking bloodmeals from either birds or mammals (Gunstream et al. 1971). *Culex tarsalis* are crepuscular/ night feeders, and spend most of their days resting under vegetation (Turell et al. 2005). The highest activity levels of host seeking females is between 10 PM and 1 AM (Bast 1961, Knight et al. 2003, Riesen et al. 1997). In the spring and early summer, females preferentially seek avian blood meals, many of which are from nestlings (Blackmore and Dow 1958). Catches of host-seeking *Cx. tarsalis* are found at highest densities in traps surrounded by elevated vegetation, and lowest over tree snags, open water, sandbars and in urban areas. In areas of southern California surrounding the Salton sea, proportions of blood meals taken from avian hosts were directly related to the density of host seeking females ($F = 140.0$, $P < 0.001$). This may be because young birds have few feathers in the nest, but quickly mature and develop defensive behaviors to reduce insect feeding as they mature (Lothrop and Riesen 2001, Bast 1961). This leads to a change in host seeking behavior by *Cx. tarsalis* from birds to mammals in the late summer and fall (Gunstream et al. 1971). Those insects that have been infected with WNV in the early summer may transmit the virus to humans and horses by this shift in feeding.

Laboratory studies indicate that 74%-100% of *Cx. tarsalis* become infected with WNV after taking blood meals with $10^{7.1}$ PFU/ml, which is a common virus titer in many North American birds (Goddard et al. 2002). These infected mosquitoes have an estimated WNV transmission rate of 81 and 91% after ingesting blood-meals containing $10^{6.5}$ and $10^{7.3}$ of virus plaque-forming units respectively (Turell et al. 2002b). A female *Cx. tarsalis* requires 35-40 days between egg cycles, and in northern climates they average 2.6-2.9 generations per season (Buth et al. 1990). This requires female mosquitoes to acquire an infected blood meal in her first gonotrophic cycle, survive at least 35 days, and then probe a susceptible host such as a human, horse or sage-grouse to transmit virus.

Culex tarsalis must either be re-infected with WNV each spring while taking a bloodmeal, undergo diapause as an infected adult or vertically transmit virus from gravid female to egg. Laboratory studies have shown vertical transmission from infected females to F_1 progeny with a minimum mosquito infection rate of 6.9/1000; however, this mechanism was not seen in all *Cx. tarsalis* samples tested, and may change between local populations (Goddard et al. 2003). This overwintering mechanism is most likely coupled with others such as reservoir hosts and infectious migratory bird, with variations in composition between regions.

Culex tarsalis is the primary vector for several encephalitic diseases including western equine encephalitis, Saint Louis encephalitis in the western United States and

West Nile virus (Knight et al. 2003). These pathogens are amplified in the enzootic cycle between birds and mosquitoes, most likely among passeriform bird species. Encephalitic diseases can affect humans and domestic mammals; however, they are dead end hosts to the pathogen, not developing high enough viremias to infect subsequent feeding mosquitoes.

Aedes vexans. *Aedes vexans* is a floodwater mosquito commonly found around flood irrigation systems and spring snowmelt locations in across North America (Knight et al. 2003). This species of mosquito is a crepuscular/ night feeder that prefers to take blood meals on large mammals such as cattle and white-tailed deer, and is rarely collected with evidence of an avian blood meal (Gunstream et al. 1971, Turell 2005). Females of this species lay individual eggs in moist soils subject to flooding. Floodwater mosquitoes, such as *Ae. vexans,* have dessicant-proof egg shells that allow an embryo to survive long periods in a dry environment. Eggs with this adaptation can remain viable for several years and will be stimulated to hatch when the right environmental and physical conditions such as flooding and snowmelt occur (Clements 1992). These eggs must undergo a period of dessication prior to inundation in a low oxygen environment as well as exposure to cold to stimulate hatching (Bates 1970).

Laboratory and field-testing indicate the *Ae. vexans* is not a primary vector of WNV in North America although studies indicate that they do transmit the pathogen at low rates (Turell et al, 2001). *Aedes vexans* is not an ornithophagic mosquito, and thus is unlikely to obtain WNV from a viremic bird. Laboratory testing has shown that even after being orally challenged with an infected blood meal, these insects were refractory to infection with dissemination rates of 8%. Of those insects where virus passes through the midgut, 100% were able to transmit virus to a new host, and would be a potential vector in the field (Turell et al. 2001). *Aedes vexans* can transmit western equine encephalitis virus in the western United States. These cases are also incidental as WEE is amplified by avian hosts in the same manner as WNV except when secondary amplification cycles occur involving small mammals such as hares (*Lepus americanus* Erxleben*)* and ground squirrels (*Spermophilus richardsoni* Elegans*)* (Knight et al. 2003).

Aedes dorsalis *Aedes dorsalis* is another floodwater mosquito that is often attracted to ephemeral areas with high salt contents for oviposition (Knight et al. 2003). This species of mosquito is found as adults throughout the summer in the western and northeastern United States and southern Canada (Darcie and Ward 1981). This species requires habitat that is relatively wet, and is common in areas flooded by snowmelt and irrigation events in dryer climates. Host-seeking females are considered opportunistic blood feeder, and take a majority of their blood meals from large mammals. They prefer to feed at night, but they will feed during the day if a suitable host enters their resting area (Turell et al. 2005).

Aedes dorsalis is not considered a primary vector of WNV in North America but is involved in WEE transmission in some parts of their range (Gunstream et al. 1971, Turell et al. 2005). Research in California indicates that *Ae. dorsalis* as well as *Ae. melanimon* and *Ae. campestris* can perpetuate a secondary transmission cycle of WEE among mammals, especially lagomorphs (Riesen et al. 1998). This species of mosquito has had reared larvae test positive for WEE at low rates, indicating vertical transmission which would allow for virus overwintering capabilities.

Aedes melanimon. *Aedes melanimon* is another floodwater mosquito found across the western United States and southwestern Canada (Darsie and Ward 1981). This species of mosquito prefers to lay its eggs in irrigated cropland and flooded vegetated areas with gonotrophic cycle length varying between 4 and 5 days (Jensen and Washino 1991). Female *Ae. melanimon* feed on mammals including cattle and humans; preferentially host-seeking at dusk. This species of mosquito has high adult survivorship and abundance across the summer, along with a short gonotrophic cycle length all of which contribute to the increased probability of obtaining and disseminating a pathogen by an individual vector (Goddard et al. 2002).

The U.S. Centers for Disease Control (CDC) considered *Ae. melanimon* a competent vector for WNV in the United States although they are not considered a primary vector of this pathogen (CDC 2006, Goddard et al. 2002). *Aedes melanimon* has been implicated as a secondary vector of WEE in parts of California by contribution to the amplification and transmission of a secondary virus cycle in cottontail rabbits (*Sylvilagus floridanus*) in WEE outbreak years (Jensen and Washino 1991). The primary vector for WEE in the western U. S. is *Culex tarsalis*, with wild bird populations serving as the basic viral reservoir (CDC 2006). *Culex tarsalis* may also feed on mammalian hosts and transmit WEE, providing an opportunity for *Ae. melanimon* to acquire the WEE pathogen. *Ae. melanimon* that obtain a bloodmeal on WEE infected mammalian hosts can quickly transmit the WEE pathogen through the susceptible host population including horses and humans; thus creating a secondary transmission cycle absent of primary vectors and hosts.

Mosquito Control Strategies

There are many tactics used for mosquito control in the United States including chemical, biological and physical control mechanisms. Each of these tactics has positive and negative attributes that should be assessed on a case by case basis before being implemented. These attributes include cost, environmental effects, duration of control and ease of use.

Biological controls include the introduction and use of natural mosquito predators to maintain mosquito populations at a reduced level. This includes the use of invertebrate and vertebrate predators such as coleoptera adults and larvae, odonata adults and larvae as well as several predatory fish species. Invertebrate predators such as odonata naiads and notonectids can significantly reduce larval mosquito populations in habitats that are greater than 1 month old, and become increasingly effective at controlling mosquito populations in mature ponds (Riesen et al. 1989, Walton et al. 1990). *Mesocyclops longisetus* Thiebaud and *Macrocyclops albidus* Jurine have been introduced in Louisiana rice fields, marshes and ditches to effectively control *Anopheles* spp. and *Culex quinquefasciatus* (38.4 ± 1.9 and 28.7 ± 3.6 larvae eaten per predator per day) (Marten et al. 1994). While these insects may not eliminate mosquito populations, they may be used to control populations in small aquatic habitats.

Vegetation management in larval mosquito habitats is also a viable mosquito control strategy, especially in man-made or intensively managed aquatic habitats. Methods used in vegetation management include burning aboveground plant material, intermentally thinning, deepening of shallow areas to reduce emergent vegetation and turning soils of ephemeral habitats during dry seasons. In general, opening densely vegetated areas reduces mosquito habitat while increasing the habitats of mosquito predators and wildlife species (De Szalay and Resh 2000, Batzer and Resh 1992, Jiannino and Walton 2004). Specifically, if densely vegetated areas are modified to contain small hummocks of emergent vegetation dispersed within deepened open water, mosquito refuge is decreased while predator habitat is increased. This results in adult mosquito emergence 100- and ten-fold lower in hummock and thinned treatment than densely vegetated control treatmets (Thullen et al. 2000). This practice allows for mosquito management while maintaining wildlife habitat without the use of pesticides or labor-intensive annual treatments.

Larvivorous fish have been used extensively across the United States for mosquito control purposes for more than 50 years with varying effects (Reference). The most commonly stocked larvivorous fish is the mosquitofish (*Gambusia affinis* Baird and Girard), but there has been some interest in the use of native fishes for mosquito control purposes (Knight et al. 2003). Mosquitofish are good larvivorous predators, even in densely vegetated conditions; however, they do not over-winter well in cool climates making them difficult to maintain in some areas (Cech and Linden 1987). In laboratory trials, *Gambusia affinis* consumed *Cx. tarsalis* at a significantly higher rate in vegetated trials than un-vegetated habitats ($P < 0.001$), even among other prey items including *Daphnia pulex* and *Hyalella azteca* (Linden and Cech 1990). Native fishes that have been tested for larvivorous activity include the Sacramento blackfish (*Orthodon microlepidotus* Ayres), *Psuedomugil signifier* Knar, *Gambusia holbrooki* Girard and the killifish *Rivulus marmoratus* Poey with varied results (Taylor et al. 1992, Willems et al. 2005). Many of these fishes are effective predators at the juvenile stage, and then move on to larger prey as they grow. These species may be valuable in an integrated pest management program where the young of the year are allowed to control mosquito populations at a given period of their development, and then other control measures are used for the subsequent portion of the mosquito season.

Pesticide use, including adulticides and larvicides, is common in urban areas with high mosquito populations, and has been used as a preventative measure in parts of the PRB. Larviciding products are more effective at controlling mosquito populations because larvae are in a confined area compared to widely dispersed like adults. Products such as *Bacillus thuringiensis* var. *israelensis* (Bti) are microbial larvicides that disrupt the insect's digestive system, and provide a 90-100% reduction in *Ae. vexans* and *Culex* spp., however, they are only effective in the larval stage (Berry et al. 1987, Russel et al. 2003). Monomolecular films are also used as a larviciding material, controlling mosquito larvae by creating a thin film on the water surface that disrupts the insect's ability to obtain oxygen through its siphon. Larviciding oils are highly effective in habitats with little emergent vegetation and little wind, and have the added benefit of being toxic to mosquito pupae as well as larvae of all instars (Lampman et al. 2000). Products such as Golden bear and methylated soy oil have LD_{50} activity of 3.6 and 3.8 $\mu l / 54\ cm^2$ respectively and have an activity time of more than 16 hours in the field (Lampman et al. 2000).

Mosquito adulticides are often distributed as a mist or aerosol, using aerial application, truck foggers or backpack foggers in areas of high adult mosquito density (CDC 2006). Some products that are commonly used by the mosquito control industry are Pyrethrins and 5% malathion (AMCA 2006). These products can be very effective;

however they require specific environmental conditions for proper use including wind speed, temperature and humidity and do not have long term treatment effects. These conditions often make adulticides less effective that larval treatments, and many mosquito abatement districts choose to use these products as a back-up to larval treatments.

Ponds from coal bed natural gas development in the Powder River basin vary in shape, size, vegetation cover and maturity. Regardless of their individual mosquito production, as a whole they greatly increase the potential for mosquito production in this region. Recent research comparing the mosquito production of various pond types in Delaware indicate that shallow sided, highly vegetated habitats produce the largest number of mosquito larvae overall (Gingrich et al. 2006). While some species such as *Ae. vexans* are more productive in those sites that have fluctuating water levels. Mosquito production in the Powder River basin will most likely be highest in those habitats that remain wet throughout the season, and have a high density of vegetation around the shorelines. Those CBNG ponds that fit this description may be very productive, while newer ponds may take time to develop these mosquito production characteristics. Finding ways to reduce mosquito production in existing ponds, and modify the design of future ponds to reduce their utility as larval mosquito habitat can greatly decrease the overall mosquito production of the Powder River basin, and reduce the risk of WNV transmission among humans, livestock and wildlife in this region.

CHAPTER 2

ADULT MOSQUITO PRODUCTION AND WEST NILE VIRUS INFECTION RATES IN NATURAL, AGRICULTURAL AND COALBED NATURAL GAS PONDS OF THE POWDER RIVER BASIN, WYOMING

Introduction

West Nile virus was first detected in Goshen County, Wyoming on 18 August 2002, resulting in 96 equine, 2 human and 17 avian cases by the end of the year. An epidemic occured in 2003, with 393 human cases and 9 fatalities, 230 positive horses, and 182 confirmed bird deaths (Table 1) (Wyoming Department of Health 2006). Of those cases, 23.4% of the human and 19.5% of the equine reports were from Sheridan, Johnson and Campbell counties, all within the geographic boundaries of the Powder River Basin (PRB). The PRB has been under development for coal bed natural gas (CBNG) extraction for the past 16 years, with the majority of development taking place after 1996. This development includes the creation of effluent CBNG ponds. Prior to 2003 quantitative or qualitative data regarding mosquito production had been collected from these ponds. However there is concern over the potential they may produce putative vectors of WNV and have a negative impact on human, equine, and wildlife health.

The 2003 WNV outbreak included the first reported case of WNV in a greater sage-grouse (*Centrocercus urophasianus*; “sage-grouse”) near Spotted Horse,

Table 1. 2002 and 2003 West Nile Virus infections in Wyoming by County. The counties of the Powder River Basin (*italics*) account for 30% of the human WNV cases in Wyoming in 2002, and 70% in 2003 (Wyoming Department of Health 2006).

County	Human Infections		Human Deaths		Horse Infections		Avian Infections		Total Infections	
	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003
Albany	0	5	0	0	0	5	0	19	0	29
Big Horn	0	4	0	0	8	22	0	1	8	27
Campbell	***0***	***71***	***0***	***1***	***9***	***15***	***1***	***16***	***10***	***102***
Carbon	0	1	0	0	2	3	0	2	2	6
Converse	0	19	0	0	0	16	0	2	0	37
Crook	0	9	0	0	1	2	0	0	1	11
Fremont	0	24	0	1	4	54	0	7	4	85
Goshen	2	89	0	3	41	3	13	64	56	156
Hot Springs	0	4	0	0	0	4	0	1	0	9
Johnson	***0***	***8***	***0***	***0***	***1***	***13***	***2***	***2***	***3***	***23***
Laramie	0	31	0	1	11	15	0	26	11	72
Lincoln	0	0	0	0	0	0	0	0	0	0
Natrona	0	28	0	0	4	11	0	24	4	63
Niobrara	0	4	0	0	1	2	0	0	1	6
Park	0	6	0	0	4	27	0	6	4	39
Platte	0	62	0	2	4	7	1	10	5	79
Sheridan	***0***	***13***	***0***	***0***	***4***	***17***	***0***	***5***	***4***	***35***
Sublette	0	1	0	0	0	2	0	0	0	3
Sweetwater	0	0	0	0	0	2	0	3	0	5
Teton	0	0	0	0	1	0	0	0	1	0
Uinta	0	0	0	0	0	0	0	0	0	0
Washakie	0	2	0	0	0	4	0	1	0	7
Weston	0	12	0	1	1	6	0	0	1	18
Total	2	393	0	9	96	230	17	189	115	812

Wyoming, causing a 75% decline in the local radio collared population (Naugle et al. 2004). These mortalities were closely associated with sage-grouse habitats undergoing development for CBNG extraction, including the development of holding ponds for effluent water extracted in the drilling process. This research program was developed to quantify the differences in mosquito populations when aquatic habitats (e.g., CBNG ponds) are increased on the landscape, and the infection rates of WNV vectors in this region. I hypothesized that the wide distribution and abundance of CBNG impoundments in the PRB will not significantly contribute to mosquito production, specifically *Culex tarsalis* in this region.

My primary objective in 2004 was to quantify the adult mosquito populations in five different types of aquatic habitats that were suspected of producing mosquitoes in the Powder River Basin, Wyoming. In 2005, I continued to sample adult mosquito populations in four of the 2004 study sites. I also compared larval mosquito production and vegetation habitat characteristics in each of these study sites to test for differences in larval mosquito production in the available aquatic habitats in the PRB (Chapter 3).

Materials and Methods

Field Methods.

Experimental Design. In 2004 and 2005 adult mosquitoes were collected using battery operated CO_2-baited CDC miniature black light traps (John W. Hock Company, Gainsville, FL). Lights were removed from the traps to exclude non-mosquito fauna.

Traps with approximately 1 kg dry ice were set out in the evening and programmed to turn on at dusk and remain running until the catch was retrieved the next morning. Upon retrieval, adult mosquito samples were transported on wet ice until they could be euthanized with tri-ethylamine and stored at -10°C for later processing (identification and virus assay).

Individual trap sites were randomly selected from aquatic habitats identified using hardcopy USGS EROS data center landsat telocomposits 7,4,3 band combinations (red, green, blue) each study area. These color bands highlight riparian habitats when viewing satellite imagery maps. (Randy McKinley USGS, personal communications). Forty- five trap sites were selected in 2004 between five different study areas in Campbell and Johnson counties, Wyoming. These sites included natural (7 sites), and agricultural water sources (8 sites), sagebrush steppe (2 study areas, 20 sites) and a combination of mature and new coal bed natural gas ponds (10 traps). Adult mosquitoes were sampled twice weekly from 11 July – 9 September with some missing samples due to weather and landowner restrictions.

Adult mosquitoes were captured from twenty trap locations in 2005 in Campbell and Johnson Counties, Wyoming. The total number of trap sites was reduced in 2005 because sagebrush steppe study areas were omitted to allow time for larval sampling. These trap locations were in four different study areas including natural water sources, irrigated agriculture, mature CBNG ponds and new CBNG ponds. Each site was randomly selected from those sampled in 2004 for a total of 5 ponds per study site. Light traps were placed in habitats between emergent vegetation and flooded grasses whenever possible based on the vegetation characteristics at each individual pond. When these habitats were not available, light traps were placed within 2 m of the shoreline near shallow water. Light traps were set bi-weekly in each study area from 15 May - 23 August. Larval samples were taken the day adult traps were set.

Study Sites.

My research area was split into five blocks in 2004, each representing a unique aquatic habitat in the PRB. These sites included; 1) developed CBNG (new and mature), 2) irrigated agriculture, 3) sagebrush steppe under CBNG development, 4) and 5) undeveloped sagebrush steppe (Figure 1). In 2005, I modified the design and selected 1) mature CBNG ponds, 2) new CBNG ponds, 3) irrigated agriculture and 4) sagebrush steppe under CBNG development. These study areas were chosen for their current land use, proximity to radio-collared sage-grouse habitats, landowner cooperation and aquatic habitat resources. A detailed description of each site is as follows:

Sagebrush Steppe under CBNG Development. Sagebrush steppe under CBNG development (natural water sources) were sampled in 2004 and 2005 and included springs, drying river beds, oxbow lakes and stock ponds. Qualifying stock ponds were not artificially filled from anthropogenic sources (e.g., CBNG water). These natural ponds were part of the PRB landscape prior to CBNG development in northeastern Wyoming. The ponds used in this block were in a study area located 24 km south of Buffalo, Wyoming off Interstate 90 (13T 0390639, 4917115, elevation 1.22 km) in land that was privately grazed by cattle. Water sources in this area are ephemeral. They are filled with runoff from snowmelt and rain water early in the season and then become dry in mid to late summer. Several small rainstorms occurred throughout the summer, allowing these aquatic habitats to stay wet into August in 2005, but there were no large rain events in either field season to fill natural depressions to early spring levels.

Aquatic vegetation was sparse around natural water sources in northeastern WY due to the ephemeral nature of natural springs in this dry environment. Average vegetation cover around the natural water sources I sampled was 63%, which included various upland grasses, sage brush (*Artimesia* spp.) and sparse areas of cattail (*Typha* spp.). Upland grasses in the PRB include bluebunch wheatgrass (*Pseudoroegneria spicata*), western wheatgrass (*Agropyron smithii*), prairie junegrass (*Koeleria macrantha*), blue grama (*Bouteloua gracilis*), Japanese brome (*Bromus japonicus*), cheatgrass (*Bromus tectorum*) and crested wheatgrass (*Agropyron cristatum*).

Undeveloped Sagebrush Steppe: CX Ranch. This site was north of Sheridan, Wyoming on the Montana/ Wyoming border (13T 0348842, 4990002, elevation 1.12 km). Upland sagebrush-steppe habitat in the PRB was dominated by Wyoming big sagebrush (*Artemisia tridentata wyomingensis* Beetle and A. L. Young) and intermixed native and non-native grasses such as bluebunch wheatgrass (*Pseudoroegneria spicata* Pursh), western wheatgrass (*Agropyron smithii* Rydb), prairie junegrass (*Koeleria macrantha* Ledeb), blue grama (*Bouteloua gracilis* Vasey), Japanese brome (*Bromus japonicas* Thunb), cheatgrass (*Bromus tectorum* L.) and crested wheatgrass (*A. cristatum* L.). Plains silver sagebrush (*Artemisia cana cana* Pursh) was also present in drainages but at much lower abundance. This sagebrush-steppe habitat has limited CBNG development. The few CBNG ponds that are present are approximately 1 acre in size, shallow, and vegetation surrounding the pond margin is sparse and subject to heavy cattle use. Light traps were set in 2004 near naturally occurring water sources (5 traps), and in upland sage areas (3 traps) where sage-grouse were radio-tracked in high densities in 2003 and 2004. This area was not sampled in 2005.

Undeveloped Sagebrush Steppe: Padlock Ranch. This study area is north of Sheridan Wyoming on the Montana/ Wyoming border and east of the CX ranch (13T 0380795, 4984181, elevation 1.16 km). Those areas that are in Wyoming are currently being heavily developed for CBNG extraction, although no CBNG ponds are currently in this area. Naturally occurring water sources include man-made stock ponds, overflowing stock tanks and one naturally occurring ephemeral pool. The sites of proposed CBNG ponds are known in this study area, and several of our 2004 light traps were placed where CBNG ponds will be located once gas extraction starts. This area was not sampled in 2005.

Irrigated Agricultural Water Sources. Agricultural water sources included small ponds and ditches from flood irrigated agricultural such as hay and alfafa. Study locations were (1) 32 km south of Buffalo, Wyoming on interstate 25 (13T 0361201, 4897075, elevation 1.55 km), (2004 and 2005) and (2) 8 km east of Buffalo, Wyoming on Wyoming highway 16 (2005 only). Water sources for flood irrigation included Upper Crazy Woman Creek, and Clear Creek in privately managed fields. In 2004, two flood irrigation events occurred the weeks of May 27th and June 25th (Julian dates 147, 176). In 2005, one flooding event occurred from 8 June – 10 June, based on the regular irrigation practices of the landowner (Julian date 159 – 161). After each irrigation event, water persisted in 3 of 5 ponds throughout the season, while the remaining 2 evaporated within two weeks (personal observational). Aquatic vegetation in agricultural water sources were predominately cattails (*Typha* spp.) with various rushes (*Juncaceae* spp.).

Mature Coal Bed Natural Gas Ponds: Mature coal bed natural gas ponds were located around Spotted Horse Wyoming, on Wyoming highway 16 (13T 0436498, 4948103, elevation 1.23 km). Mature CBNG ponds received effluent CBNG water for ≥ 5 years and vegetation covered more than 50% of the shoreline. Many of these ponds were previously used as stock ponds by private landowners and were excavated and enlarged to accommodate larger water influxes from CBNG development. Effluent water from CBNG development was added to these ponds at various rates, maintaining relatively stable water levels throughout the field season. Vegetation cover ranged from 45.6% to 89% between ponds, including sedges, rushes, forbes and flooded upland grasses, with an average vegetation cover of 54.5%.

New Coal Bed Natural Gas Ponds: New coal bed natural gas ponds were also located near Spotted Horse Wyoming, on Wyoming highway 16 (13T 0433045, 4949482, elevation 1.2 km). These ponds received effluent CBNG water for ≤ 5 years and vegetation covered less than 50% of the shoreline. Several of these ponds were also former stock ponds, and were recently excavated for effluent water storage. Other ponds were constructed specifically for CBNG water use and were occasionally used for livestock watering. These ponds were also continuously inundated with water from CBNG wells and maintained relatively constant water levels with the exception of one pond (Smith pond) who's water level fluctuated several feet over the course of the summer.   Average vegetation cover per sampling point was 21%, and was predominately flooded upland grasses, algae and forbes.

The CX upland sagebrush-steppe and padlock upland sagebrush-steppe study sites were combined to represent one upland sagebrush habitat block in the final statistical analysis after preliminary statistical tests indicated no significant differences between these study sites for variables tested.

Laboratory Methods.

Mosquito samples were stored at -30°C and sorted on a laboratory chill table (BioQuip 1431) using a 63x – 500x stereomicroscope. All mosquito specimens collected in 2004 were identified to genus using the key of Darci and Ward (1981), with putative WNV vectors in the *Culex* or *Aedes* genera identified to species for Padlock and CX Ranch upland sagebrush-steppe areas by members of USDA ARS arthropod-borne animal disease research laboratory (ABADRL) in Laramie, Wyoming. *Aedes* and *Culex* mosquitoes captured from other study areas in 2004 and all study areas in 2005 were sorted to species.

RNA extractions for WNV were conducted on pools of female mosquitoes in 2004 and 2005 by USDA ARS ABADRL. A maximum of 50 and minimum of 20 specimens were tested per pool with a total of 923 pools in 2004 and 244 in 2005. Those light trap collections that contained < 20 mosquitoes of the same species were pooled with other samples for the same trapping location in a given month. If 20 insects were not collected from a trap site in a month, the pool was run with < 20 specimens.

RNA extraction was conducted with the RNeasy 96 kit (Qiagen, Valencia, CA). Samples were ground in liquid nitrogen, mixed with 1 mL buffer RLT and centrifuged at 8000 x *g* for 10 minutes. Half of the supernatant was stored at -80 °C, and the remaining was used in the extraction according to manufacturer's specifications. Approximately 50 µL of eluate was recovered per sample and stored at -20 °C until used in the TaqMan assay. RT-PCR was run (Lanciotti et al. 2000) on the ABI Prism 7000 sequence detection system with TaqMan one step RT-PCR master mix reagents (Applied Biosystems, Foster City, CA, USA). Primer and probe combinations (DNA Technologies Inc., Coralville, IA, USA) were then synthesized (Lanciotti et al. 2000, Lanciotti and Kerst 2001). Positive samples from the WNENV primer/ probe were tested with the WN3'NC primer/ probe set. Pools were considered positive when CT values were <37, and the normalized fluorescent signal (Rn) was 2 x greater than the average of eight non-template controls for both primer/ probe sets.

Statistical Methods.

Data from the 2004 and 2005 field seasons were analyzed separately due to differences in study designs and data collection protocols. Differences in adult mosquito abundance between habitat types were analyzed in SAS PROC MIXED with a generalized mixed effect linear model. In 2004, the sagebrush-steppe study areas were combined to represent one upland sagebrush steppe habitat after an initial PROC MIXED model was run and no significant differences in mosquito populations were found between sampling sites. Because sequential mosquito counts can be serially-correlated and mosquito counts estimated for the same habitat closer in time are more likely to be correlated than measures more distant in time, I modeled the appropriate covariance structure that best represented the data in SAS PROC MIXED (Littell et al. 1996, 1998). The covariance structure is derived from variances at individual times and correlations between measures at different times on the same habitat (Littell et al. 1998). I used a compound symmetry (CS) error structure where all measures at all times have the same variance and all pairs of measures on the habitat have the same correlation (Littell et al. 1996). SAS PROC MIXED is a generalization of a standard linear model and data are permitted to exhibit correlation and nonconstant variability (SAS 8.2 online doc.). I used the REPEATED statement in PROC MIXED to model the covariation within habitats, which accounts for the violation of independence of the observations on the same pond at different times (Littell et al. 1998). The RANDOM statement was used to model the variation between habitats, which accounts for heterogeneity of variances from individual ponds (Littell et al. 1998). The random effects factor was the sub-sample of ponds within treatment group that were randomly chosen from all available ponds in the study area. In this manner my results are able to extrapolate to all ponds in the study area. All other factors in the model were fixed effects. Maximum likelihood methods were then used to fit a mixed-effects (both random and fixed effects) general linear model in SAS PROC MIXED.

Minimum infection rates of mosquito pools were calculated using the Pooled Infection Rate add-in for Microsoft Excel® (Biggerstaff 2006). Infection rates were first calculated for each species, and then re-grouped and analyzed by study area and study site for those species found to have positive pools in a given year.

Weather data were obtained from the United States National Weather Service archival climatological data for Sheridan, Wyoming (National Weather Service 2006).

Average monthly temperatures from May - August were recorded, including the departure from normal. Precipitation data were recorded as monthly totals including the departure from normal, as well as the number of days with 0.01, 0.10, 0.50 and 1.00 inches or more of rainfall.

Results

2004 Mosquito Collections

38,543 adult mosquitoes representing 10 taxonomic groups was sorted and pooled for WNV testing in 2004 from 554 trap nights. *Culex tarsalis* accounted for 37% of the total population, followed by *Ae. dorsalis* with 31.4% of the population. Other species that were identified from the collection in 2004 included *Ae. vexans* (16.7%), *Ae. melanimon* (10.9%), *Psorophora* spp. (1.6%), *Ochleratatus* spp, (1.9%), *Cu. inornata* (1%), *Cx. pipiens* (<1%), *Culiseta* spp. (<1%), *Anopheles* spp. (<1%) (Figure 2).

Total mosquito collections in 2004 varied among study sites (DF = 3, F = 3.00, *P* = 0.03), and weeks (*P* = 0.0001), with highest collections in May and June. Overall more mosquitoes were collected from irrigated agriculture sites in 2004 than any other study area with an average of 171.59 (SE = 26.95) specimens collected per trap night. CBNG and n areas averaged 109.00 (SE = 24.37) and 163.14 (SE = 27.2) specimens per trap night, respectively (Figure 3). Sagebrush-steppe study sites had the lowest average mosquito counts of all study sites, with a mean of 101.99 (SE = 17.7) and were

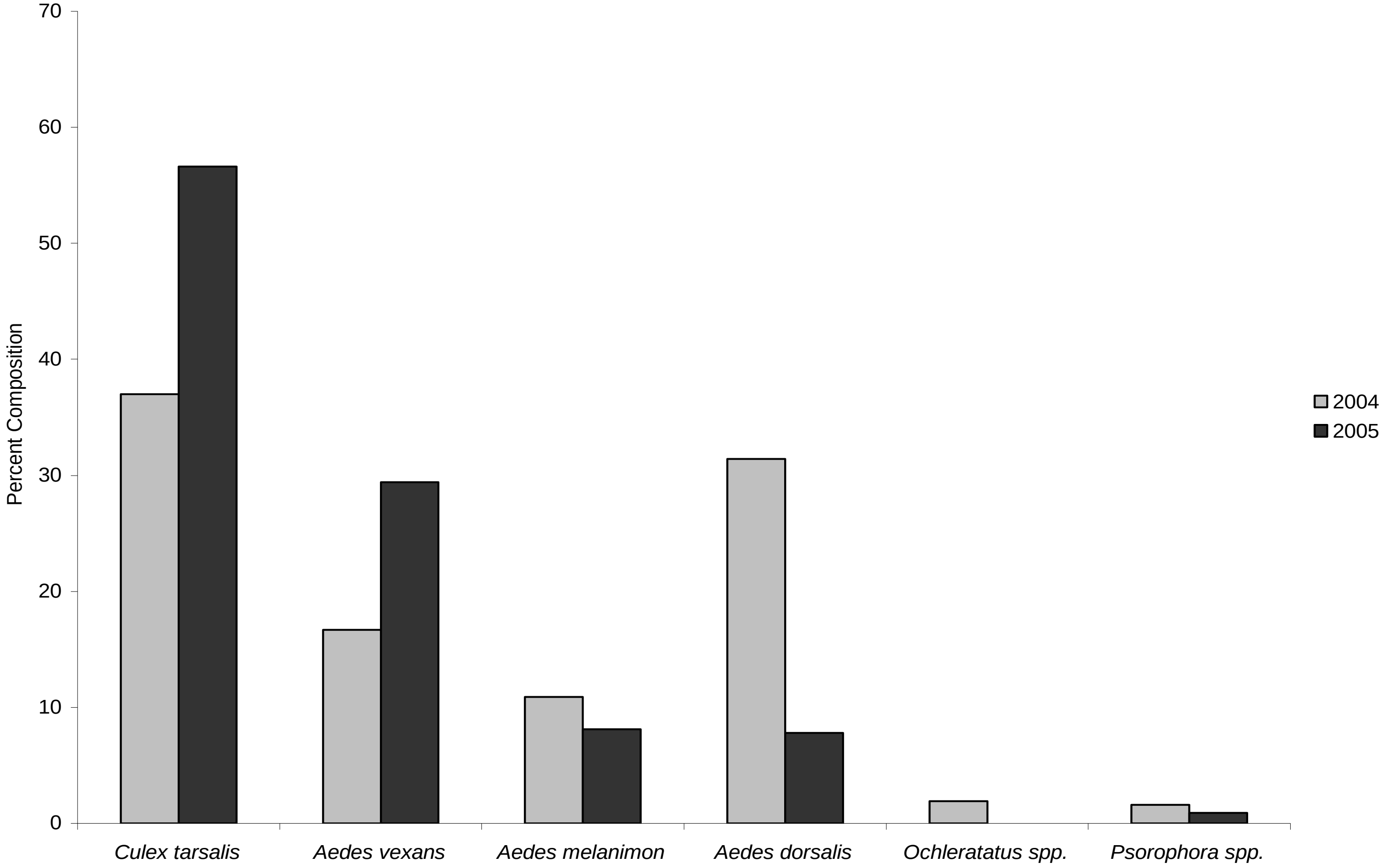

Figure 2. Percent composition of adult mosquito species collected by CDC black light traps, Powder River basin, Wyoming 2004 and 2005.

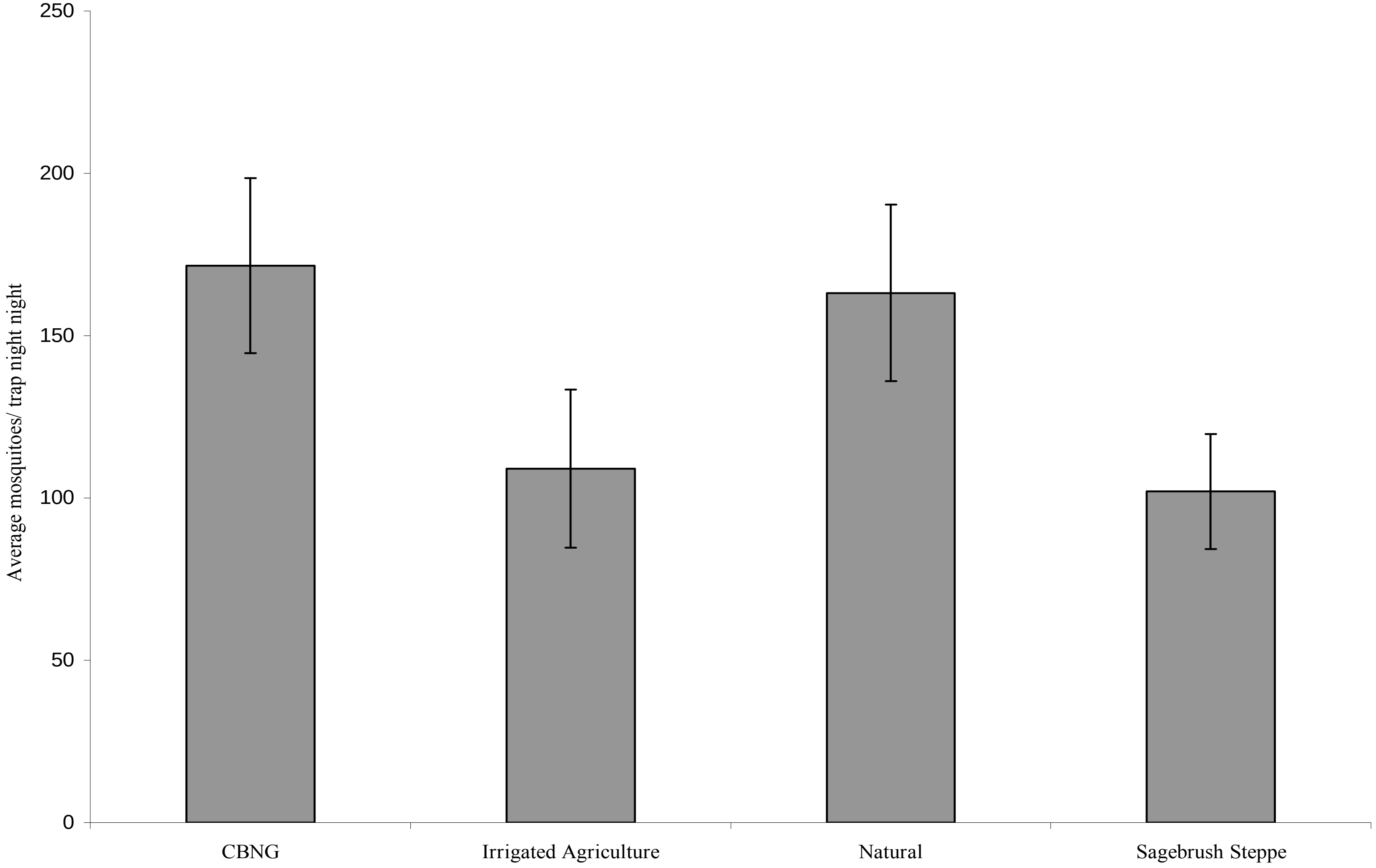

Figure 3. Average mosquitoes collected per trap night by study area, Powder River Basin, Wyoming 2004.

significantly lower than mosquito populations from natural ($P = 0.013$) and irrigated agricultural sites ($P = 0.03$).

Culex tarsalis collections in 2004 differed (3 df, F = 10.27, $P < 0.0001$) between the five study areas sampled. They were significantly higher in irrigated agricultural sites than natural or sage-steppe study areas ($\mu = 44.3$, SE = 6.91, $P \leq 0.007$) (Figure 4, Figure 5). *Culex tarsalis* populations were the lowest in sagebrush steppe sites ($\mu = 10.06$, SE = 4.92). Sagebrush steppe populations were significantly lower than all other populations sampled ($P \leq 0.05$), I was surprised to collect any adult mosquitoes in this area considering that these traps were set in upland sagebrush steppe rather than near aquatic habitats.

Culex tarsalis collections in 2004 varied by week (DF = 8, F = 4.75, $P < 0.0001$). The highest mean population estimates for the entire PRB were found at week 7 ($\mu = 48.17$, SE = 6.57, $P = 0.0001$), and the lowest population estimate were found at week 12 ($\mu = 2.47$, SE = 8.52, $P = 0.77$). Differences in least square means indicate a significant difference between weeks 4 and 7 ($P = 0.001$), 6 and 7 ($P = 0.006$), and 8 - 12 and 7 ($P \leq 0.01$) (Table 2). No differences were found between other weeks sampled.

Aedes vexans was most abundant in irrigated agriculture areas ($P < 0.0001$) with significantly higher populations than any other sampled habitat (Figure 5). Mean population sizes in agricultural areas were 58.1 mosquitoes per trap night (SE = 10.07). There were no significant trends by week found for this species of mosquito across the PRB ($P = 0.48$), likely due to low *Ae. vexans* collections in CBNG, sagebrush steppe and natural areas (Table 2).

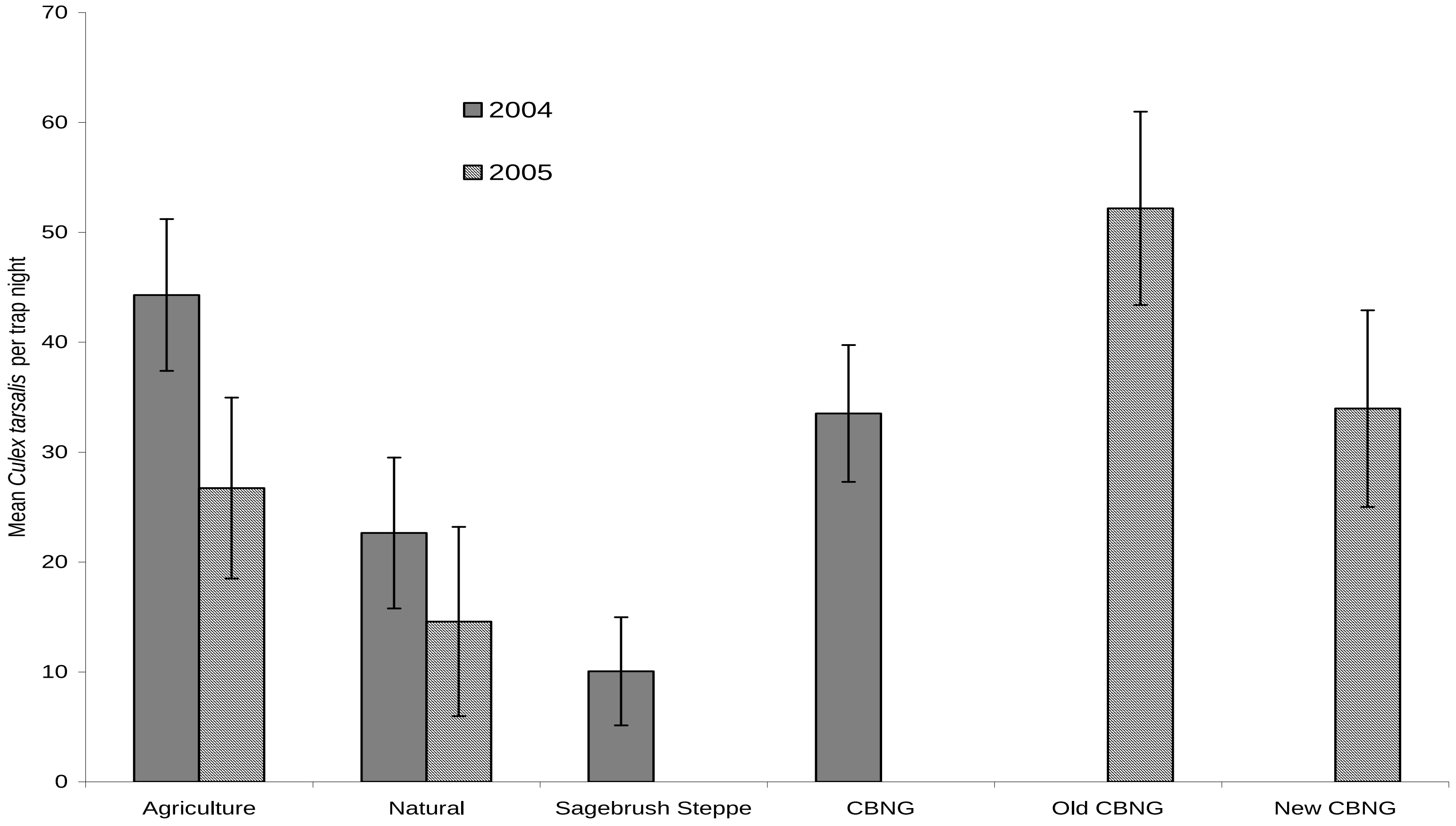

Figure 4. Mean *Culex tarsalis* per trap night by study site in the Powder River basin, Wyoming, 2004 and 2005.

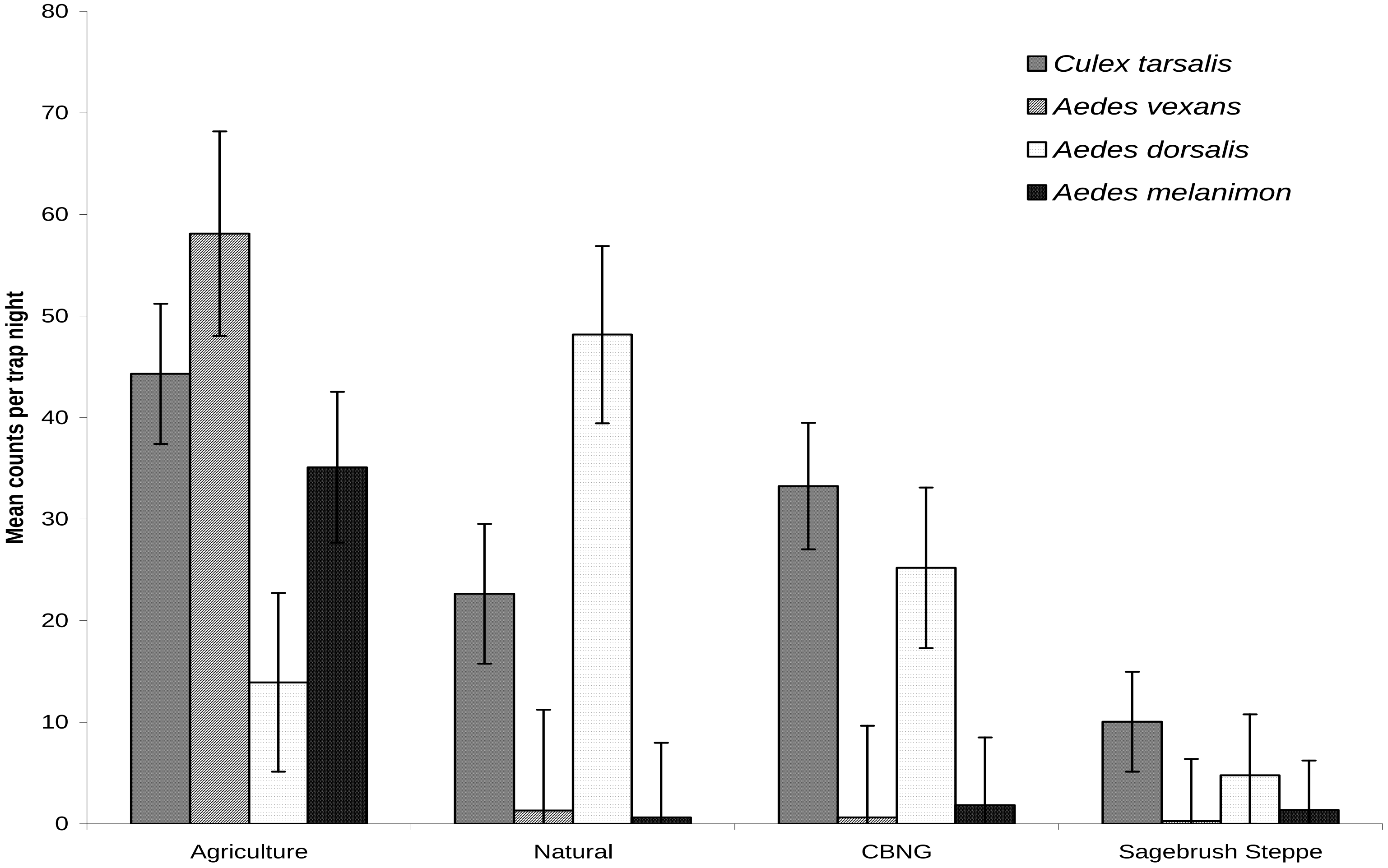

Figure 5. Means and standard errors by study area for the four most abundant mosquito species collected in the Powder River Basin, Wyoming, 2004.

Table 2. Mean counts of 4 species of adult mosquitoes by week in the Powder River Basin of Montana and Wyoming 2004.

Julian date	134 Week 4	148 Week 5	162 Week 6	176 Week 7	190 Week 8	204 Week 9	218 Week 10	232 Week 11	246 Week 12
Culex tarsalis									
CBNG	N/A	5.50	N/A	66.50	30.30	22.38	37.81	56.25	101.69
Agriculture	0.67	1.37	13.67	35.70	54.12	N/A	77.90	7.70	12.10
Natural	0.75	0.50	3.50	9.00	17.07	20.86	75.21	9.71	17.50
Sagebrush	0.00	0.34	13.25	4.11	14.22	4.68	25.25	6.99	10.35
Aedes melanimon									
CBNG	N/A	0.00	N/A	56.00	40.90	19.88	104.44	32.88	12.25
Agriculture	1.22	0.00	0.00	2.10	4.06	N/A	0.75	0.80	0.10
Natural	1.25	1.14	0.07	0.00	1.43	0.14	5.57	1.86	0.43
Sagebrush	1.13	0.44	0.25	1.11	0.55	0.08	0.35	0.20	2.88
Aedes vexans									
CBNG	N/A	0.00	N/A	485.50	41.50	77.88	79.44	24.50	39.88
Agriculture	0.00	0.00	0.00	1.50	5.35	N/A	0.45	0.10	0.30
Natural	0.00	0.00	0.29	0.29	0.21	0.00	1.86	3.86	5.93
Sagebrush	0.00	0.00	0.00	0.23	0.30	0.03	0.20	0.15	0.30
Aedes dorsalis									
CBNG	N/A	0.25	N/A	13.25	1.10	4.88	4.25	16.88	36.19
Agriculture	1.11	10.42	37.83	47.80	92.88	N/A	25.15	5.45	2.15
Natural	1.25	0.14	18.64	5.71	10.29	3.29	183.86	28.14	92.29
Sagebrush	39.50	1.10	0.25	4.40	4.58	0.50	0.90	1.90	15.40

Abundance of *Ae. dorsalis* was significantly higher in natural aquatic habitats ($\mu = 48.17$, SE = 8.73, $P \leq 0.04$) than any other study area (Figure 5). *Aedes dorsalis* collections were similar between irrigated agriculture and CBNG ($P = 0.32$). Sagebrush steppe areas supported the lowest population of *Ae. dorsalis* ($\mu = 4.77$, SE = 6.00) which was significantly lower than natural or CBNG sites ($P = 0.03$, $P < 0.0001$) (Table 2). Weekly abundances of *Ae. dorsalis* from the entire PRB were highest in mid-summer ($P = 0.043$) (Julian date 213), with abundances decreasing in late August and September likely due to ephemeral larval habitats in natural areas and cool summer temperatures.

The majority of the *Ae. melanimon* population in 2004 was found in the agricultural sites, with an average of 33.66 specimens per trap night (SE = 5.79). All other study sites averaged less than 1.2 specimens per trap night, and were not found to be a significant source for this species. No by-week trends were found for *Ae. melanimon* in the 2004 field season (Table 2).

Culex pipiens were rarely caught in 2004, with no significant difference between study areas, and a maximum average collection of 0.04 in the agricultural study site (SE = 0.02). Other species of mosquitoes captured representing <1% of the total population included *Cx. pipiens, Ae. campestris, Ae. implicates, Ae. trivittatus, Ae. nigromaculus, Ae. c. canadensis, Ae. provocans, Ae. cataphylla, Ae. idahoensis, Ae. hendersoni, Cu. inornata Culiseta* spp,. and *Anopheles* spp.

2005 Mosquito Collections

6,469 adult mosquitoes representing 16 taxonomic groups were sorted and pooled for WNV testing in 2005 from 160 trap nights. From these samples *Cx. tarsalis* was the most abundant mosquito collected, representing 56.6% of the total mosquito population. Other species that were identified include *Ae. vexans* representing 29.4% of the population, *Ae. melanimon* (8.1%), *Ae. dorsalis* (7.8%), *Ae. campestris* (<1%), *Ae. implicates* (<1%), *Anopheles* spp. (<1%), *Psorophera* spp. (<1%), *Ae. trivittatus* (<1%), *Ae. nigromaculus* (<1%), *Ae. c. canadensis* (<1%), *Cx. pipiens* (<1%), *Ae. provocans* (<1%), *Ae. cataphylla* (<1%), *Ae. idahoensis* (<1%), and *Ae. hendersoni* (<1%).

Total mosquito populations were significantly different from one another at the $P = .10$ level in 2005 ($P = 0.069$), with irrigated agriculture areas producing the highest total mosquito counts over the field season ($\mu = 107.63$, SE = 23.34). These irrigated sites were significantly different from natural and old CBNG sites ($P = 0.05$, $P = 0.02$), with most of the specimens in this area identified as *Ae. vexans* followed by *Cx. tarsalis*, *Ae. melanimon* and *Ae. dorsalis* (Figure 6). Significant differences were found between weekly total mosquito production (DF = 8, $P = 0.004$), with week 5 – 7 having higher total mosquito counts than any other week sampled (Julian date 162 – 178) (Figure 7).

Culex tarsalis were the most abundant total mosquito collected in 2005 (Figure 2), with old CBNG sites producing significantly more mosquitoes than irrigated agriculture or natural water sources ($\mu = 33.9$, SE = 8.94, $P \leq 0.03$) (Figure 6). Weekly population counts were significant for *Cx. tarsalis* in 2005, with weeks 5 – 8 (Julian date 162 – 188) producing more mosquitoes than all other weeks sampled (DF = 8, F = 11.3, $P \leq 0.008$) (Figure 7, Table 3). Week six had the largest average catch of all weeks sampled, with mean counts of 86.29 *Cx. tarsalis* per trap night (SE = 9.47).

Table 3. Mean counts of adults of 4 species of mosquitoes by week in the Powder River Basin of Montana and Wyoming 2005.

Julian date	134	148	162	176	190	204	218	232	246
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Culex tarsalis									
Old CBM	N/A	0.00	0.00	5.40	66.60	103.00	121.50	55.20	54.50
New CBM	N/A	0.00	0.00	3.60	75.60	66.00	52.40	41.80	24.00
Natural	0.00	0.00	0.00	2.20	11.00	31.40	64.60	5.75	N/A
Agriculture	0.00	0.20	0.00	22.00	20.60	145.40	34.00	2.67	0.00
Aedes melanimon									
Old CBM	N/A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
New CBM	N/A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Natural	0.00	0.00	0.00	0.60	0.20	0.00	0.00	0.25	N/A
Agriculture	0.00	0.00	0.00	7.00	21.00	108.60	4.00	0.00	0.00
Aedes vexans									
Old CBM	N/A	0.00	0.00	0.00	0.80	2.40	0.67	0.60	18.00
New CBM	N/A	0.00	0.00	3.60	75.60	66.00	52.40	41.80	24.00
Natural	0.00	0.00	0.00	2.20	11.00	31.40	64.60	5.75	N/A
Agriculture	0.00	0.20	0.00	22.00	20.60	145.40	34.00	2.67	0.00
Aedes dorsalis									
Old CBM	N/A	0.00	0.00	0.40	5.80	3.20	2.67	3.20	4.50
New CBM	N/A	0.00	0.00	4.60	12.20	8.60	1.20	7.60	0.00
Natural	0.00	0.40	0.00	48.00	2.60	5.80	3.20	12.00	N/A
Agriculture	0.00	2.20	0.00	8.00	2.40	0.00	0.40	0.50	1.00

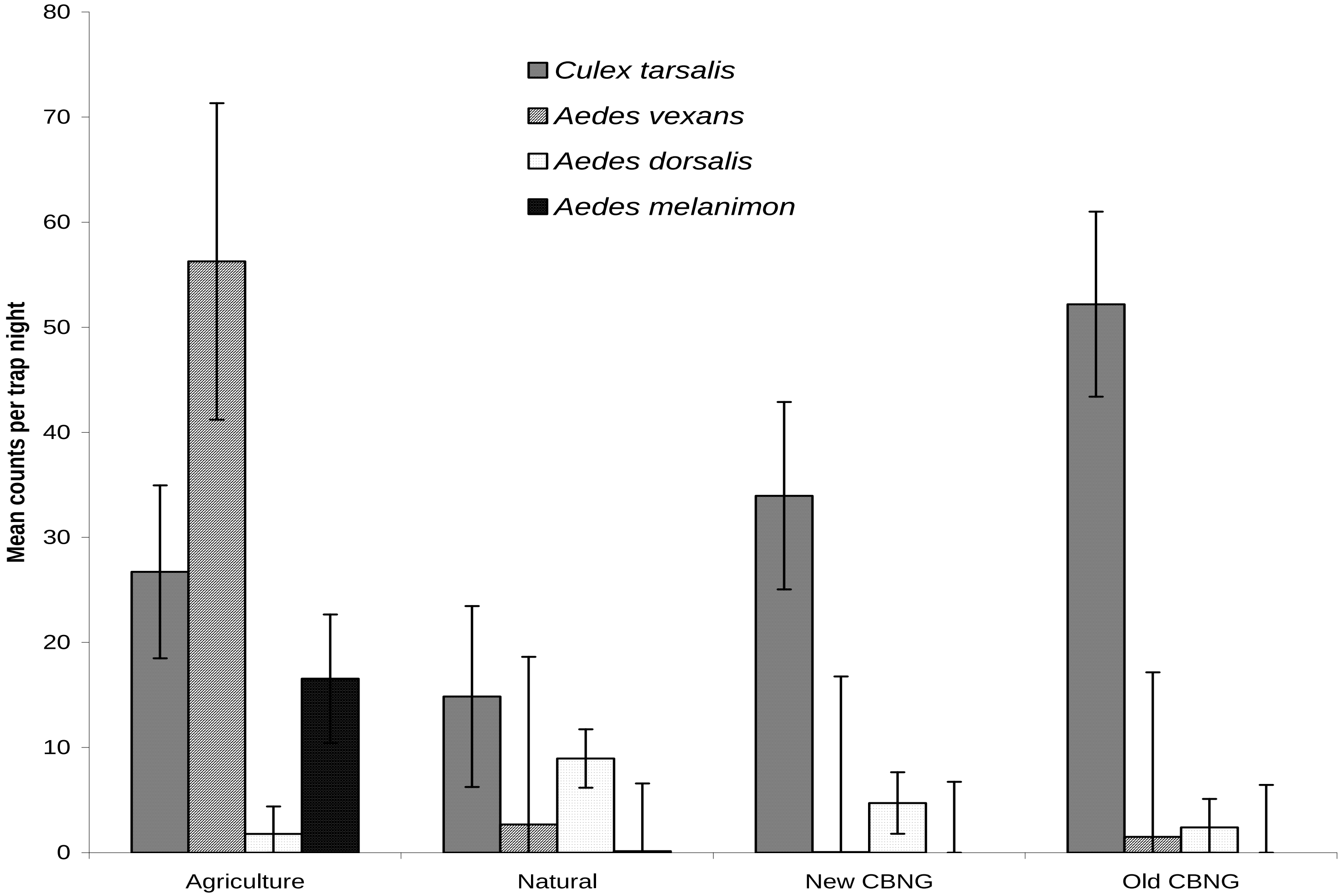

Figure 6. Means and standard errors by study area for the four most abundant mosquito species collect in the Powder River Basin, Wyoming, 2005.

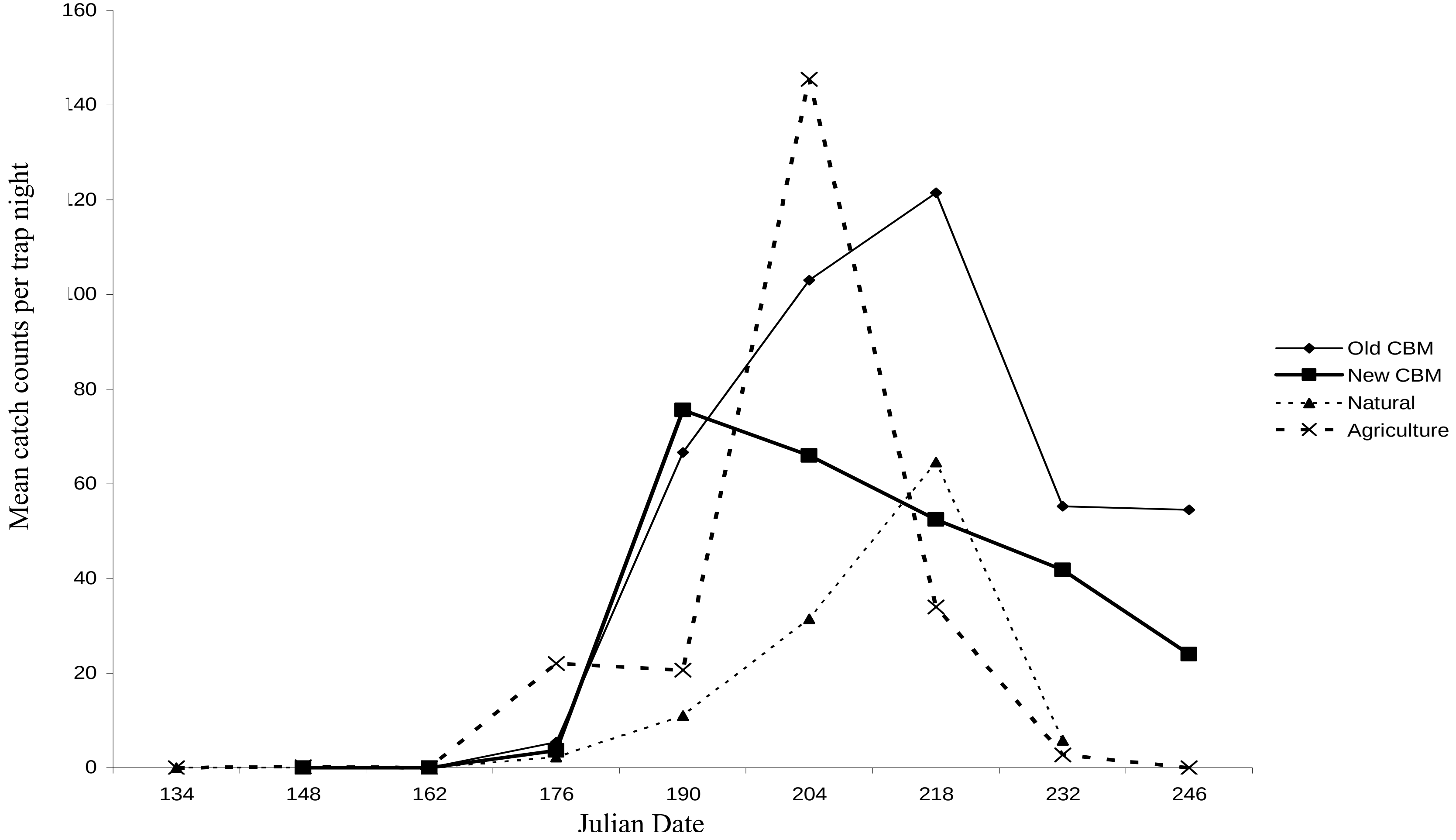

Figure 7. *Culex tarsalis* mean catch counts over time by study area, Powder River basin, Wyoming, 2005.

Aedes vexans were most abundant in irrigated agricultural areas in 2005, similar to 2004 sampling. Mean counts of *Ae. vexans* in agricultural areas were 56.3 mosquitoes per trap night, which was significantly higher than all other study sites sampled (Figure 6) ($P \leq 0.015$). While irrigated agricultural areas were significantly more productive for *Ae. vexans* than other study site, similarly to 2004, there was no significant weekly population trend seen in 2005 ($P = 0.41$) (Table 3).

Abundances of *Ae. dorsalis* in 2005 were much lower than 2004 samples, with no significant differences between study areas (Figure 6) ($P = 0.24$). The highest abundances were around natural water sources, as in 2004, however mean catches were much lower ($\mu = 8.95$, SE = 2.78), with no significant differences from other study areas. No significant weekly trends were seen in *Ae. dorsalis* populations in 2005 ($P = 0.26$) (Table 3).

Aedes melanimon population trends were similar in 2005 to the previous year samples, with abundances higher in irrigated agriculture than other sampled water sources (Figure 6). In 2005 these differences were not significant ($P = 0.15$). There were no significant weekly trends for *Ae. melanimon* in 2005 ($P = 0.47$) (Table 3), likely due to the reduced irrigation practices in 2005 from that seen in 2004.

Mosquito Infection Rates.

A total of 923 and 244 pools of insects were tested for WNV using PCR assays in 2004 and 2005 respectively, with 16 pools resulting in positive detections. Species that were tested for WNV included *Cx. tarsalis* (241, 125 respectively), *Ae. vexans* (52, 22), *Ae. provocans* (1- 2005), *Ae. nigromaculus* (2, 1), *Ae. melanimon* (38, 8), *Psorophera* spp. (10- 2004), *Ochleratatus* spp. (21- 2004), *Culiseta* spp. (8- 2004), *Ae. implicates* (1- 2005), *Ae. dorsalis* (124, 11), *Ae. campestris* (1- 2005) and the biting midge *C. sonorensis* (428, 75). All the positive pools detected were *Cx. tarsalis*, with minimum infection rate of 1.22 per 1000 from 2004, and 0.84 per 1000 from 2005 (Table 4).

There was a geographic difference where infected pools of mosquitoes were collected 2004 and 2005. Of the 12 infected pools found in 2004, 8 were from agricultural areas, 2 were from CBNG and 2 were from CX sagebrush steppe with minimum infection rates of 2.90, 0.60 and 1.48 per 1000 respectively. In 2005 all the positive pools detected were from CBNG areas. Two infected pools were found at old CBNG ponds with an infection rate of 0.99, and 2 infected pools were detected in new CBNG areas with an infection rate of 1.96. This widespread distribution of WNV positive pools reflects the distribution of vector species throughout northwestern Wyoming and southeastern Montana.

Weather Data

Average monthly temperature and rainfall data for Sheridan, WY, May - August 2004 and 2005 indicate normal to below average temperatures in 2004 and 2005 (National Weather Service 2006). 2004 average temperatures ranged from 52.0 to 68.7 °F in 2004, and 51.0 - 72.0 °F in 2005 (Table 5). Departures from normal temperatures were -3.2 °F in 2004 and -0.1 °F in 2005. Average monthly rainfall in 2004 ranged from 0.56 - 1.72 inches in 2004 and 1.01 - 6.18 inches in 2005. Departures from normal rainfall was -2.17 inches in 2004 and +4.99 inches in 2005, indicating major changes in

Table 4. Mosquito infection rates for adult samples collected in the Powder River basin, Wyoming in 2004 and 2005. *Culex tarsalis* was the only species of mosquito detected with WNV by PCR assay in 2004 and 2005.

Year	Species	Infection Rate	Lower Limit	Upper Limit	Number Pools	Number Positive Pools	Number Individuals
2004	*Cx. tarsalis*	1.22	0.66	2.07	239	12	10,120
2005	*Cx. tarsalis*	0.84	0.27	2.03	123	4	4,804

Culex tarsalis infection rates

	Group	Infection rate	Lower Limit	Upper Limit	Number Pools	Number Positive Pools	Number Individuals
2004	Agriculture	2.90	1.36	5.52	63	8	2,936
	Natural	0.00	0.00	2.23	38	0	1,637
	CBNG	0.60	0.11	1.98	79	2	3,338
	CX Sagebrush steppe	1.48	0.27	4.87	36	2	1,372
	Padlock Sagebrush steppe	0.00	0.00	4.21	23	0	837
2005	Agriculture	0.00	0.00	3.35	29	0	1,065
	Natural	0.00	0.00	5.17	18	0	663
	Old CBNG	1.96	0.36	6.43	29	2	1,030
	New CBNG	0.99	0.18	3.26	47	2	2,043

Table 5. Average monthly temperature and rainfall data for Sheridan, WY, May - August 2004 and 2005 (National Weather Service 2006). Departures from normal temperatures were -3.2 °F in 2004 and -0.1 °F in 2005. Departures from normal rainfall was -2.17 inches in 2004 and +4.99 inches in 2005, indicating major changes in total rainfall between 2004 and 2005 field seasons.

Month	Year	Average Monthly Temperature (°F)	Departure from Normal (°F)	Total Monthly Precipitation (inches)	Departure from Normal (inches)	Days with total rainfall ≥ (inches)			
						0.01	0.10	0.50	1.00
May	2004	52.9	0.4	0.73	-1.68	11	2	0	0
	2005	51.0	-1.5	6.18	3.77	12	6	3	3
June	2004	60.6	-1.0	1.16	-0.86	9	3	1	0
	2005	62.2	0.6	2.94	0.92	10	4	3	0
July	2004	68.7	-0.1	1.72	0.61	11	6	1	0
	2005	72.0	3.2	1.01	-0.10	4	2	1	0
August	2004	65.7	-2.5	0.56	-0.24	7	1	0	0
	2005	65.8	-2.4	1.2	0.40	11	5	0	0

total rainfall between 2004 and 2005 field seasons. A total of 52 days accumulated > 0.01 inch of total rainfall between May and August 2004, with zero days accumulating >1.00 inch total rainfall. The 2005 field season included 64 days with > 0.01 inch total rainfall, with three days accumulating >1.00 inch total rainfall in May 2005.

Discussion

The Powder River Basin of Wyoming is currently undergoing landscape scale changes in land use and development due to the production of coal bed natural gas. Satellite imagery shows that CBNG development has had a 2-fold increase in road, 2-3x increase in powerlines, 5x increase in total ponds in ranching areas with a 9x increase in total area of water, and a 2x increase in ponds and water in agricultural fields (Naugle et al. in press). Further imagery indicates that these ponds have contributed to a significant increase in potential *Cx. tarsalis* habitat across this region (75%) (Zou et al. 2006). *Culex tarsalis*, the vector responsible for transmitting WNV in northeastern Wyoming, is a native species of mosquito to the PRB (Hayes 2005, Turell et al. 2005); however their population levels have increased in some areas due to human development in both agriculture and CBNG fields. This in combination with my research data allows me to reject my hypothesis that CBNG development has increased mosquito production in the PRB including the WNV vector *Cx. tarsalis*.

In 2004 *Cx. tarsalis* was the most abundant mosquito collected across the PRB and was second in abundance to *Ae. vexans* in 2005. *Culex tarsalis* populations were highest in irrigated agriculture and CBNG sites, both of which are artificially supplemented with water throughout the summer. These sites were vegetated by sedges, rushes, forbes and flooded upland grasses. Many of these ponds also included inlets and outlets, which were significant production areas for *Cx. tarsalis* larvae in 2005 (Chapter 3). *Culex tarsalis* populations have been observed in southern California with high densities around irrigated agriculture (Riesen et al. 1992), and are known to be one of the first species to colonize wastewater ponds in the southwestern United States (Walton et al. 1990, Fanara and Mulla 1974). Our *Cx. tarsalis* collections show similar patterns to those observed in anthropognic water sources in California, with the highest catch counts in Wyoming observed around irrigated and CBNG habitats.

In 2004 high populations of *Cx. tarsalis* were observed in agricultural sites, followed by sites under CBNG development. This summer saw below average precipitation in northeastern Wyoming (-41.7% average, National Weather Service 2006) and subsequently our study sites had a 2-fold increase in irrigation of hay fields (Sparo Zezas, personal communications). In contrast, rainfall in 2005 was 4.99 inches above the seasonal average, with normal seasonal temperatures and irrigation practices. This was reflected in adult mosquito populations with total mosquito production in irrigated agricultural areas increasing by 27% above average under drought conditions, and *Cx. tarsalis* production increasing by 39%. In comparison, natural sites saw a 10% decrease in *Cx. tarsalis* production from 2005 to 2004. These mosquitoes have been observed under drought conditions in California, and have demonstrated similar trends, with increased populations in irrigated agriculture during a dry year (1990) (Riesen et al. 1992). Overall, drought conditions may facilitate increased mosquito production in agricultural areas by increasing flood irrigation habitats when naturally occurring habitats are drying down due to lack of precipitation.

Seasonal trends in mosquito populations for both the 2004 and 2005 field season were strongest in *Cx. tarsalis* populations across the PRB. These populations increased over the course of the spring and summer, with peak population the week of 22 July (x = 86.29 per trap). Similar population trends have been observed in California with peak *Cx. tarsalis* populations the first week of July (Isoe and Millar 1995, Knight et al. 2003). No other strong weekly trends were seen in other species of mosquitoes collected in the PRB. *Aedes vexans* were slightly more abundant in the early spring, with no significant differences found between sampling weeks in 2004 or 2005.

West Nile virus mosquito infection rates varied between study years and study sites across the Powder River basin. In 2003, female *Cx. tarsalis* caught in CDC light traps tested positive for WNV with an infection rate of 7.16 per 1000, and *Culicoides sonorensis* were found with a WNV infection rate of 2.31 per 1000 (Naugle et al. 2004). In 2004 and 2005, study areas with the highest adult *Cx. tarsalis* population also had the highest mosquito infection rates, with agricultural sites having infection rates of 2.90 in 2004, and old CBNG sites had infection rates of 1.96 in 2005. *Culex tarsalis* average 2.6- 2.9 generations per season in northern climates, with infected females needing to survive a minimum of 35 days to infect a susceptible host and continue amplifying WNV in the environment (Buth et al. 1990).

Landscape changes due to CBNG development and irrigated agriculture in the PRB have created habitats with significantly higher mosquito populations than natural landscapes of northeastern Wyoming. CBNG ponds placed in upland sagebrush steppe habitat have created areas with significantly more mosquitoes than the original landscape, including the WNV vector *Cx. tarsalis*. These mosquitoes have been detected with WNV in 2003, 2004 and 2005 and WNV has been documented in greater sage grouse in CBNG fields. Modifications to current water usage practices will likely be required to mitigate the threat of WNV to human health and wildlife.

CHAPTER 3

COMPARITIVE LARVAL MOSQUITO PRODUCTION IN NATURAL, AGRICULTURAL AND COAL BED NATURAL GAS PONDS OF THE POWDER RIVER BASIN, WYOMING

Introduction

The effects of energy development on the economy, environment and wildlife populations of western North America is an issue of concern as new energy resources are explored across the west. The PRB coal seam boundary which spatially defines where CBNG development occurs likely is ~ 2.4 million ha; roughly the size of New Hampshire. Within this area the Bureau of Land Management (BLM) has already authorized plans to drill 51,000 CBNG wells on federal mineral holdings in the PRB of Wyoming and the potential exists for another 15,000 in Montana (BLM 2003 a, b). Coal bed natural gas is currently being extracted for commercial use in the Powder River basin by the natural gas industry at the rate of 23,304,764 m^3 per day (Department of Energy 2002). Methane extraction includes the removal of groundwater to allow confined gases to flow to well heads. This groundwater is discharged into existing cattle ponds, newly constructed ponds, or surface drainages (Clark et al. 2001). Coal bed natural gas development and associated infrastructure in the PRB has caused rapid, large-scale changes to sagebrush habitats of Montana and Wyoming. The potential impacts that could result from the high density of wells, power lines, roads, increased vehicle traffic, pipelines, compressor stations, and water storage ponds within a gas field this size is of concern to wildlife managers tasked with conservation of sensitive species. Since 1999, an estimated 19,000 CBNG well heads have been constructed in the PRB, with 20,000 more projected in the future, each of which will produce discharge water that must be held in CBNG ponds, or re-injected into the aquifer (Department of Energy 2002).

Coal bed natural gas ponds vary in shape, age and structure creating varied types of aquatic habitats in a region that has previously been considered semi-arid (Hemstrom et al. 2002, Walker et al. 2004). These ponds are potential habitats for mosquito production, including the mosquito *Culex tarsalis*, the main vector for West Nile virus (WNV) in the western United States (Hayes 2005, Turell et al. 2005, Zou et al. 2006).

Coal bed natural gas development has affected several species of wildlife native to the PRB (Daszak et al. 2000, Marra et al. 2004), including the greater sage-grouse (*Centrocercus urophasianus*) (Naugle et al. 2004, 2005, Walker et al. 2004). The new networks of roads, power lines, pipelines, compressor stations and wellheads from energy development result in cumulative impacts that are detrimental to sage-grouse survival (Holloran 2005, Aldridge and Boyce In Press). Along with these habitat changes, the introduction of new pathogens to the sage-grouses native range may cause population declines that, when compounded, are beyond the scope of recovery for this species. The introduction of WNV to the PRB reduced late summer survival of female sage grouse by 80% in some areas in 2003. Additional vectors of WNV in the PRB from CBNG ponds may increase WNV sage grouse mortality in this region.

Populations of adult *Cx. tarsalis* mosquitoes have been found throughout the PRB including in natural, agricultural and CBNG habitats. This species was positive for WNV in select areas of the PRB and is the likely vector of this pathogen to human, equine and wildlife species (Hayes 2005, Turell 2005). Migratory flights of host-seeking or ovipositional-site-seeking female *Cx. tarsalis* have been found to travel up to 17.7 km in California (Bailey et al. 1965), indicating that females caught in a CO_2 baited light trap may have emerged in a different aquatic habitat than where they were collected as adults. To identify where mosquitoes are being produced in the PRB and the specific habitats preferred for larval mosquitoes I sampled four different types of aquatic habitats including CBNG, natural and irrigated agriculture.. I hypothesized that the type of habitat created by CBNG development would produce mosquitoes equivalent to or better than natural and agricultural water sources.

Materials and Methods

Study Sites.

To determine the taxa and numbers of mosquitoes produced in these areas five different habitats were sampled. These included natural water, irrigated agricultural water, new CBNG ponds, mature CBNG ponds and CBNG pond outlets. Aquatic habitats sampled for adult mosquitoes were also sampled for mosquito larvae production. A complete description of these study sites is found in Chapter 2 (pages 29 - 33). Coal bed natural gas outlets were sampled for larval production separately from the CBNG ponds. These areas were not sampled for adult mosquitoes because they are contiguous with the ponds.

These outlets are formed by water seeping under the earthen dam created to hold CBNG water. Neither age nor vegetation type of the contributing CBNG pond was included in the classification of CBNG outlets. Outlets were treated as a separate block in the analysis, as they had different vegetation and shoreline characteristics, and they produced mosquitoes independently of their contributing CBNG pond. These outlets were small areas, generally less than 50 m in length and 3 m in width and no more than 46 cm in depth. Water levels were relatively stable throughout the 2005 field season, although outlet lengths were often reduced during hot, dry weather. Average vegetation cover was 40% in late August, predominately covered by rushes, sedges, flooded upland grasses and emergent wetland grasses.

Field Methods.

Mosquito larvae were collected bi-weekly from 13 May – 24 August, 2005 in each of the five habitat blocks (Figure 1). Each block contained five randomly selected aquatic habitats which were sampled at 20 points along a transect at 5 m intervals. Each point was sampled four times using a 350 ml standard dipper. A sample was taken at 0.5 m intervals in each of the cardinal directions while I faced the body of the pond to be sampled with the shoreline behind me. All larvae collected from a sampling points were pooled and concentrated into 20 ml vials and preserved in 95% alcohol for processing.

I characterized pond vegetation on 3-17 August 2005 when vegetation had matured enough to be accurately identified to major taxanomic groups (e.g., rushes, sedges, flooded upland grasses and forbes). I used standard 46 x 46 cm Daubenmire (1959) frames to sample each larval sampling point for vegetation variables including plant cover (%), cover type and plant type. Cover variables included emergent, submergent, open water and flooded upland vegetation. Plant type variables included algae, forbes, grasses, rushes, sedges woody plants and open water. I converted categorical estimates of plant cover to percentages using methods developed by Daubenmire (1959) (1 = 2.5%, 2 = 15%, 3 = 37.5%, 4 = 62.5%, 5 = 85%, 6 = 97.5%) for each larval sampling point, and averaged these values for each pond, and for each study site.

Weather data obtained from the United States National Weather Service archival climatological data for Sheridan, Wyoming (National Weather Service 2006). Average monthly temperatures from May - August were recorded, including the departure from normal. Precipitation data was recorded as monthly totals including the departure from normal, as well as the number of days with 0.01, 0.10, 0.50 and 1.00 inches or more of rainfall.

Laboratory Methods.

Second, third and fourth stage larvae were counted and identified to genus and/ or species (Darsie and Ward 1981). *Aedes* and *Culex* larvae were identified to species; *Culiseta* and *Anopheles* were identified to genus. First instar and pupae were recorded but were not identified due to lack of species keys for this region. All specimens were stored in 70% ethanol for future reference.

Statistical Methods.

For data analysis on mosquito production between aquatic habitats, mean values were calculated for each mosquito species from the 20 points sampled per pond to avoid pseudoreplication (Hulbert 1984). Data analysis conducted to assess the impact of different aquatic vegetation characteristics between pond types used each larval sampling point individually, as vegetation characteristics could vary from point to point within a pond.

Larval production of mosquitoes between pond types was analyzed in SAS PROC MIXED with a generalized mixed effect linear model (Littell et al. 1996). Number of mosquito larvae per time period was log transformed $\ln(x + 1)$ to meet the assumption of normality. Because sequential larval counts can be serially-correlated and larval counts estimated for the same pond closer in time are more likely to be correlated than measures more distant in time, I modeled the appropriate covariance structure that best represented the data in SAS PROC MIXED (Littell et al. 1996, 1998). The covariance structure is derived from variances at individual times and correlations between measures at different times on the same pond (Littell et al. 1998). I used a compound symmetry (CS) error structure where all measures at all times have the same variance and all pairs of measures on the pond have the same correlation (Littell et al. 1996). SAS PROC MIXED is a generalization of a standard linear model and data are permitted to exhibit correlation and non-constant variability (SAS 8.2 online doc.). I used the REPEATED statement in PROC MIXED to model the covariation within ponds, which accounts for the violation of independence of the observations on the same pond at different times (Littell et al. 1998). The RANDOM statement was used to model the variation between ponds, which accounts for heterogeneity of variances from individual ponds (Littell et al. 1998). The random effects factor was the sub-sample of ponds within treatment group that were randomly chosen from all available ponds in the study area. All other factors in the model were treated as fixed effects. Maximum likelihood methods were then used to fit a mixed-effects (both random and fixed effects) general linear model in SAS PROC MIXED.

Timing of larval production between aquatic habitats for each of the four most abundant species was assessed using a 1-way ANOVA blocked by week. I used a 1-way ANOVA to assess differences in larval populations on a week-by-week basis because these were only within week comparisons, and ponds were not repeatedly sampled within weeks.

I also used a 1-way ANOVA to assess whether the production of *Cx. tarsalis* was related to vegetation characteristics in the four habitat types that were sampled. I used *Cx. tarsalis* because it is the most abundant mosquito species in the PRB and is known to vector WNV in the western U.S. Only larval counts taken the week that vegetation characteristics were measured were used in analyses.

Results

Mosquito Populations.

A total of 6,483 mosquito larvae were captured and identified from 12,636 individual dips. The dominant species identified across all study sites was *Cx. tarsalis*, which accounted for 47.8% of the individual larvae collected (Figure 8). *Culiseta* spp. represented 20.8% of the collections, followed by *Ae. vexans* (4.2%), *Aedes dorsalis* (3.1%), *Ae. melanimon* (2.3%) and *Ae. campestris* (0.1%). Unidentified 1st instar larvae and pupae accounted for 20.9% and 0.08% of the total collection, respectively.

Culex tarsalis production was significantly different at the 90% level (df = 4, P = 0.09) between the five sampled aquatic habitats. Post-hoc tests showed that *Cx. tarsalis* production was similar across all types of CBNG and natural sites ($P \geq 0.41$, Figure 9). *Culex tarsalis* production was lowest in agricultural sites, with a mean count of 0.47 larvae per sampling point (post hoc $P = 0.03$) (Table 6). *Culex tarsalis* showed strong seasonal differences ($P < 0.0001$) with a peak in larval populations the week of 18 July (Julian date 184) (Figure 10). *Culex tarsalis* production increased precipitously from mid-June to mid-July, (Julian date 142 – 184) and sustained high production through mid-August (Figure 10). The habitat type that contributed most to this peak was CBNG outlet ponds (μ=141.59, SE=1.71, $P = 0.03$; Figure 11). *Culex tarsalis* populations in new CBNG, old CBNG and natural sites also increased the week of 18 July, but with no differences between group means ($P \geq 0.95$), and to a lesser extent when compared to CBNG outlet ponds ($P = 0.03$).

Production of *Culiseta* differed ($P = 0.05$) between the five sampled aquatic habitats. *Culiseta* production was similar in agricultural, natural and CBNG outlets ($P \geq 0.001$), and was lowest in new and old CBNG sites ($P = 0.196$ and $P = 0.053$, Table 6). Unlike other species, *Culiseta* did not show strong seasonal differences in 2005, but timing of production was variable between aquatic habitats ($P = 0.09$). *Culiseta* populations in CBNG outlets and natural sites peaked in mid-summer (Julian date 142 - 184; Figure 9).

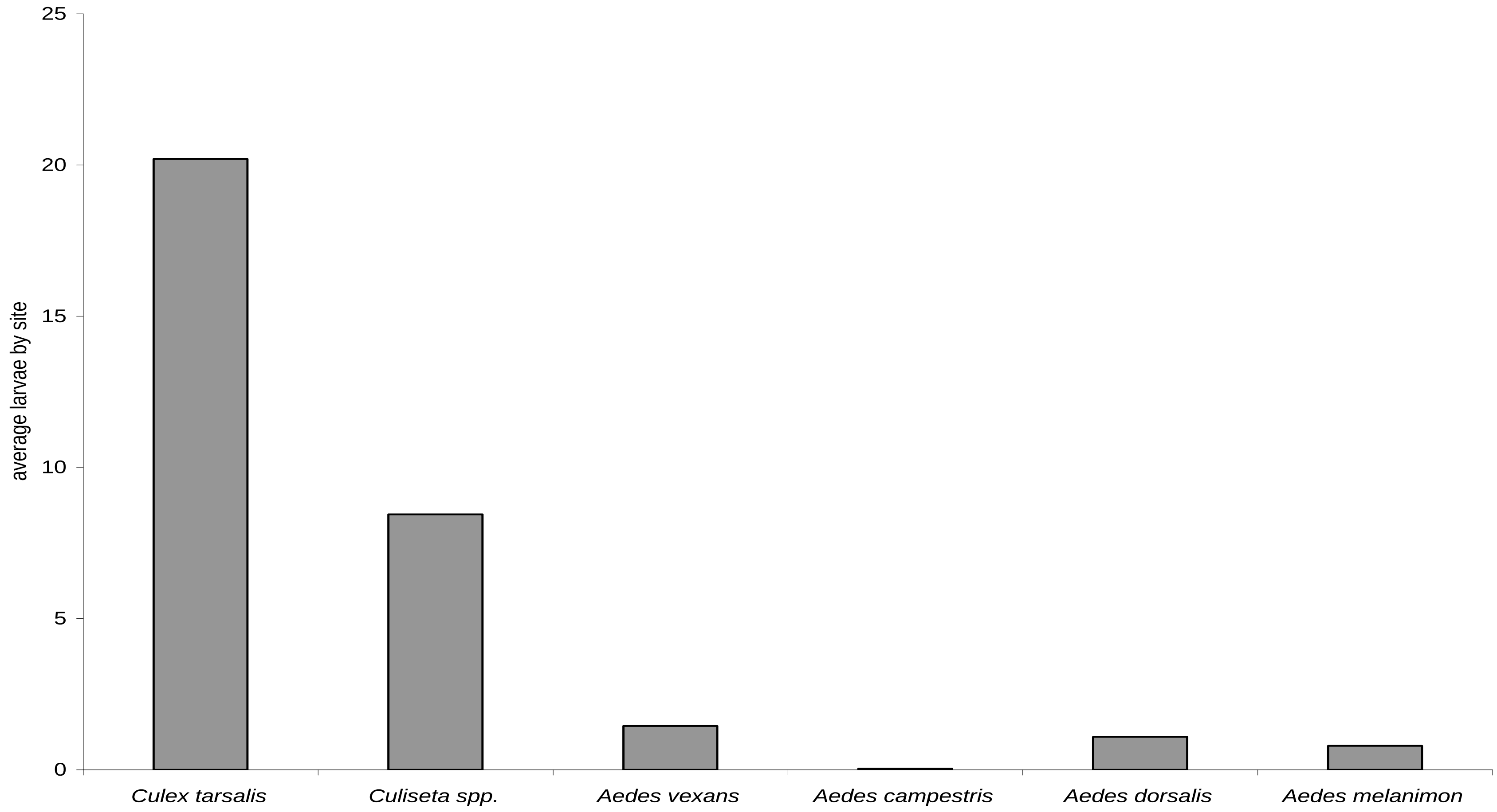

Figure 8. Mosquito larvae collected by taxon in the Powder River Basin, Wyoming, 2005. *Culex tarsalis* were the most abundant species collected, followed by *Culiseta* spp. and *Aedes vexans*.

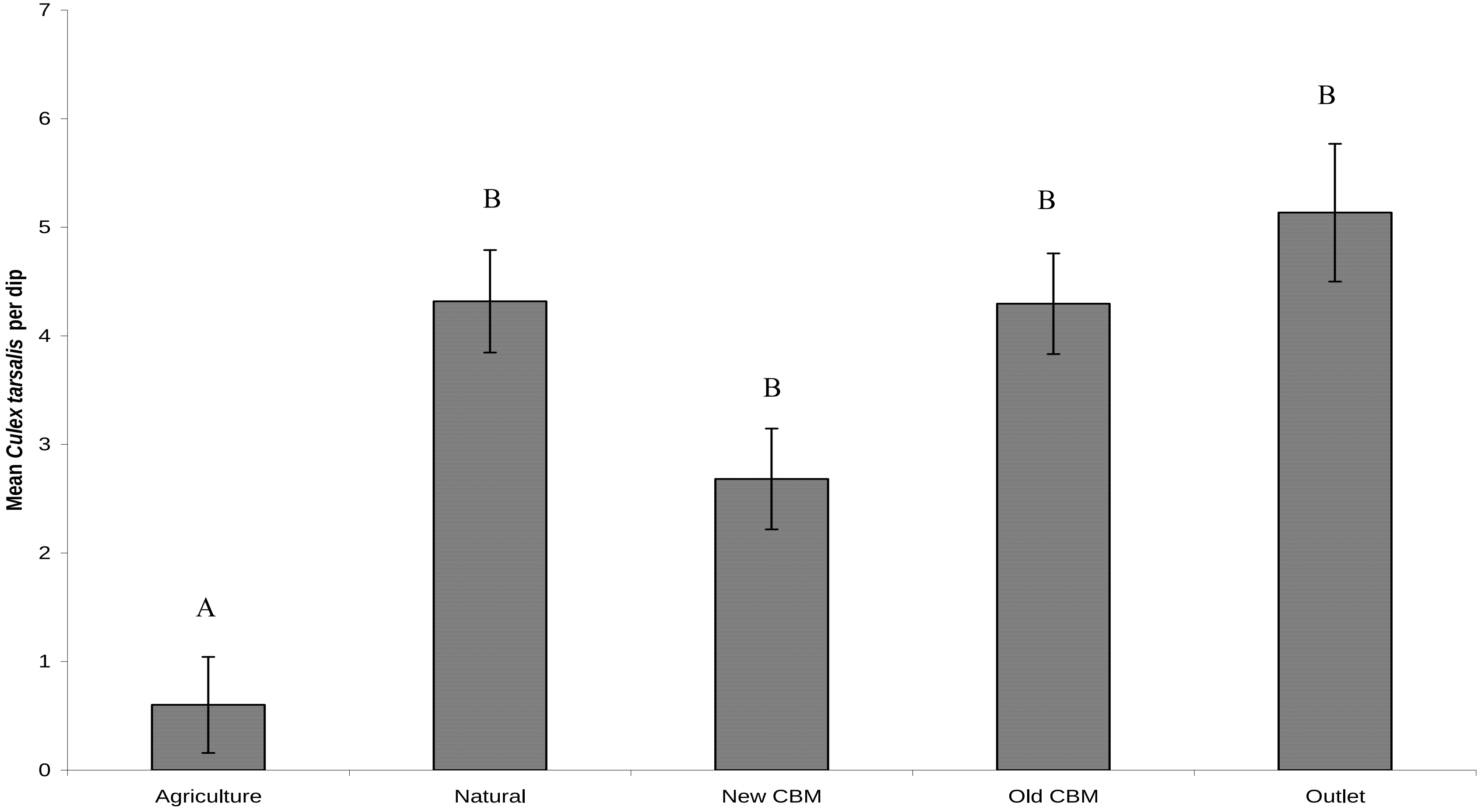

Figure 9. Mean larval production (SE bars) of *Culex tarsalis* per dip from 5 aquatic habitats types in the Powder River Basin, Wyoming, 2005. (Statistical differences > 0.05 denoted by letters).

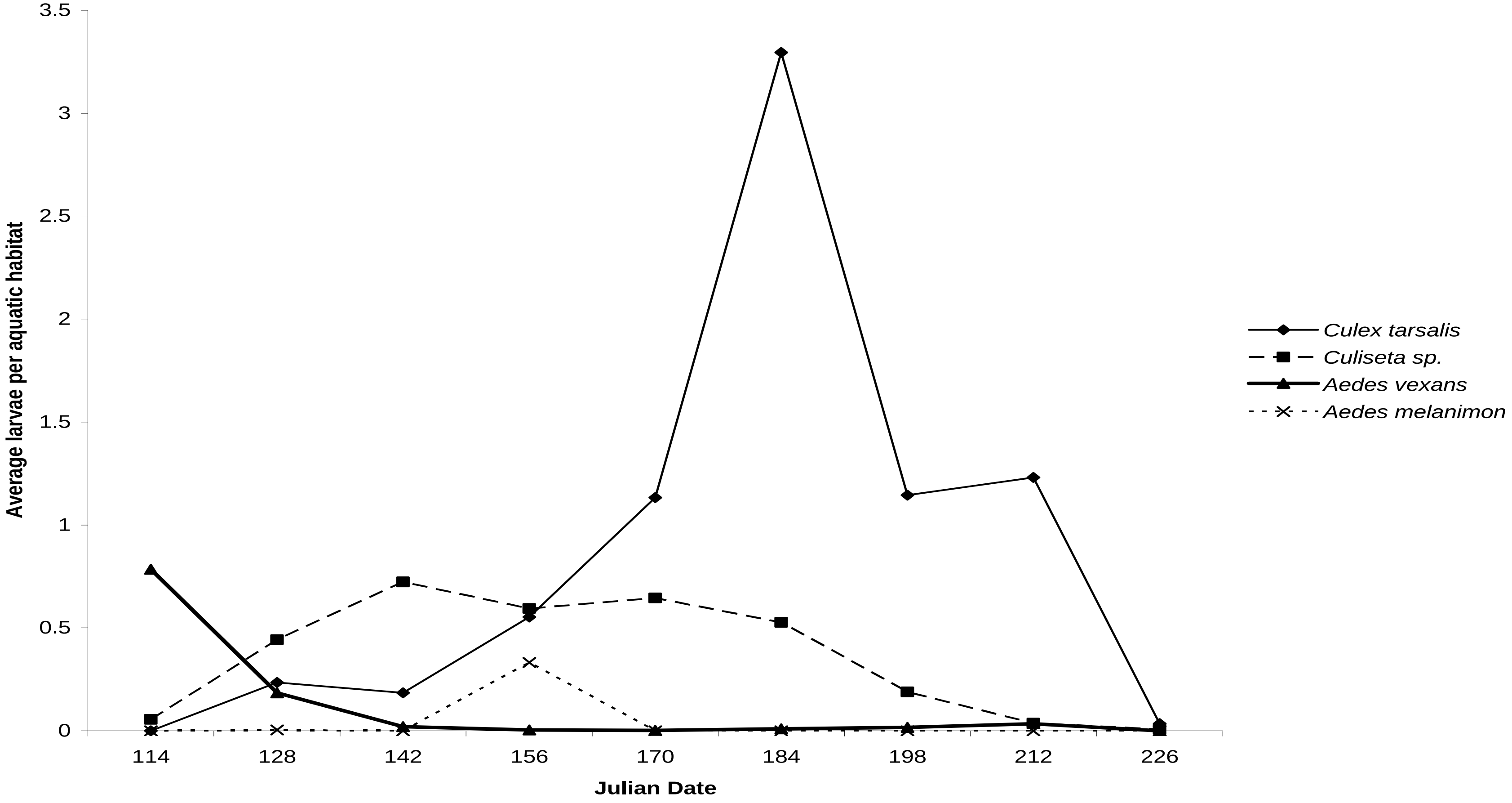

Figure 10. Timing of larval production for four species of mosquitoes in the Powder River Basin, WY, 13 May – 24 August, 2005.

Table 6. Weekly larval mosquito mean counts per dip (SE) by study area for the four most abundant larval species collected, Powder River basin Wyoming, 2005.

Julian Date	128 Week 1	142 Week 2	156 Week 3	170 Week 4	184 Week 5	198 Week 6	212 Week 7	226 Week 8	Season
					Culex tarsalis				Total
Agriculture	0.00(0)	0.38(.71)	0.25(.67)	0.38(1.01)	1.13(1.32)	1.07(1.16)	1.43(1.21)	0.37(.85)	.47(.33)
Natural	0.00(0)	2.84(.71)	1.06(.67)	5.07(1.01)	7.32(1.57)	32.55(1.37)	29.45(1.43)	1.45(2.17)	4.28(.43)
New CBNG	na	0.64(.71)	0.32(.67)	0.78(1.01)	5.48(1.32)	13.97(1.16)	7.01(1.21)	2.06(1.07)	2.97(.43)
Old CBNG	na	2.93(.71)	2.33(.67)	2.76(1.01)	1.96(1.32)	23.85(1.16)	1.30(1.21)	13.01(1.07)	4.12(.43)
CBNG Outlet	na	0.00(1.00)	0(.94)	1.72(1.47)	10.87(1.97)	141.59(1.71)	3.85(1.78)	13.67(1.57)	5.18(.51)
					Aedes vexans				
Agriculture	0.80(1.25)	0.64(.60)	0.00(.25)	0.00(.10)	0.15(.07)	0.43(.14)	0.43(.21)	0.53(.21)	.36(.12)
Natural	1.69(1.25)	4.44(.60)	0.48(.25)	0.25(10)	0(.07)	0.15(.15)	0.19(.24)	0.00(.43)	.70(.12)
New CBNG	na	0.38(.60)	0.15(.25)	0(.10)	0(.07)	0(.14)	0.15(.21)	0(.25)	.09(.12)
Old CBNG	na	1.65(.60)	0.25(.25)	0(.10)	0(.07)	0(.14)	0(.21)	0(.25)	.20(.12)
CBNG Outlet	na	0(.84)	0(.32)	0(.14)	0(.07)	0(.18)	0(.29)	0(.34)	0(0)
					Aedes melanimon				
Agriculture	0(0)	0(0)	0(0)	1.54(.59)	0(0)	0(0)	0(0)	0(0)	.20(.07)
Natural	0(0)	0.22(.10)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	.03(.07)
New CBNG	na	0(.10)	0(0)	0(.59)	0(0)	0(0)	0(0)	0(0)	0(0)
Old CBNG	na	0(.10)	0(0)	0(.59)	0(0)	0(0)	0(0)	0(0)	0(0)
CBNG Outlet	na	0(.10)	0(0)	0(.83)	0(0)	0(0)	0(0)	0(0)	0(0)
					Culiseta spp.				
Agriculture	0.59(.43)	1.90(1.02)	4.99(1.18)	2.96(.97)	3.23(.90)	0.78(.87)	5.61(.51)	0.63(.22)	2.02(.32)
Natural	0.25(.43)	2.23(1.02)	0.97(1.18)	2.10(.97)	6.46(1.06)	6.55(1.01)	0.57(.59)	0(.45)	1.67(.36)
New CBNG	na	1.31(1.02)	1.56(1.18)	0.15(.97)	0(.90)	0.15(.87)	0(.51)	0(.26)	.38(.32)
Old CBNG	na	2.80(1.02)	2.61(1.18)	0(.97)	0(.90)	0.59(.87)	0(.51)	0(.26)	.54(.32)
CBNG Outlet	na	0(1.48)	0(1.74)	3.60(1.41)	10.20(1.30)	6.63(1.24)	0(.71)	0.82(.35)	1.56(.39)

Agricultural sites produced two population peaks, one in early summer and another later in the year; both peaks coincided with the release of irrigation water on fields. The second peak produced more larvae of *Culiseta* in agricultural sites ($P = 0.02$) than in any other habitat type at that time of the year (Table 6).

Production of *Aedes vexans* differed ($P = 0.030$) between the five sampled aquatic habitats. Production was highest in natural habitats ($P = 0.030$), intermediate in agricultural and new and old CBNG sites, and absent from CNBG outlets (Table 6). Timing of production varied seasonally ($P = 0.0005$) and was highest across all habitat types in late May (Table 6). Natural water sources produced the highest mean *Ae. vexans* counts per dip on 22 May, 2005 (Julian date 142), likely due to flooding from snowmelt and spring rain events.

Production of *Aedes melanimon* was similar in agricultural and natural sites ($P = 0.27$); no larvae were captured in CBNG habitats of any type (Table 6). Timing of *Ae. melanimon* production varied seasonally ($P = 0.085$) with a peak in early summer (26 June, Julian date 177) (Table 6).

Larval Habitat Use.

Production of *Cx. tarsalis* differed ($P = 0.056$) between the four vegetative cover types (Figure 13). Production was higher in flooded upland vegetation than in open water, emergent or submergent cover types ($P < 0.00001$); very few larvae were collected from open water habitats that lacked vegetative cover ($\mu = 0.02$, SE = 0.08) (Figure 13).

Production of *Cx. tarsalis* also differed ($P = 0.01$) between plant types encountered during larval sampling (Figure 12). *Culex tarsalis* production was highest in forbes (μ= 1.03, SE = 0.12) followed by flooded upland grasses (μ= 0.88, SE = 0.11). Open shoreline with no vegetation, non-vegetated sampling points and those with woody plant cover harbored almost no larvae over the 2005 sampling season, and were not good predictors for *Cx. tarsalis* larval habitats.

Weather Data

Average monthly temperature and rainfall data for Sheridan, WY, May - August 2004 and 2005 indicate normal to below average temperatures in 2004 and 2005 (National Weather Service 2006). 2004 average temperatures ranged from 52.0 to 68.7 °F in 2004, and 51.0 - 72.0 °F in 2005 (Table 3). Departures from normal temperatures were -3.2 °F in 2004 and -0.1 °F in 2005. Average monthly rainfall in 2004 ranged from 0.56 - 1.72 inch in 2004 and 1.01 - 6.18 inches in 2005. Departures from normal rainfall was -2.17 inch in 2004 and +4.99 inch in 2005, indicating major changes in total rainfall between 2004 and 2005 field seasons. A total of 52 days accumulated > 0.01 inch of total rainfall between May and August 2004, with zero days accumulating > 1.00 inch rainfall. The 2005 field season included 64 days with > 0.01 inch total rainfall, with three days accumulating >1.00 inch total rainfall in May 2005.

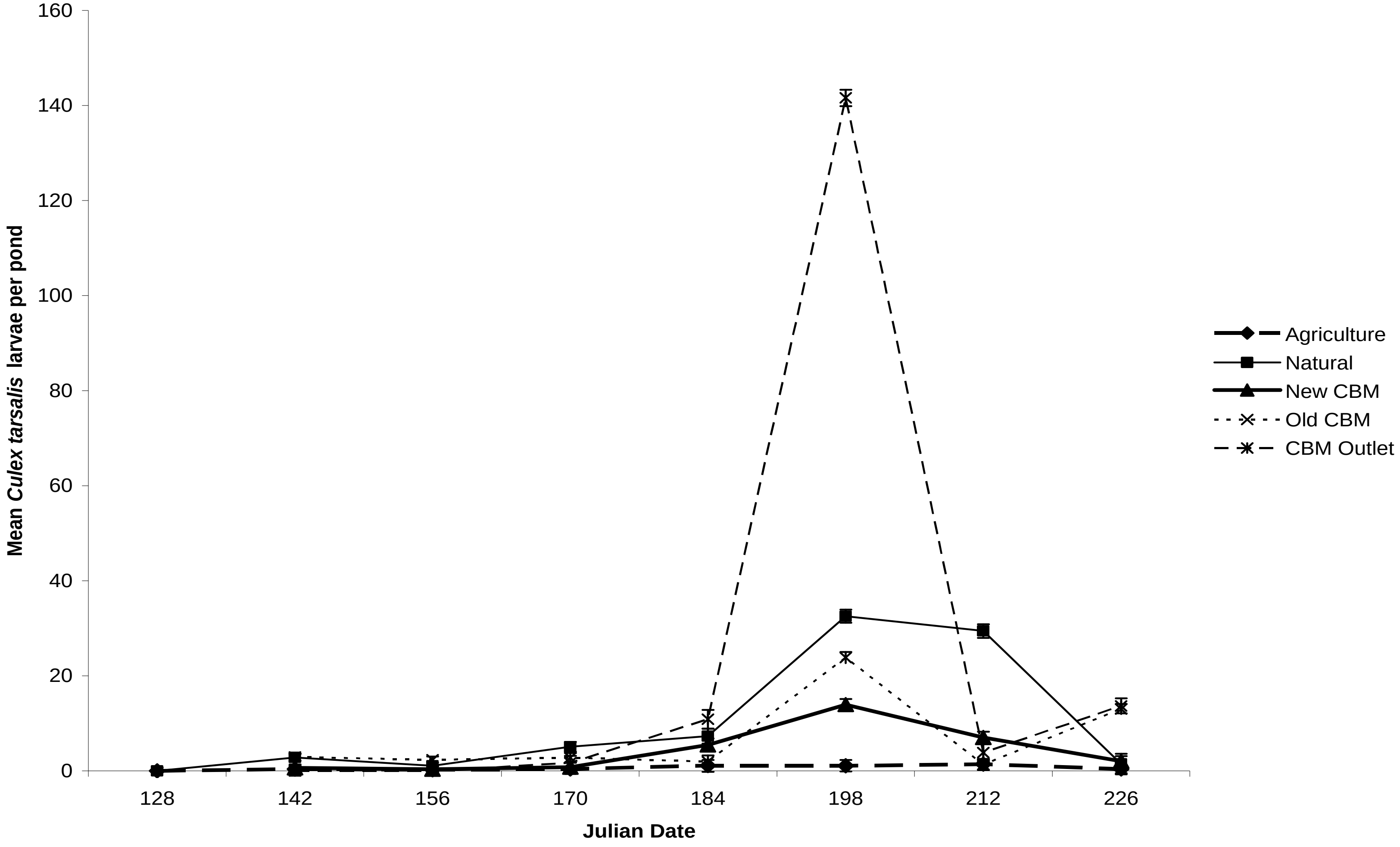

Figure 11. *Culex tarsalis* production over time by aquatic habitat in the Powder River basin, Wyoming, 2005.

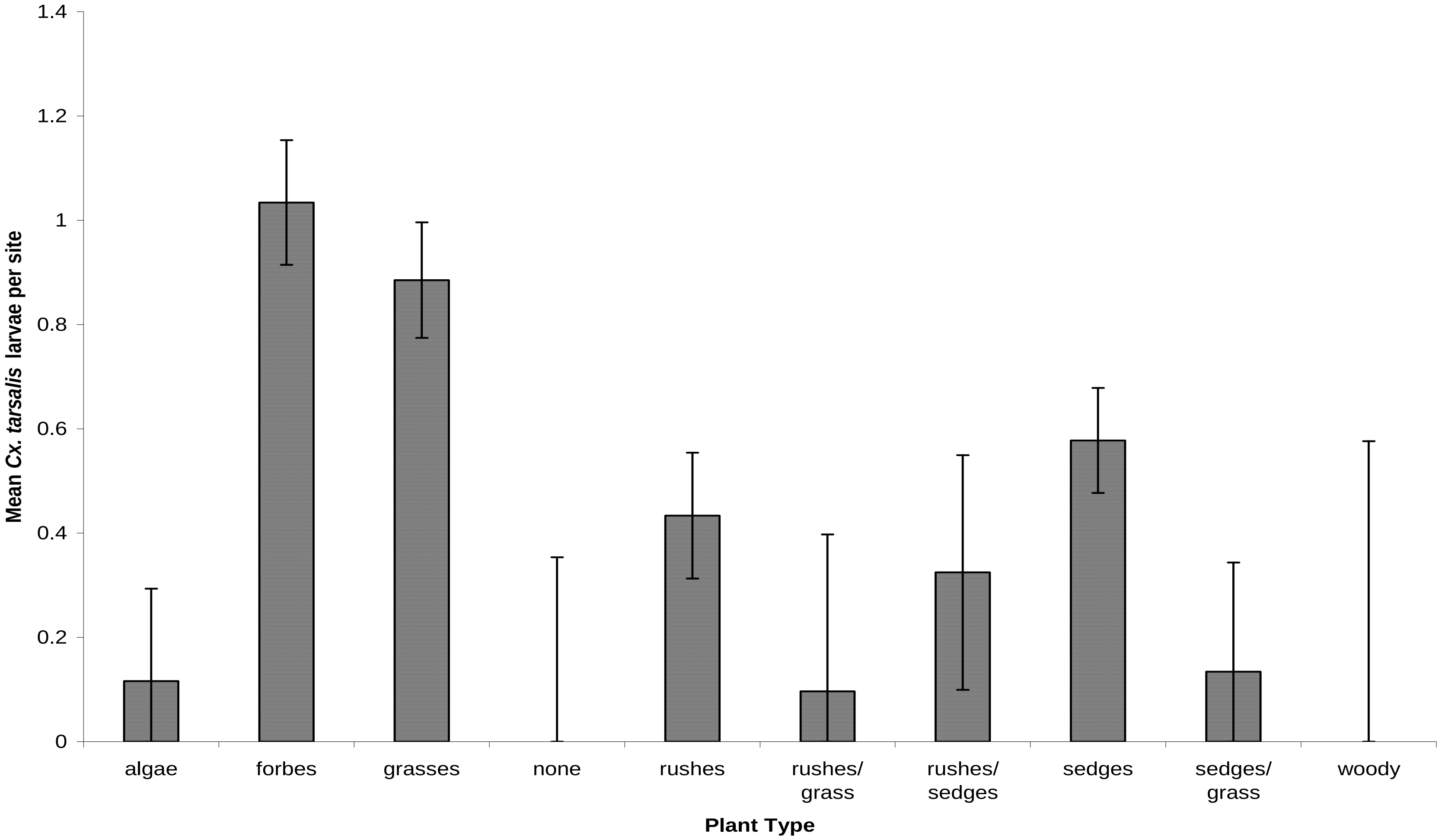

Figure 12. *Culex tarsalis* production by local habitat plant type across the Powder River basin, Wyoming for the week of 4 August 2005 (Julian date 216).

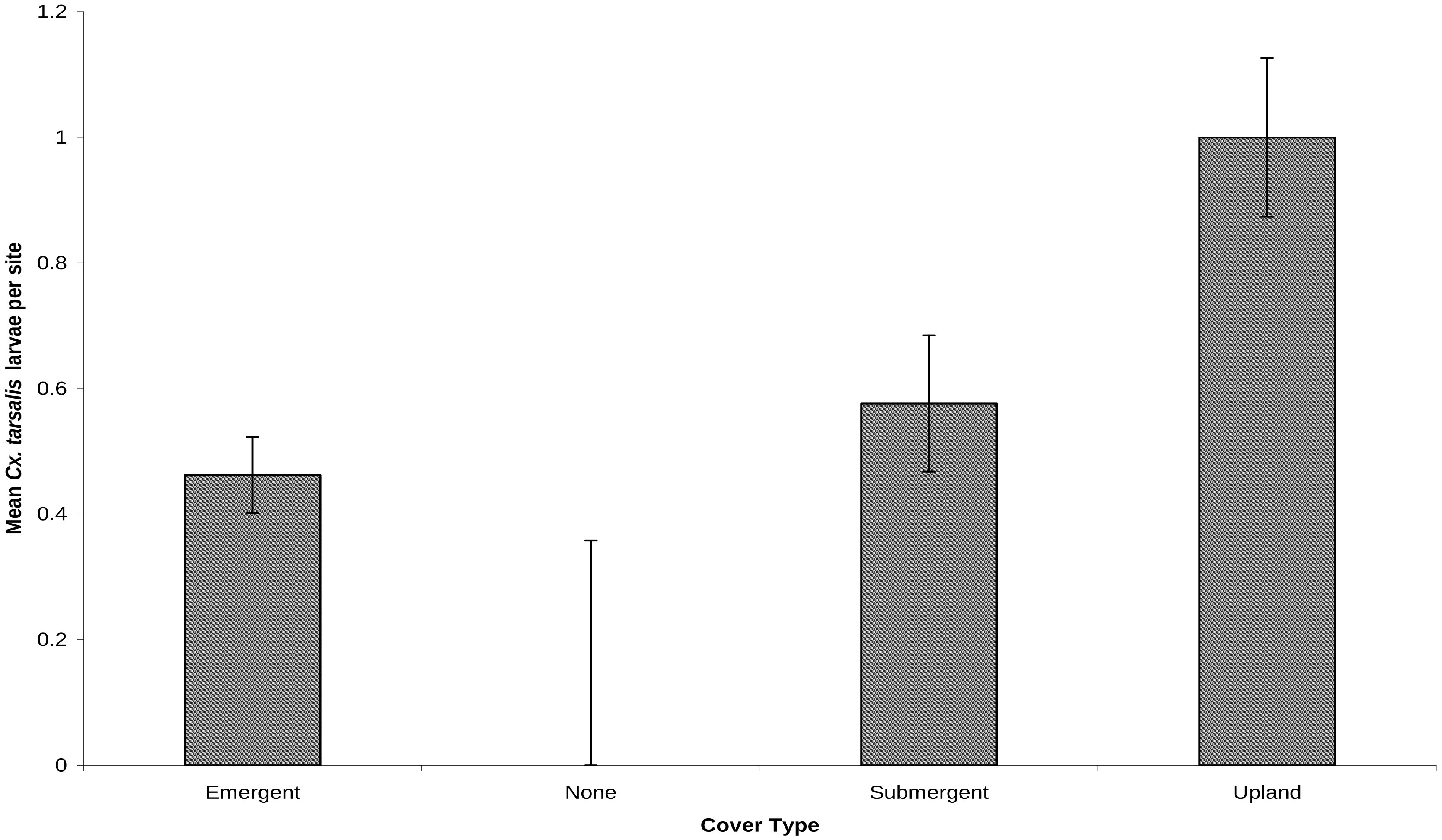

Figure 13. *Culex tarsalis* production by local habitat cover type across the Powder River basin, Wyoming for the week of 4 August 2005.

Discussion

New and mature CBNG ponds are producing *Cx. tarsalis* larvae similar to or above levels occurring in natural water sources in northeastern Wyoming. These site are also producing *Cx. tarsalis* over longer intervals than natural sites with peak larval production the week of 18 July (Julian date 198). This is comparable to *Cx. tarsalis* production in Nebraska, where the first larvae were found on 25 May, with peak production the week of 11 July (Julian date 191) (Edmunds 1955). The most productive areas for *Cx. tarsalis* larvae were CBNG pond outlets, which have been observed to fluctuate in water level in 2005 (observational data). In other areas *Cx. tarsalis* have been found in high abundances in freshly flooded ponds in Southern California, with peak populations several days after flooding ($x = 7$) (Beehler and Mulla 1995). Fluctuating water levels of CBNG ponds and pond outlets are similar flooded habitats to those studied in California, and are providing ovipositional sites for *Cx. tarsalis* above natural levels for this region.

High larval production of *Cx. tarsalis* in CBNG sites is consistent with high capture rates of adult *Cx. tarsalis* in light traps in 2005, showing that increased larval populations equate to an increased abundance of host-seeking vectors that spread WNV. Study areas with the highest adult *Cx. tarsalis* population also had the highest mosquito infection rates in 2004 and 2005, with mature CBNG sites having infection rates of 1.96 infected mosquitoes per 1000 in our 2005 study. In 2003, the U. S. Geological Survey indicated that 70% of WNV cases in humans in Wyoming were from the PRB, which accounts for approximately 11% of the counties in the state. That same year, survival of sage-grouse in natural gas fields in the Spotted Horse area of the PRB showed a 75% decline due to WNV infection, and currently have little ability to develop antibodies to this pathogen (Naugle et al. 2004, 2005, Walker et al. 2004).

Coal bed natural gas ponds do not currently produce significant amounts of *Ae. vexans* which are known vectors for Rift Valley Fever (RVF) in Eurasia and Africa (Ba et al. 2005). They also do not produce significant *Ae. melanimon*, which vector Western Equine Encephalitis (WEE) and Saint Louis Encephalitis (SLE) in the western hemisphere (Jensen and Washino 1991). Larvae of *Ae. vexans* or *Ae. melanimon* were most abundant in natural and irrigated agricultural sites, likely because these sites are ephemeral, providing muddy substrate for egg oviposition. I recommend that these habitats be closely monitored if the risk of RVF, WEE or SLE increases regionally.

Field studies in southern California indicated that *Cx. tarsalis* prefer aquatic habitats surrounded by grasses and annual vegetation with high populations of protozoans, bacteria and vegetation decay (Beehler and Mulla 1995, Fanara and Mulla 1974). Vegetation and high primary productivity provide food and cover for larval mosquitoes, making them an important component for ovipositional sites. My vegetation assessment indicates that both new and mature CBNG ponds as well as natural water sources are fulfilling these requirements for *Cx. tarsalis* habitats. Recent research using Landsat satellite imagery from the PRB found that CBNG development has resulted in a 75% increase of potential larval habitat for *Cx. tarsalis* (Zou et al. 2006). My larval sampling indicates that CBNG sites are potential larval habitats for *Cx. tarsalis*. As such

CBNG ponds are producing mosquitoes at a rate at or above natural water sources in this region.

Culex tarsalis do not prefer open water habitats as oviposition sites throughout their range (Jiannino and Walton 2004). In the PRB I found no *Cx. tarsalis* larvae in open water habitats throughout the 2005 field season. Modifying existing CBNG ponds to reduce aquatic vegetation and steepen shorelines may reduce *Cx. tarsalis* production in this region without providing habitats for other disease vectors such as *Cu. sonorensis*. Habitat modifications for *Cx. tarsalis* production have been used with some success in wastewater treatment ponds in southern California (Batzer and Resh 1992, DeSzalay and Resh 2000, Thullen et al. 2002). Coal bed natural gas ponds provide us a unique opportunity to experiment with habitat manipulation practices as vegetation can be completely removed from these areas without reducing the efficiency of the site as in a wastewater treatment facility.

Management Recommendations

I recommend a multi-dimensional approach to reduce mosquito production from CBNG ponds across the PRB (AMCA 2006). A three-pronged approach for mosquito control of *Cx. tarsalis* at CBNG sites would include 1) limit construction of new CBNG ponds for primary source reduction, 2) site modifications to new CBNG sites and retro-fitting existing ponds to reduce larval production. and 3) initiate mandatory use of larval control methods at existing CBNG sites.

The most effective way to reduce future mosquito production is to limit construction of additional CBNG ponds. One way to limit the number of newly created CBNG ponds is to re-inject water produced during the extraction process into sub-surface voids after gas is removed. If new CBNG ponds are not eliminated, then modifications such as the ones listed below to new and existing ponds would likely reduce mosquito production from these habitats. Following are seven distinct site modifications that if adhered to would minimize exploitation of CBNG ponds by *Cx. tarsalis:*

1. Overbuild the size of ponds to accommodate a greater volume of water than is discharged. This will result in un-vegetated and muddy shorelines that breeding *Cx. tarsalis* avoid (De Szalay and Resh 2000). This modification may reduce *Cx. tarsalis* habitat but could create larval habitat for *Culicoides sonorensis*, a vectorfor blue tongue disease, and should be used sparingly (Schmidtmann et al. 2000). Steep shorelines should be used in combination with this technique whenever possible (Knight et al. 2003).

2. Build steep shorelines to reduce shallow water (>60 cm) and aquatic vegetation around the perimeter of impoundments (Knight et al. 2003). Construction of steep shorelines also will increase wave action that deters mosquito production, and create more permanent ponds that are a deterrent to colonizing mosquito species like *Cx. tarsalis* which prefer newly flooded sites with high primary productivity (Knight et al. 2003).

3. Maintain the water level below that of rooted vegetation for a muddy shoreline that is unfavorable habitat for mosquito larvae. Rooted vegetation includes both aquatic and upland vegetative types. Avoid flooding terrestrial vegetation in flat terrain or low lying areas. Aquatic habitats with a vegetated inflow and outflow separated by open water produce 5 -10 fold less Culex mosquitoes than completely vegetated wetlands (Walton and Workman 1998). Wetlands with open water also had significantly less stage III and IV larval instars which may be attributed to increased predator abundances in open water habitats (Walton and Workman 1998).

4. Construct dams or impoundments that restrict down slope seepage or overflow by digging ponds in flat areas rather than damming natural draws for effluent water storage, or lining constructed ponds in areas where seepage is anticipated (Knight et al. 2003). Seepage and overflow results in down-grade accumulation of vegetated shallow water areas that support breeding mosquitoes.

5. Line the channel where discharge water flows into the pond with crushed rock, or use a horizontal pipe to discharge inflow directly into existing open water, thus precluding shallow surface inflow and accumulation of sediment that promotes aquatic vegetation.

6. Line the overflow spillway with crushed rock, and construct the spillway with steep sides to preclude the accumulation of shallow water and vegetation.

7. Fence pond site to restrict access by livestock and other wild ungulates that trample and disturb shorelines, enrich sediments with manure and create hoof print pockets of water that are attractive to breeding mosquitoes.

The third and final part of my three-pronged approach is to initiate the use of larval control methods at CBNG ponds that have been sampled and are positive for mosquito larvae. Treating CBNG ponds with larvicides such as *Bacillus thuringiensis* var. *israelensis* (Bti) have been shown to provide a 90-100% reduction in *Ae. vexans* and *Culex* spp. larvae in California, and these materials could be used in CBNG ponds to control larvae during weeks of peak densities (Berry et al. 1987, Russel et al. 2003). Other products such as methylated soy oils (LD_{50} 3.8μl/cm^2) and insect growth regulators (81% pupal inhibition over 5 weeks) can also be effectively used to control larvae with additional cost and personnel training in application procedures (Lampkin et al 2000, Woodrow et al. 1995).

Larvicide treatments of CBNG ponds should be conducted by certified pesticide applicators that have been trained to identify mosquito breeding habitats in the field, and can efficiently distribute larviciding materials according to product guidelines. The key to managing mosquito production with larvicide materials is to place the product in areas of high larval densities (Berry et al. 1987). Trained field personnel will need to visit potential mosquito production areas on a weekly or bi-weekly basis to check for mosquito production. Treatment will then need to be administered when 1) appropriate larval densities are found (ex. 5 larvae per dip) and 2) when larvae sampled are in a target genus (ex. *Culex* spp.). When larvicides are applied they should be used in concentrations according to product guidelines, and only in aquatic areas that are know larval mosquito habitats including flooded upland grasses and emergent aquatic vegetation.

Lastly, additional research is being conducted to assess the efficacy of using native larvivorous fishes to control mosquito population CBNG ponds. It is possible that a combination of water re-injection, CBNG pond modification and larvivorous fishes could be used to reduce the overall mosquito production without the need for a long-term labor-intensive mosquito management programs surrounding CBNG development. Until this is known, this three-pronged approach to managing mosquito production is prudent to reducing the risk of disease to humans and wildlife in the PRB.

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