AN ABSTRACT OF THE DISSERTATION OF

Jill S. Townzen for the degree of Doctor of Philosophy in Environmental Science on August 12, 2008
Title: Examining Parameters of Vectorial Capacity for Mosquitoes Associated with Stormwater Catch Basins in Corvallis, Oregon

Abstract approved:

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This research examines two of parameters of vectorial capacity for mosquitoes associated with catch basins in Corvallis, Oregon. The parameters of interest were determining 1) abundance of the mosquito species associated with the catch basins and 2) feeding patterns of local mosquito species. Three species of mosquitoes were collected from Corvallis catch basins: *Culex pipiens* L., *Culex stigmatosoma* Dyar, and *Culiseta incidens* (Thomson). Over 32 weeks of sampling 60 catch basins in Corvallis in 2004, a total of 1,920 catch basin visits were made and 79,760 immature mosquitoes were collected. Emerging mosquitoes were collected from 20 catch basins in southern Corvallis for 22 days in August 2006 and from 20 catch basins in northern Corvallis for 20 days in September 2006. Based on the numbers of mosquitoes collected from the 20 catch basins sampled, an estimated 138,484 female *Cx. pipiens* emerged from the all of the catch basins in the southern area and 84,432 emerged from the northern catch basins. Molecular analysis of the bloodmeals from *Cx. pipiens*, *Cx. stigmatosoma*, and *Cs. incidens* collected in Corvallis parks and greenspaces found

that the two *Culex* spp. fed primarily on avian hosts and *Cs*. *incidens* fed primarily on mammalian hosts. Based on the abundance and host feeding pattern data collected, all three mosquitoes could be involved in epizootic and epidemic transmission of mosquito-borne encephalitis, including West Nile virus, in Corvallis, if the virus were present.

Examining Parameters of Vectorial Capacity for Mosquitoes Associated with Stormwater Catch Basins in Corvallis, Oregon

by
Jill S. Townzen

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Jill S. Townzen, Author

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CONTRIBUTION OF AUTHORS

Dr. Andrew V. Z. Brower assisted with the design of Chapters 4 and 5 and provided funding and access to the laboratory resources necessary to conduct this research.

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CHAPTER 1. INTRODUCTION

RESEARCH OVERVIEW

Recent changes to the Clean Water Act, including stricter enforcement of stormwater discharge regulations, are increasing the numbers of stormwater management structures built in urban areas. One such structure, the sumped catch basin, is designed to hold water, inadvertently providing habitat for ovipositioning and immature mosquitoes. This project investigated two of the parameters of vectorial capacity for mosquitoes associated with urban stormwater catch basins by addressing the following questions:

1) Are the catch basins in Corvallis suitable habitat for mosquito larvae?
    a. If so, which species and how many (Chapters 2 and 5)?
2) What is the feeding habit of the mosquito species associated with the catch basins (Chapters 3 and 4)?

Field investigations will provide data that may be used to determine potential risk from mosquitoes associated with catch basins. Though the study was conducted in Corvallis, Oregon, it will provide baseline data for use in other areas of the Pacific Northwest. Additionally, new techniques for studying the feeding habit of insect vectors were developed.

BACKGROUND ON VECTOR-BORNE DISEASE

In the late 1890s, mosquitoes in the genus *Anopheles* were shown to transmit malaria parasites, and just a few years later a mosquito in a different genus, *Aedes*

aegypti (Linnaeus), was implicated in the transmission of Yellow Fever. Since then, a great deal of resources have been devoted to modeling and controlling mosquito populations and mosquito-borne disease. These efforts are complicated due, in part, to the diversity of mosquitoes. World wide there are over 42 genera and 3550 recognized species of mosquito (WRBU 2005). Mosquitoes manifest this diversity in numerous ways, one of the most obvious is appearance; they can range from the bright metallic coloring of *Sabethes cyaneus* (Fabricius) to the drab brown of *Culex pipiens* (Linnaeus). More importantly, however, they also vary in oviposition habitat, feeding habit and ability to transmit disease. For example the larvae of *Culex apicalis* Adams are commonly found in fresh water wetlands, the adult stage feeds primarily on reptiles and is generally not thought to be involved in disease transmission. In contrast, immature *Aedes albopictus* (Skuse) are commonly collected from artificial containers including tires and buckets; adults readily feed on humans and are competent vectors of several diseases. All of these differences play a role in modeling mosquito-borne disease transmission.

Beginning in 1909 with work conducted by Ronald Ross, there is a nearly one hundred year history of mathematically modeling the mosquito-borne disease malaria. In the 1950s Ross' work was expanded upon by George Macdonald and the resulting model, commonly called the Ross-MacDonald malaria model, is the basis for much of the mosquito-borne disease research being conducted today. The Ross-MacDonald model mathematically predicts the basic reproduction rate of malaria, that is, the number of subsequent cases of malaria that may arise from a single case. It is from

the Ross-MacDonald model that the mathematical equation for vectorial capacity was developed (Garrett-Jones 1964).

Malaria is an ideal mosquito-borne disease to model because of its relatively simple transmission cycle. The malaria parasite requires both a mosquito and human host to reach reproductive maturity, no intermediate host, and relatively few species of mosquitoes are involved. Vectorial capacity, as derived from the Ross-MacDonald model for the basic reproduction rate of malaria, represents the number of malaria infections that a population of vectors could distribute per case per day at a set place and time. The mathematical expression for vectorial capacity from Garrett-Jones (1964) is:

$$C = -\frac{ma^2p^n}{\log p} \quad ,$$

where m is the density of the vector relative to the human hosts, a is the man-biting habit of the vector, p is the probability of daily survival of the vector and n is the intrinsic incubation period of the malarial parasite. This quantity will obviously be variable by geographic area and time; it is therefore site specific and calculated from field studies. The man-biting habit a can be calculated from the human blood index of the mosquitoes and the frequency at which the mosquito feeds. Human blood index, the proportion of a mosquito's bloodmeals taken from humans, is variable by mosquito species and affected by host availability. It is generally estimated in the field. Feeding frequency is species-specific, though it may also vary with temperature, and most easily estimated from laboratory calculations. The man-biting habit is expressed twice in the equation because the mosquito must feed twice; first to pick up

the parasite, assuming the first host is infected, and then again to infect a new host. Finally, the probability of daily survival *p* is self explanatory, and again species specific and estimated from laboratory calculations. One benefit of using the vectorial capacity model to study vector-borne disease is that it models potential disease transmission; presence of a pathogen is not a necessary component of the model (Smith and McKenzie 2004).

Numerous authors (Bruce-Chwatt 1976**,** Bailey 1982**,** Nedelman 1984 and others) have published extensive reviews of malaria modeling research, and it is not my intent to review that work again here. My goal is to explore how that research can be applied to the investigation of vectorial capacity for local mosquitoes and diseases other than malaria. Arboviruses, short for arthropod-borne viruses, are endemic in many areas of the United States and the introduction of new arboviruses is no longer just a possibility. These vector-borne diseases have transmission cycles that are much more complex than that of malaria.

The transmission cycle for most arboviruses includes an enzootic (non-human) cycle and an epidemic (human) cycle. For example, the enzootic cycle of Yellow Fever involves monkeys, while birds are the primary reservoirs for St. Louis encephalitis. In both cases there is a mosquito, or group of mosquito species (enzootic vectors), involved in the enzootic cycle and a different mosquito, or group of mosquitoes (bridge vectors) involved in the epidemic cycle. Zoonotic vectors typically have a narrow range of hosts they will take a bloodmeal from. For example, *Culiseta melanura* (Coquillett), a zoonotic vector of Eastern equine encephalitis, feed primarily on birds which are the zoonotic reservoir for the disease. When large

numbers of enzootic reservoirs become infective, bridge vectors may pick up the virus from an infected animal and transmit it to a human the next time it feeds. An example of a bridge vector for Western Equine encephalomyelitis is *Culex tarsalis* Coquillett, which feed indiscriminately on birds and mammals. This complexity makes it much more difficult to model patterns of disease transmission for these viruses. In the case of an arbovirus in which humans are dead end hosts, meaning that humans do not have a viremia high enough to infect more mosquitoes, a high man biting habit is not as important as bloodmeal index that involves both the enzootic reservoir and humans.

The introduction of a new mosquito-borne disease to the United States is no longer just a potential. In the summer of 1999 West Nile virus (WN), an arbovirus not previously seen in the United States, was detected in New York City, New York. West Nile virus is an encephalitis virus that can cause a swelling of the brain. Severe cases can lead to long term disability or death. In the first two years after its appearance there were 149 reported human cases of the disease and 18 deaths. The newly introduced virus has since spread westward and has now been detected in all of the lower 48 states. It is currently the most widespread arbovirus in the United States, and may have long lasting effects. Researchers in New York found that only 37% of patients diagnosed with the neuroinvasive form of WN in 1999 were fully recovered after 12 months (Klee et al. 2004). In 2002 there were 4156 human cases of West Nile virus in the United States and the short-term medical costs associated with neuroinvasive cases alone has been estimated at $63.1 million (Zorhabian et al. 2004). The seriousness and quick spread of the disease has garnered intense media attention, making the disease the most important arbovirus in the US.

A retrospective cluster analysis of cases of WN during 2002 in Chicago, Illinois, found an association between cases and man-made catch basins that were not being treated for mosquitoes (Ruiz et. al. 2004). While there is no direct evidence at this time that catch basins, or the mosquitoes that oviposit in them, are responsible for the spread of WN, it does indicate that the relationship between mosquitoes and catch basins requires further study.

URBAN DEVELOPMENT AND MOSQUITOES

The oviposition habitats of mosquitoes are nearly as diverse as the mosquitoes themselves. Immature mosquitoes can be found in almost every type of standing water. Laird (1988) classified 11 different types of habitat: flowing streams, ponded streams, lake edges, swamps and marshes, shallow permanent ponds, shallow temporary ponds, shallow temporary pools, intermittent ephemeral puddles, natural containers, artificial containers, natural subterranean waters, and finally artificial subterranean waters. Each of these categories can, in turn, be broken into sub-categories based on size, water quality and seasonal appearance. The number of different larval habitats that a single species of mosquito will inhabit is another area where mosquitoes exhibit great diversity. Some species prefer a very specific immature habitat, for example *Deinocerrites cancer* Theobald, commonly called the crabhole mosquito because of its obligate immature habitat. Others are much less restricted. For example *Culiseta incidens* (Thomson) can be found everywhere from lake and stream margins to puddles and artificial containers. The larval habitat of interest to this paper is the stormwater catch basin. These are artificial containers

designed to manage stormwater, however, because of their design they also provide habitat for immature mosquitoes.

An increase in mosquito habitat in urban areas has the potential to increase the risk of mosquito-borne disease. In 1987 Congress amended the Clean Water Act to cover stormwater discharges into natural waterways of the United States (EPA 2003). The amendment required the Environmental Protection Agency to establish programs to phase in stricter stormwater quality regulations beginning in 1990. One way to increase the water quality of stormwater is to impound it before it is released. The goal of cleaner water is admirable; however it is also important to realize that impounding stormwater to "clean" it may be inadvertently increasing mosquito oviposition habitat in urban areas.

In 1998 the California Department of Health Services Vector-Borne Disease Section, began a three year study examining stormwater best management practices (BMPs) for mosquito production. The initial study examined the variety of BMPs used in California (CDHS 2002) then expanded to include the practices of seven areas of the United States outside of California through onsite visits and questionnaires sent out to vector and mosquito control programs (CDHS 2001). The study focused primarily on large BMPs, and concluded that those with sumps or catch basins were likely to contribute to mosquito production (CDHS 2001). The study also found that location of the BMP played a role in the numbers of mosquitoes produced at a site. Unfortunately, this study did not specifically look at street catch basins, a commonly used BMP that can be quite numerous in urban areas.

Even though catch basins are designed to hold water (Figure 1.1) they are often overlooked as sites for mosquito oviposition. Catch basins are designed to impound water with an outflow pipe located above the bottom of the basin, creating the sump. Sump depth varies depending on the amount of rainfall anticipated in the first ten minutes of a storm. Impoundment of the water allows particulate matter washed off streets to settle out.

Several authors (Geery and Holub 1989, Knepper 1992, McCarry 1996, Pfuntner 1978, and Siegel 1999) have examined catch basins for mosquito larvae. However, the focus of these papers is generally on the control of mosquitoes that are found in them. Only two, Geery (1989) and Pfuntner (1978), further investigated the relationship between mosquitoes and catch basins. Pfuntner (1978), in an effort to increase the efficacy of control methods, evaluated the amount of time it took two mosquito species common in Southern California catch basins, *Culex quinquefaciatus* Say and *Culex stigmatasoma* Dyar, to reach the adult stage after eggs were laid in a catch basins. Geery and Holub (1989) evaluated seasonal abundance of mosquitoes associated with catch basins in Cook County, Illinois, but made no effort to determine if production was the same between the two study areas. The importance of catch basins as mosquito habitat is underscored by Ruiz et al. (2004). In this paper a geospatial cluster analysis of the West Nile virus cases in Chicago, Illinois, 2002, found a positive association between cases and untreated catch basins. More research is needed to further explore the mosquito-catch basins relationship as it relates to where the catch basins are located and how the catch basins are designed.

Catch basins are not a new phenomenon, and they have a long history of use, especially on the East Coast. On the other hand, their use on the West Coast is steadily increasing. Corvallis, Oregon, is an ideal location to conduct this research for two reasons. First, there is currently no mosquito control in the city, so the mosquitoes in the catch basins are not artificially suppressed. Second, though sumped catch basins have been used here for nearly 30 years their numbers are rapidly increasing. Corvallis spans approximately 14 square miles, has a population of nearly 53,000 people, and is surrounded by rural agricultural land. The city covers a diverse landscape from the wooded hills on the north and west sides of the city to the flood plains of the Willamette and Mary's rivers in the southern region. The city is also home to nearly 1,700 acres of park lands. This diverse landscape increases the hosts and habitat available to mosquitoes in different areas of the City. Currently, there are over 153 miles of storm drains in Corvallis that include over 7500 sumped catch basins (Figure 1.2), an increase of more than over 1500 sumped catch basins since the start of this project in 2004 (Corvallis 2004). This represents a significant amount of potential larval mosquito habitat as well.

The City of Corvallis utilizes two types of catch basins (Figure 1.3). The first is approximately 58.5 centimeters by 76.5 centimeters for an available surface area of 4475.25 square centimeters (1.3.A). The second is smaller 34 centimeters by 54 centimeters or 1836 square centimeters of surface area (1.3.B). The depth from the top of the catch basin to the surface of the water and the depth of the water can vary for both designs. Another, more obvious, difference in the design of the two catch basins is the grate. The smaller of the two has an open grate built horizontally into the

surface of the street, while the larger of the two catch basins has a metal plate built into the sidewalk that allows access to the catch basins; the grate is built into the vertical curb edge of the sidewalk. Many of the agencies responsible for installation and maintenance of catch basins have two misconceptions regarding their potential to produce mosquitoes: 1) catch basins are dry by mid summer and 2) oil from car engines prohibits mosquito production. This project provides valuable information that can be used to educate stormwater managers on the potential risks associated with mosquitoes and catch basins.

BITING HABIT

Historically a variety of tests have been designed to analyze bloodmeals taken by individual mosquitoes. The most common methods used in the past are precipitin tests and enzyme-linked immunosorbent assays (ELISA). The precipitin test was developed in 1904 and first used in mosquito bloodmeal analysis in 1923 (Washino and Tempelis 1983). In the 1980's ELISA became popular for bloodmeal identification. Direct, indirect and sandwich ELISAs have been used (Chow et al. 1993) to detect avian and mammalian antibodies within mosquitoes, in some cases seven days after the mosquito has fed. Though ELISA can be both sensitive and specific, they are time-consuming to perform and the reagents used are sometimes difficult to obtain. Modern molecular polymerase chain reaction (PCR) techniques provide a test that is more sensitive, specific and easier to perform than the tests used in the past.

Recent PCR protocols have been developed to amplify *cytochrome b* (Cyt *b*) sequences for bloodmeal identification. Boakye et al. (1999) developed a PCR heteroduplex assay that was able to detect and identify mosquito bloodmeals 72 hours post ingestion. Ngo and Kramer (1999) used four order specific cytochrome b primers to identify avian bloodmeals in mosquitoes. However, there is some discussion on whether or not CytB is the appropriate gene to use. The current push for "DNA barcoding", the use of a short segment of cytochrome oxidase subunit I (COI) to identify all animal life, by Hebert and others (2003) suggests that this might be an appropriate gene region to use for bloodmeal identification. In fact mosquito bloodmeal analysis is an ideal application of the barcoding effort; as more organisms are barcoded the use of COI sequences in bloodmeal analysis will identify a wider range of hosts. Protocols were developed (Chapter 3) to use amplify CytB and COI sequences to identify the vertebrate hosts of mosquitoes collected in Corvallis (Chapter 4). GenBank does not have equal species coverage for the species and genes in their database, the use of two gene regions minimizes the numbers of gaps in the data. Having results for two gene regions will also serve as a check on sequence identification and provide an indication or contamination or other problems when the putative identities of bloodmeals from two gene regions do not match.

RESEARCH STRATEGY

This project evaluates the relative density and biting habit components of vectorial capacity by investigating bloodmeal index and size of the population. First the mosquito populations utilizing stormwater catch basins as immature habitat are

characterized (Chapters 2 and 5). Once the species utilizing the catch basins have been identified molecular techniques were used to determine what those species are feeding on in Corvallis (Chapters 3 and 4).

The first step in characterizing the mosquitoes associated catch basins was to determine if the Corvallis catch basins truly are habitat for immature mosquitoes, and if so which species were utilizing them and when they were present (Chapter 2). Further evaluation of the immature mosquitoes in the catch basins determined whether both catch basins support mosquitoes in similar numbers. Because the larger of the two catch basins is more protected (Figure 1.3.A) it may be a better habitat for immature mosquitoes than the smaller, more open, catch basins (Figure 1.3.B). Finally, before an attempt to measure the size of the population was made it was also necessary to determine if mosquitoes are utilizing catch basins equally in all areas of the city.

Once it was determined that catch basins are suitable habitat for immature mosquitoes the size and density of the population utilizing catch basins was be estimated based on numbers of emerging adults. Modified emergence traps were used to determine the numbers of adult mosquitoes emerging from each study catch basin over a specific period of time period (Chapter 5).

To determine the role mosquito utilizing catch basins might play in mosquito-borne disease transmission the feeding habit of mosquitoes collected in Corvallis, including those utilizing catch basins, will be determined using modern molecular techniques. Primers were developed for Cyt *b* and cytochrome COI regions of mitochondrial DNA that amplify vertebrate DNA while excluding DNA from the

mosquito (Chapter 3). Several methods were used to collect bloodfed mosquitoes for the bloodmeal study. Walk in red boxes were used as artificial resting habitat for mosquitoes in urban greenspaces and parks throughout Corvallis, and it was from these sites that the majority of the bloodfed mosquitoes were collected. However, collections were augmented with a few mosquitoes collected using carbon-dioxide baited traps, gravid traps, and black-light traps hung directly in catch basins. Only locally collected, blood-engorged, mosquitoes were used to determine the vertebrate host feeding pattern. The abdomens of individual mosquitoes were mechanically homogenized and screened for mammalian and avian DNA. When detected, amplified regions were sequenced and compared against published GenBank sequences for species identification. This information was then used to determine the vertebrate host feeding pattern for local mosquitoes (Chapter 4).

REFERENCES

Bailey NTJ. 1982. *The Biomathematics of Malaria*. Oxford University Press, New York, NY.

Boakye DA, Tang J, Truc P. 1999. Identification of bloodmeals in haematophagous Diptera by cytochrome B heteroduplex analysis. *Med Vet Entomol* 13: 282-287.

Bruce-Chwatt LJ. 1976. Mathematical models in the epidemiology and control of malaria. *Trop Geogr Med* 28: 1-8.

CDC, 2004. West Nile virus homepage. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector Borne Infectious Disease, Ft. Collins, CO. [Accessed April 2008] http://www.cdc.gov/ncidod/dvbid/westnile/index.htm. Accessed 2000 – 2004.

CDHS. 2001. A preliminary assessment of vectors associated with stormwater management structures in the United States. California Department of Health Services, Vector Borne-Disease Section, Sacramento, CA.

CDHS. 2002. A three year assessment of mosquito production in structural best management practices in Southern California. California Department of Health Services, Vector Borne-Disease Section, Sacramento, CA.

CDHS. 2004. California West Nile virus homepage. [Internet] Available from California Department of Health Services, Sacramento, CA. [Accessed November 2004] http://www.westnile.ca.gov/.

Chow E, Wirtz RA, Scott TW. 1993. Identification of blood meals in *Aedes aegypti* by antibody sandwich enzyme-linked immunosorbent assay. *J Am Mosq Control Assoc* 9: 196-205.

Corvallis, 2004. City of Corvallis GIS data. [Internet] Available from Corvallis GIS ftp site. [Accessed November 2004] ftp://ftp.ci.corvallis.or.us/pw/gis/.

Dambacher JM, Levins R, Rossignol PA. 2005. Life expectancy change in perturbed communities: derivation and qualitative analysis. *Math Biosci* 197: 1-14.

Edman JD, Taylor DJ. 1968. *Culex nigripalpus*: seasonal shift in the bird-mammal feeding ratio in a mosquito vector of human encephalitis. *Science*. 161: 67-68.

Fonseca DM, Keyghobadi N, Malcolm CA. 2004. Emerging vectors in the *Culex pipiens* complex. *Science*. 303: 1535-1538.

Garrett-Jones C. 1964. The human blood index of malaria vector in relation to epidemiological assessment. *Bull World Health Organ* 30: 241-261.

Greery PR, Holub RE. 1989. Seasonal abundance and control of *Culex* spp. in catch basins in Illinois. *J Am Mosq Control Assoc*. 5: 537-540.

Hebert PDN, Cywinska A, Ball SL, deWard JR. 2004. Biological identifications through DNA barcodes. *Proc R Soc London B Biol Sci* 270: 313-321.

Kilpatrick AM., Kramer LD, Campbell SR, Alleyne EO, Dobson AP and Daszak P. 2005.West Nile virus risk assessment and the bridge vector paradigm. *Emerg Infec Dis* 11: 425- 429.

Klee AL, Maldin B, Edwin B, Poshni I, Mostashari F, Fine A, Layton M, Nash D. 2004. Long-term prognosis for clinical West Nile virus infection. *Emerg Infec Dis* 10: 1405-1411.

Knepper RG, Leclair AD, Strickler JD, Walker ED. 1992. Evaluation of Methoprene (Altosid ® XR) sustained release briquets for control of *Culex* mosquitoes in urban catch basins. *J Am Mosq Control Assoc* 8: 228-230.

Laird, M. 1988 *The Natural History of Larval Mosquito Habitats*. Academic Press, San Diego, CA.

McCarry MJ. 1996. Efficacy and persistence of Altosid ® Pellets Against *Culex* species in catch basins in Michigan. *J Am Mosq Control Assoc*. 12: 144-146.

Nedelman J. 1985. Some new thought about some old malaria models. *Math Biosci* 73: 159-182.Niebylski, M. L. and C. L. Meek. 1992. Blood-feeding of *Culex* mosquitoes in an urban environment. *J Am Mosq Control Assoc* 8: 173-177.

ODHS. 2004. West Nile virus page. [Internet] Available from Oregon Department of Health Services, Acute and Communicable Disease Prevention [Accessed April 2008] http://www.dhs.state.or.us/publichealth/acd/wnile/index.cfm.

Pfunter AR. 1978. The development and control of *Culex quinquefasciatus* Say and *Culex peus* Speiser in urban catch basins. *Proc & Pap 46th Annul Conf Calif Mosq Control Assoc* 46: 126-129.

Ruiz MO, Tedesco C, McTighe TJ, Austin C, Kitron U. 2004. Environmental and social determinants of human risk during a West Nile virus outbreak in the Greater Chicago area, 2002. *Int J Health Geogr* [Internet] Available at http:// www.ijhealthgeographics.com/ content/3/1/8.

Siegel JP, Novak RJ. 1999. Duration of activity of the microbial larvicide Vectolex CG® (*Bacillus sphaericus*) in Illinois catch basins and waste tires. *J Am Mosq Cont Assoc* 15: 366-370.

Smith DL, McKenzie FE. 2004. Statics and dynamics of malaria infection in Anopheles mosquitoes. *Malar J* [Internet] Available at www.malariajournal.com/content/3/1/13.

Smith JL, Fonseca DM. 2004. Rapid assays for identification of members of the *Culex* (Culex) *pipiens* complex, their hybrids and other sibling species (Diptera: Culicidae). *Am J Trop Med Hyg* 70: 339-345.

Tempelis CH. 1975. Host-feeding patterns of mosquitoes with a review of advances in analysis of bloodmeals by serology. *J Med Entomol* 11: 635-653.

Tempelis CH, Reeves WC. 1964. Feeding habits of one Anopheline and three Culicine mosquitoes by the precipitin test. *J Med Entomol* 1: 148-151.

Tempelis CH, Washino RK. 1967. Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley Callifornia, with notes on other species. *J Med Entomol* 4: 315-318.

Terry J. 2004. Oregon State Public Health Laboratory, Virology/Immunology Section. Personal Communication June 15, 2004.

Townzen KR. California Department of Health Services, Vector-Borne Disease Section. Personal communication June 2005.

USGS. 2004. West Nile virus maps. [Internet] Available from United States Geological Survey [Accessed November 2004]http://westnilemaps. usgs.gov/.

Vinogradova EB. 2000. *Culex pipiens pipiens Mosquitoes: Taxonomy, Distribution, Ecology, Physiology, Genetics, Applied Importance and Control*. Pensoft Publishing. Sofia, Moscow.

Washino RK, Tempelis CH. 1983. Mosquito host bloodmeal identification: methodology and data analysis. *Annu Rev Entomol* 28: 179:201.

WRBU. 2005. 2001 Systematics Catalog of the Culicidae [Internet] Available from Walter Reed Biosystematics Unit [Accessed November 2005] http://www.mosquitocatalog.org/main.asp

WHO. 2004. Integrated Vector Management. World Health Organization Regional Office for the Eastern Mediterranean. Cairo.

Zavaleta JO and Rossignol PA. 2004. Community level analysis of risk of vector-borne disease. *Trans R Soc Trop Med Hyg* 98: 610-618.

Zohrabian A, Meltzer MI, Ratard R, Kaafee B, Molinari NA, Kakoli R, Scott II RD, Peterson L. 2004. West Nile virus economic impact, Louisiana, 2002. *Emerg Infec Dis* 10: 1736-1744.

Figure 1.1. General catch basin design.

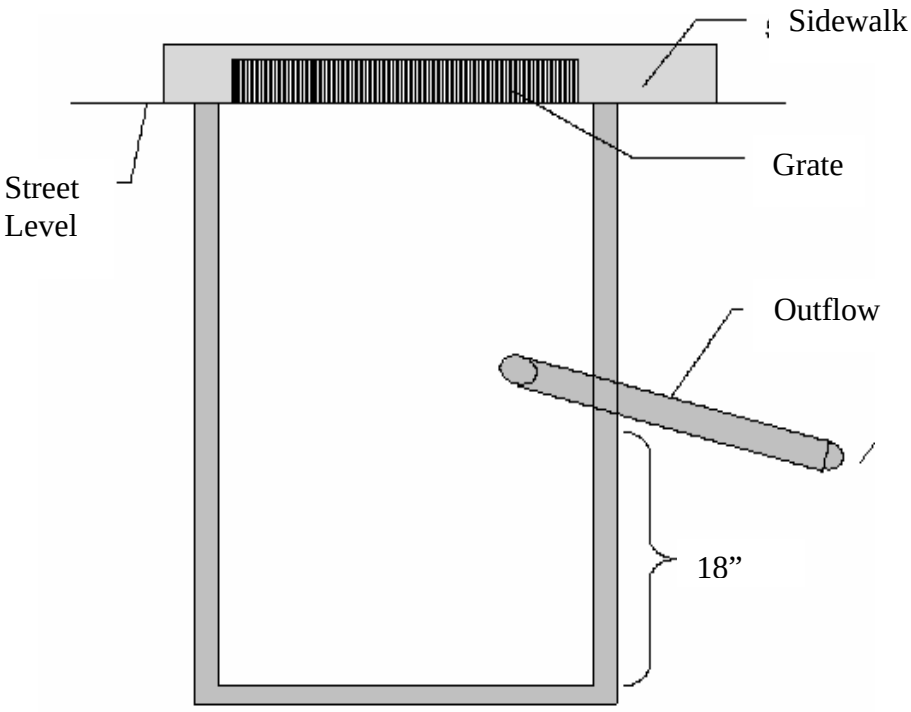

Figure 1.2. Location of catch basins in the City of Corvallis, OR.

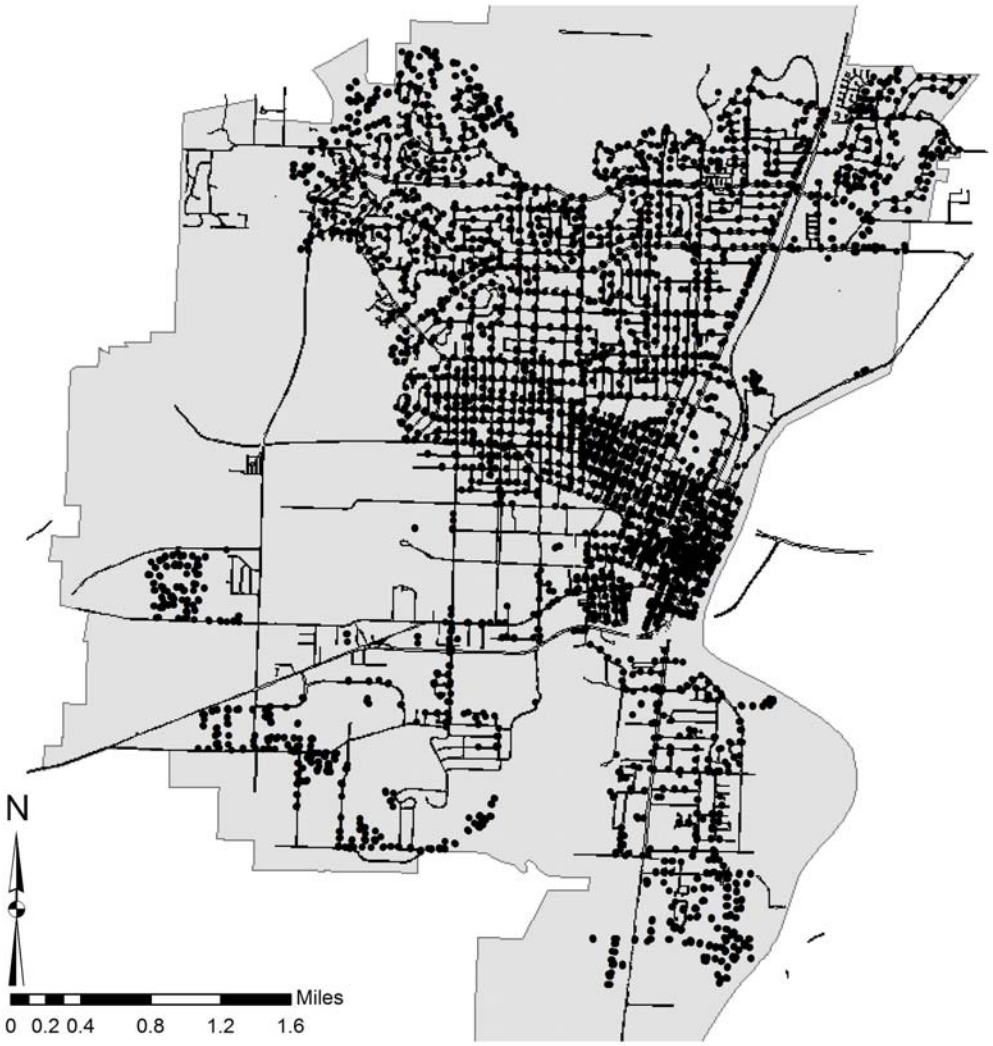

Figure 1.3. The two catch basin designs used in Corvallis, Oregon. A, the larger more protected catch basin and B, the smaller more open catch basin.

A.

B.

CHARACTERIZATION OF STORMWATER CATCH BASINS AS HABITAT FOR IMMATURE MOSQUITOES IN CORVALLIS, OREGON

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ABSTRACT

Stormwater management structures, including sumped catch basins, can be a source of nuisance and vector mosquitoes in urban and suburban areas across the United States. Corvallis, Oregon, is a city of over 50,000 people that does not currently manage mosquitoes. The primary goal of this study was to determine the species diversity and phenology of mosquitoes that use the catch basins in Corvallis as immature habitat. Sixty sumped catch basins were sampled weekly for mosquitoes from March through October 2004. In all, 1,920 samples were collected, which contained a total of 79,760 mosquitoes. *Culex pipiens* constituted nearly 83% of the mosquitoes sampled. Two other species were also routinely collected: *Culiseta incidens* (10%) and *Culex stigmatosoma* (7%). None of the parameters studied (location, catch basin design, nor environmental conditions) affected the presence of larval mosquitoes.

Key Words: Immature mosquito habitat, urbanization, Clean Water Act, larval control, temperature,

INTRODUCTION AND BACKGROUND

When it rains in urban areas, stormwater flows over impervious surfaces and carries away trash, oil, and other common pollutants. Without proper water management this runoff flows directly into natural waterways, resulting in an estimated 50% of water pollution in the United States (Copeland 2006). Although the Federal Water Pollution Control Act of 1948, commonly called the Clean Water Act (CWA), marked the first earnest attempt in the United States to mandate curtailing water pollution, it was not until 1987 and the Water Quality Act that the CWA was amended to include regulations for managing nonpoint sources, including stormwater runoff in urban areas. The Water Quality Act required medium to large municipalities (cities with populations over 100,000 people) to manage nonpoint sources within seven years. Smaller municipalities were allowed an additional nine years to become compliant with the act (Copeland 2006). As a result of tightening regulations governing stormwater discharges, there has been an increasing reliance on manmade structures to impound runoff in urban and suburban areas.

Efforts to manage stormwater focus on permanently retaining runoff, temporarily detaining runoff and filtering runoff (Smith and Shisler 1981, Metzger et al. 2003). Management structures come in a variety of designs that can mitigate pollution from sediments, heavy metals, and other debris (Metzger et al. 2003). Stormwater catch basins are management structures associated with the street drains found in many cities throughout the country. While they are smaller than many other stormwater structures used in urban areas, they are also more numerous. Many street drains lead directly into storm drains, sewer systems or a combination of both.

However, enforcement of stormwater regulations has increased the use of sumped catch basins, which are designed with an outflow pipe located above the bottom of the basin (the sump) in order to impound water (Kronenwetter-Koepel 2005 provides a good illustration of a sumped catch basin). During rain events particulates and other pollutants are washed off impervious services and into the basins. When properly maintained, impounding water in catch basins removes to 10 to 25% of particulate matter and lead from stormwater runoff, and it has been suggested that larger sumps would enhance pollution control (Pitt et al. 1999).

Any basin that contains standing water is potential habitat for oviposition and development of immature mosquitoes. While the association between mosquitoes and stormwater management structures is not new (Munstermann and Craig 1977, Smith and Shisler 1981), there has been a recent renewed interest in the topic due to the increased interest in stormwater management in general and worry over the introduction of new mosquito-borne disease (Kronenwetter-Koepel 2005, Gingrich et al. 2006, Rey et al. 2006, Butler et al. 2007, Henn et al. 2008, Kwan et al. 2008, Metzger et al. 2008). Even though catch basins are specifically designed to hold water, many of the agencies responsible for maintenance and installation of the devices are unaware of their potential role as habitat for immature mosquitoes. Two common responses to inquiries regarding mosquitoes and stormwater catch basins are: 1) catch basins are designed to go dry and 2) engine oil with kill any larvae that appear in them. Unfortunately, neither of these statements holds true, and a variety of mosquitoes have been collected from them. Although management of mosquitoes and the efficacy of control products in stormwater catch basins have been well studied

(Geery & Holub 1989**,** Knepper et al. 1992, McCarry 1996, Pfuntner 1978, and Siegel & Novak 1999), there is a paucity of information on the general ecology of mosquitoes in catch basins.

Mosquito control programs need to have a firm understanding of the relationship between mosquitoes and stormwater structures in order to have an open dialogue with water managers about potential nuisance and disease issues (Metzger et al. 2003). While several Oregon Vector Control Districts include management of mosquitoes in catch basins as a part of ongoing control programs, the population dynamics and species that use them as immature habitat have not been extensively studied. In areas without mosquito control, understanding the role these structures may play in urban mosquito production is an important step to determine whether or not control should be initiated. Currently there are no mosquito control activities conducted in the City of Corvallis, Oregon, making it an ideal location to study undisturbed populations of mosquitoes.

The objectives of surveying catch basins for mosquito larvae are two fold: first, to identify which mosquito species inhabit catch basins in Corvallis; and second, whether environmental or physical differences between catch basin designs and among locations affected mosquito presence. A better understanding of the ecology of mosquito communities inhabiting these bodies of water will allow more precise estimation of vector potential and fine tuning of management practices. By meeting these objectives, one can determine whether these structures are utilized by mosquitoes implicated in transmission of mosquito-borne disease and, if possible,

prioritize catch basin mosquito control based on the location, age or catch basin design.

MATERIALS AND METHODS

Corvallis, Oregon, is about 90 miles south of Portland, in the Willamette Valley. The city has a population of nearly 53,000 people and encompasses 14 square miles. Much of the land surrounding the city is agricultural. At the time of the study, the City of Corvallis maintained just over 6000 sumped catch basins as a part of its stormwater management program. There are two different catch basins designs used in Corvallis (Figure 2.1). One design features a small grate built into the vertical curb of the sidewalk, through which water drains into a concrete lined vault built below the sidewalk and is accessible from above via a metal panel set into the sidewalk. The vault has a water surface area of 4475 cm^2 (Figure 2.1B) The second type is smaller with only 1836 cm^2 of water surface area and is set directly in the street with water draining into it via a larger open grate (Figure 2.1A). Depth from the roadway to the surface of the water and water depth is variable and not standardized.

To compare the differences in mosquito production in the two catch basin designs, areas of the city with both types were chosen for sampling: the southern end, separated from the rest of city by the confluence of the Willamette and Mary's Rivers; the northern end, built in a hilled area bordered by the McDonald–Dunn Research Forest; and the western area with the Mary's River to the east and bordering an undeveloped hilled area to the north (Figure 2.2). Sampling did not occur in the central or downtown areas of the city because these areas to not contain both catch basin designs.

Twenty catch basins were chosen from each of three areas of the city (north, south, and west) for a total of 60 catch basins sampled (Figure 2.2). The geographical areas were selected based on the proximity of newer neighborhoods (built since 2000) to older neighborhoods (built prior to 1985), the presence of sumped catch basins, and both catch basin designs. Catch basins were classified by design type and installation date. Sampled catch basins were pseudo-randomly selected using the RANDBETWEEN function in Excel 2003 (Microsoft 2002) from a list of catch basins that held water and contained no mosquitoes for the three weeks preceding the start of the study (unpublished data, Townzen). In each of the three areas, five catch basins were selected from each of the following categories: 1) small grate built within the previous five years, 2) large grate built within previous five years, 3) small grate built over 15 years earlier, and 4) large grate built over 15 years earlier

All 60 catch basins were sampled weekly from March 26, 2004 through October 31, 2004. Initially, the catch basins were sampled over two a two day period; however, once mosquitoes were routinely collected, all catch basins were sampled on the same day and in the same order every week. Two 350 ml dips of water were taken from each catch basin using a long handled polyethylene dipper (Wildco). The handle of this dipper is six feet long and connected to the cup at a 45 degree angle allowing it to easily collect samples from the catch basins. Water was allowed to settle for one minute after the catch basins was opened before taking the first dip and for 45 seconds before taking the second. Water from both samples was combined in a container from which temperature and pH were measured using a pHep temperature/pH meter (Hanna Instruments). Immature mosquitoes were concentrated from the two dips with a fine

meshed aquarium fish net and taken back to the lab in mason jars. Excess water was returned to the catch basin.

Mosquitoes were counted and sorted by species and life stage in the lab. A subset of the immature mosquitoes collected were reared to adult stage and vouchered in the Oregon State Arthropod Collection, Corvallis, OR. At the two peaks in mosquito counts, July 16th and August 15th, differences in temperature, pH, and mosquito numbers were analyzed by geographic region and grate type using one-way anova and two-sample t-tests respectively. All data were analyzed using S-Plus 6.1 (Insightful 2002) and Excel (Microsoft 2002). Weather data were accessed from the Oregon Climate Service monthly data website for Corvallis (http://www.ocs.oregonstate.edu/index.html).

RESULTS

Over the 32 weeks of sampling from the 60 catch basins, a total of 1,920 catch basin visits were made, collecting 79,760 immature mosquitoes. Mosquitoes were sampled from 55 of the 60 catch basins sampled (92%) at some time during the study. Almost all of the species of mosquitoes collected belonged to three species: *Culex pipiens* Linnaeus (82.9%), *Culiseta incidens* (Thomson) (10.1%), and *Culex stigmatosoma* Dyar (7.0%) (Figure 2.3). A single *Culex tarsalis* Coquillette larva was collected on May 23. First instar *Cx. pipiens* and *Cs. incidens* were first sampled in four catch basins on April 11. Both species continued to be regularly sampled through October 31st and 24th respectively. *Culex stigmatosoma* was present for a shorter time period, with its first detection on June 27th and last collection on October 17th. When

sampling ended on October 31st, two *Cx. pipiens* pupae were collected from a single catch basin. No mosquitoes were collected from the remaining 59 sampled basins. Mosquito counts were higher in months with little or no rainfall (Figure 2.4).

On July 16th, 57 of the 60 catch basins (95%) contained water and immature mosquitoes were found in 45 of the wet catch basins (79%). Water temperature averaged 22.0 °C (95% CI 21.6 to 22.5) and average pH was 7.3 (range of 6.6 to 8.0). An average of 23, 3rd and 4th instar larvae and pupae (95% CI 14 to 32), were sampled per catch basin. There was no measurable difference in numbers by catch basin design (t-stat=0.62, df = 58, $P = 0.535$) and only moderate evidence of a difference by area (f-stat = 4.06, df = 2, 57, $P = 0.0225$).

On August 15th, 57 of 60 catch basins contained water and mosquitoes were collected from 45 (79%) of the wet basins. Ten of the 12 catch basins from which no mosquitoes were collected on July 16th had been recolonized on August 15th. The water temperature and pH in the catch basins averaged 22.8 °C (95% CI 22.3 to 23.2) and the 6.9 (range of 4.8 to 7.6) respectively. An average of 34.9 late instar larvae and pupae (95% CI 19 to 51) was collected per catch basin on this date and no evidence of a difference in numbers by either catch basin design (t-stat = 1.30, df = 58, $P = 0.1995$) or the area in which it was located (F-stat = 0.077, df = 2, 57, $P = 0.9262$) was found.

DISCUSSION

With the introduction of West Nile virus (WNV) to the United States in 1999, and its subsequent spread across the country, there has been a renewed interest

in managing mosquitoes and mosquito-borne disease. As a common source of standing water and now confirmed habitat for mosquitoes in Corvallis, that are in close association with humans, catch basins could play a role in transmission of mosquito-borne diseases. Many of the new mosquito control programs formed in response to WNV have limited budgets and must carefully allocate resources for control efforts that are both cost effective and efficacious. With this in mind, the goals of this study were to determine if immature mosquitoes utilizing catch basins in Corvallis are species that could be involved in disease transmission, and if so, whether or not it would be possible to prioritize a catch basin mosquito control strategy based on the location, age or type of catch basin. Two of the species routinely sampled from the Corvallis catch basins, *Cx. pipiens* and *Cx. stigmatosoma* have been implicated in transmission of WNV in the United States and should be the focus of control activities to manage the disease. However, prioritization of catch basin control efforts would be difficult because mosquitoes were collected equally in all three geographic areas and in both grate types.

Based on the average temperature of the water in the Corvallis catch basin in July and August, 22° and 22.7° C respectively, *Cx. pipiens* larvae would be expected to mature from egg to adult in approximately 17 to 20 days (Vinagradova 2000). The water temperature also falls within the optimal range of temperatures for adult emergence (Vinogradova 2000). Higher water temperatures would increase the maturation rate at the expense of the number of adults that would reach emergence.

Only four catch basins (7%) were completely devoid of mosquitoes for the entirety of the study. One of these catch basins had a constant flow of water into it,

from what appeared to be a broken water pipe, and the constant turbulence may have been enough to prevent oviposition. Neither measured, nor observed, differences in the three other basins explains the absence of mosquito larvae.

While the number of mosquitoes collected in a catch basin varied greatly (from 0 to 2331), high numbers of mosquitoes were collected from catch basins in all three areas of the city (Figure 2.5) and in both the older and newer neighborhoods and from the two different grate types. It is also important to remember that this method of dipping for mosquitoes is a qualitative sampling method. While it can be used to compare relative abundance among catch basins or through the course of the season, it cannot be used to quantify the actual numbers of mosquitoes in the catch basins. That being said, it is reasonable to assume dipping did not collect all of the mosquitoes in each catch basin and therefore the actual number of mosquitoes in each is likely to have been much higher than the number sampled.

Rainfall and urban "slobber," from activities such as watering lawns and washing cars, may have provided enough runoff to keep a permanent supply of standing water in 55 of the catch basins. Four of the basins that went dry at least once, contained immature mosquitoes after being refilled. It was not uncommon to collect mosquitoes from a particular catch basin for a few weeks, and then not collect larvae for a week or two. Water use from human activities may have flushed the mosquitoes out of one catch basin and into another. Many catch basins are connected to each other and it is possible that mosquitoes were flushed into another basin further down the drainage line. This could explain why catch basins could be absent of mosquitoes

one week and contain late instars and pupae the following week, well ahead of their developmental cycle.

Determining the species of mosquitoes collected from catch basins is important in determining if they could be involved in disease transmission. Mosquitoes in the genus *Culex* are the most commonly collected genus in catch basins across the United States; however, the individual species collected varies geographically. In Marshfield, WI, (Kronenwetter-Koepel et al. 2005) Chicago, IL, (Geery and Holub 1989) and Narragansett, RI, (Butler et al. 2007) catch basin sampling primarily collected *Cx. pipiens* and *Culex restuans* Theobald. In Vero Beach and Key West, FL, the dominant species collected in stormwater structures were *Culex quinquefasciatus* Say and *Culex nigripalpus* Theobald (Rey et al. 2006). On the West Coast in Riverside, CA the two dominant species collected in a 1978 study (Pfunter) were *Cx. stigmatasoma* and *Cx. quinquefasciatus*.

Two of the three species collected in Corvallis catch basins, *Cx. pipiens* and *Cx. stigmatasoma*, are competent vectors of West Nile virus (WNV) (Goddard et al. 2002 and Turell et al. 2005) and pools of both species of mosquitoes have tested positive for West Nile virus in Oregon (DeBess 2005). *Culex pipiens* is considered one of the primary enzootic vectors of WNV due to its competency to vector the disease and its predilection for avian hosts (Tempelis & Washino 1976). Additionally, Hamer et al. (2008) collected a single *Cx. pipiens* with a disseminated WNV infection that had fed on a human host. While this may be a rare occurrence, it strengthens the importance of *Cx. pipiens* in the WNC cycle and highlights its potential for epidemic transmission, in addition to enzootic transmission.

In North America, most WNV activity occurs from July through October (Hayes et al. 2005). This corresponds with peaks in mosquito abundance in the catch basins sampled in Corvallis (Figure 2.3). Mosquito abundance was highest on July 16th and August 15th. An average of 11.5 and 17.5 late instar larvae and pupae, respectively, were collected per dip of water from each catch basin, which is well above the treatment threshold for most mosquito abatement programs. Corvallis received very little rainfall between July 21st and August 15th, and the large drop in the mosquito population on August 22nd (Figure 2.3) corresponded with a greater than 2.5 centimeter rainfall event that likely flushed the mosquitoes out of the catch basins.

As urban development continues, the numbers of stormwater structures needed to manage water pollution will continue to increase. In 2004, when the study of Corvallis catch basins was initiated, there were approximately 6000 sumped basins. In four years, the population of Corvallis increased by just over 7%, and the number of sumped catch basins increased by 20%. As urbanization continues, so will the need to better understand local mosquito population dynamics, including mosquitoes associated with stormwater management structures. Introduction of mosquito-borne disease, like West Nile virus, to the area heightens the need to understand the ecology of these vectors. Our findings of high mosquito counts from catch basins in all three areas of the city studied and in both catch basin designs will be an important decision making tool if managing mosquitoes in the City of Corvallis is initiated.

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REFERENCES CITED

Butler M, Ginsberg HS, LeBrun RA, Gettman AD, Pollnak F. 2007. Natural communities in catch basins in southern Rhode Island. *Northeastern Naturalist* 14: 235-250.

Copeland C. 2006. Water Quality: Implementing the Clean Water Act. CSR Repot for Congress. [Internet] Available from National Council for Science and the Environment, Washington, D.C. [accessed March 26, 2008] www.cnie.org/ NLE/CRSreports/ 06Nov/RL33466.pdf.

Gingrich JB, Anderson RD, Williams GM, O'Connor L, Harkins K. 2006. Stormwater ponds, constructed wetlands, and other best management practices as potential breeding sites for West Nile virus vectors in Delaware during 2004. *J Am Mosq Control* Assoc. 22: 282-291.

Goddard LB, Roth AE, Reisen WK, Scott TW. 2002. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis* 8: 1385-1391.

Greery PR, Holub RE. 1989. Seasonal abundance and control of *Culex* spp. in catch basins in Illinois. *J Am Mosq Control Assoc*. 5: 537-540.

Hamer, GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, Walker ED. 2008. *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. *J Med Ent* 45: 125-128.

Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL. 2005. Epidemiology and transmission dynamics of West Nile virus disease *Emerg Infect Dis* 11: 1167-1173.

Knepper RG, Leclair AD, Strickler JD, Walker ED. 1992. Evaluation of Methoprene (Altosid ® XR) sustained release briquets for control of *Culex* mosquitoes in urban catch basins. *J Am Mosq Control Assoc* 8: 228-230.

Kronenwetter-Koepel TA, Meece JK, Miller CA, Reed KD. 2005. Surveillance of above- and below-ground mosquito breeding habitats in a rural Midwestern community: baseline data for larvicidal control measures against West Nile virus vectors. *Clin Med & Res* 1: 3-12.

Kwan JA, Riggs-Nagy JM, Fritz CL, Shindelbower M, Castro PA, Kramer VL, Metzger ME. 2008. Mosquito Production in Stormwater Treatment Devices in the Lake Tahoe Basin, California. *J Am Mosq Control Assoc* 24: 82-89.

McCarry MJ. 1996. Efficacy and persistence of Altosid ® Pellets Against *Culex* species in catch basins in Michigan. *J Am Mosq Control Assoc*. 12: 144-146.

Metzger ME, Myers CM, Kramer VL. 2003. The “dark side” of stormwater runoff management: Vectors associated with BMPs. *Proc Calif Mosq Vector Control Assoc* 70: 2-10.

Pfunter AR. 1978. The development and control of *Culex quinquefasciatus* Say and *Culex peus* Speiser in urban catch basins. *Proc & Pap 46th Annul Conf Calif Mosq Control Assoc* 46: 126-129.

Pitt R, Lilburn M, Durrans SR, Burian S, Nix S, Voorhees J, Martinson J. 1999. Guidance Manual for Integrated Wet Weather Flow (WWF) Collection and Treatment Systems for Newly Urbanized Areas (New WWF Systems. [Internet accessed March 26, 2008] Available from http://rpitt.eng.ua.edu/ Publications/ BooksandReports/Design%20for%20the%20Future/ DesignforFuture.htm

Rey JR, O’Meara GF, O’Connell SM, Cutwa-Francis MM. 2006. Factors affecting mosquito production from stormwater drains and catch basins in two Florida cities. *J Vector Ecol* 3: 334-343.

Ruiz MO, Tedesco C, McTighe TJ, Austin C, Kitron U. 2004. Environmental and social determinants of human risk during a West Nile virus outbreak in the greater Chicago area, 2002. [Internet] available at International Journal of Health Geographics. www.ij-healthgeographics.com/content/3/1/8.

Siegel JP, Novak RJ. 1999. Duration of activity of the microbial larvicide Vectolex CG® (*Bacillus sphaericus*) in Illinois catch basins and waste tires. *J Am Mosq Cont Assoc* 15: 366-370.

Smith CM, Shisler JK. 1981. An assessment of storm water drainage facilities as sources of mosquito breeding. *Mosq News* 41: 226-230.

Tempelis CH, Wasino RK. 1967. Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley, California, with notes on other species. *J Med Entomol* 3: 315-318.

Turell MJ, Dohm DJ, Sardelis MR, O’Guinn ML, Andreadis TG, Blow JA. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *J Med Entomol* 42: 57-62.

Figure 2.1. Photographs of the small (A) and large (B) catch basin designs found in Corvallis, OR.

A.

B.

Figure 2.2. Map of Corvallis, OR, showing the locations of the study catch basins. The circles and triangles represent the larger and smaller catch basins respectively.

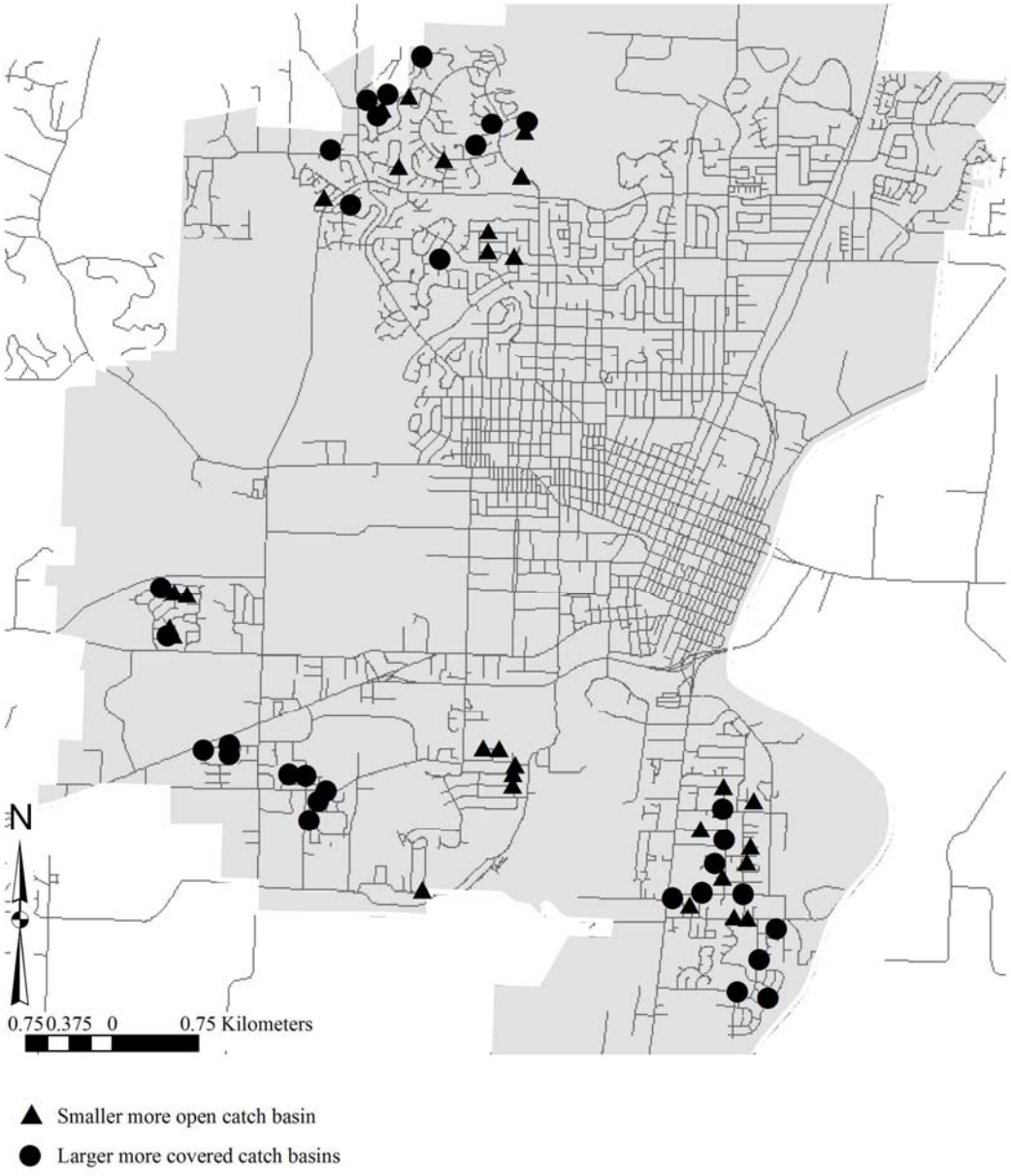


Figure 2.3. Numbers of mosquitoes collected by date and species in Corvallis, OR, 2004.

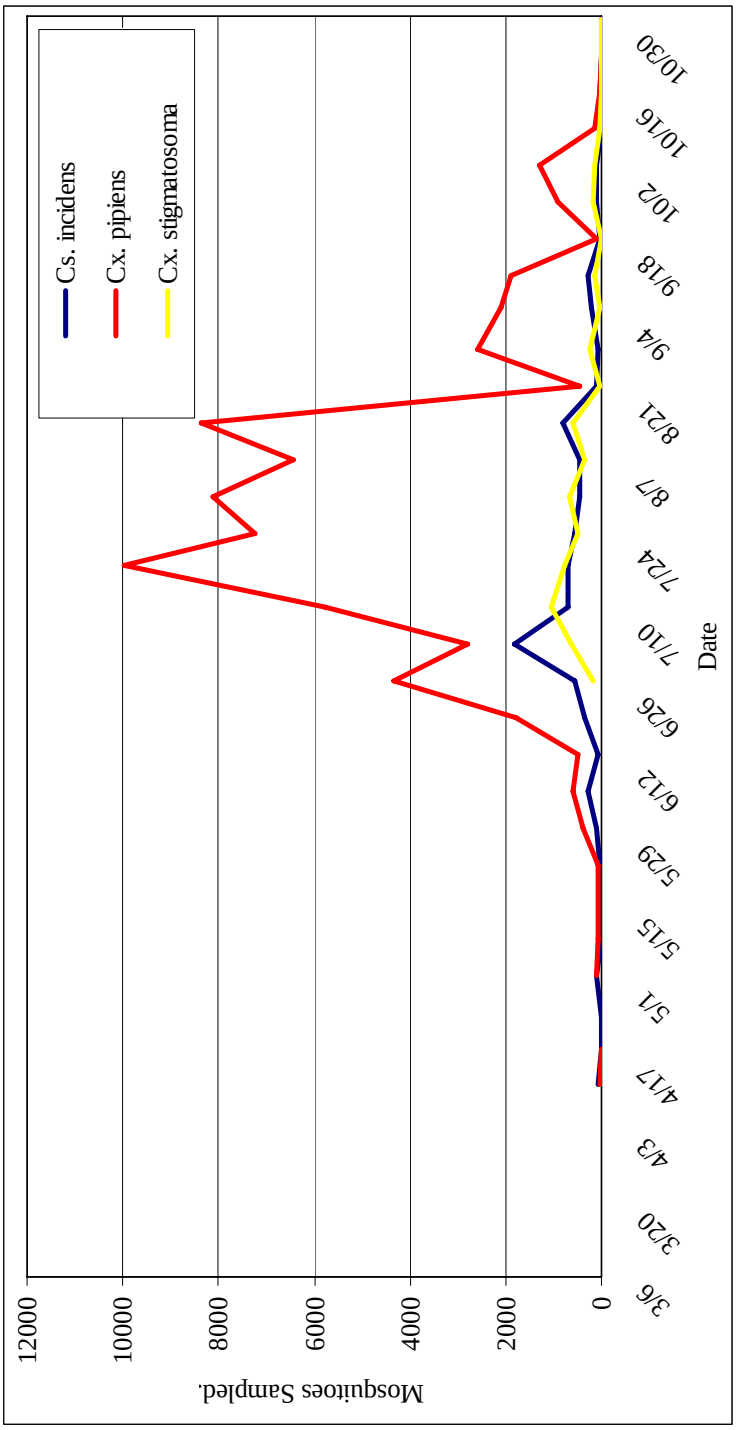


Figure 2.4. Total numbers of mosquitoes collected by date and average weekly rainfall in Corvallis, OR, 2004.

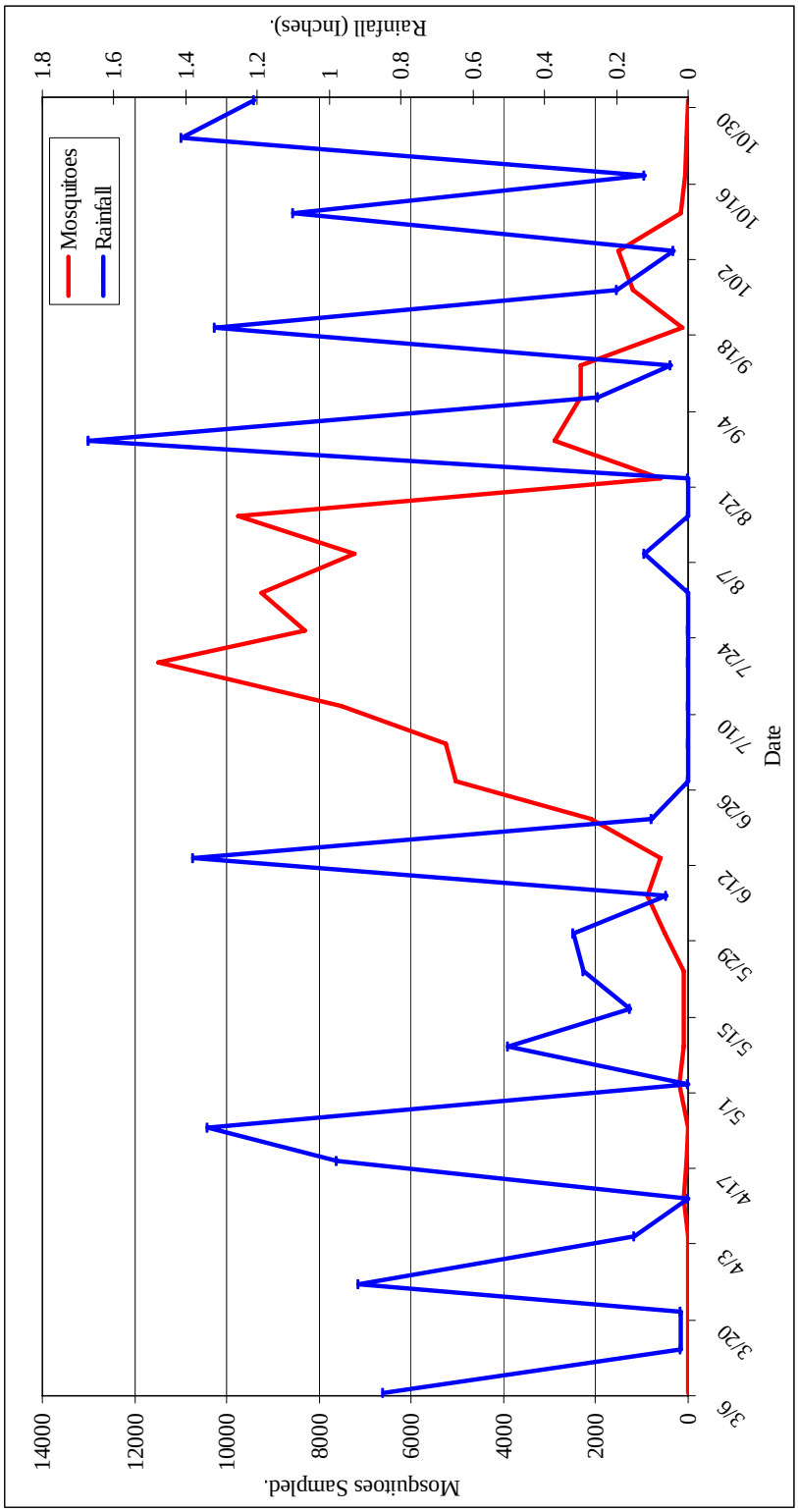
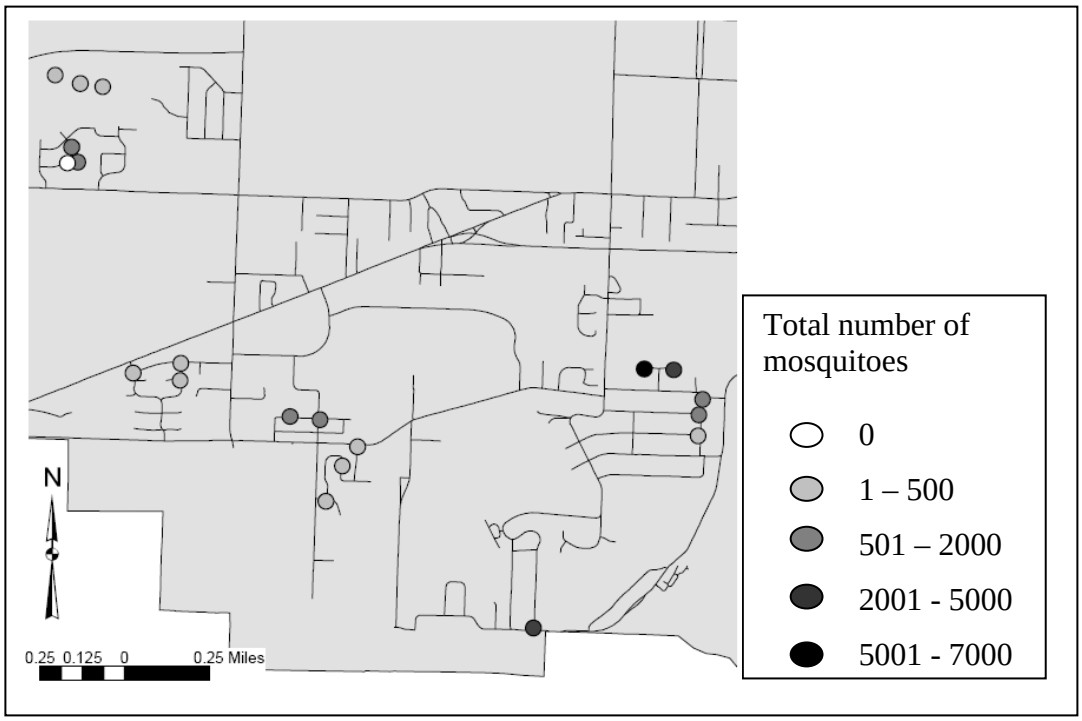


Figure 2.5. Total number of mosquitoes collected from each catch basin in north (A) west (B) and south (C) Corvallis, OR, marc through October 2004.

A.

B.



C.


N
0.2 0.1 0 0.2 Miles
Total number of mosquitoes
0
1 –500
501 – 2000
2001 - 5000
5001 - 7000

IDENTIFYING SOURCES OF MOSQUITO BLOODMEALS USING *CYTOCHROME OXIDASE SUBUNIT I* AND *CYTOCHROME B*

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ABSTRACT

Primer pairs were designed, and protocols developed, to selectively amplify segments of vertebrate mitochondrial *cytochrome oxidase subunit 1* (COI) and *cytochrome b* (Cyt *b*) mtDNA from the bloodmeals of mosquitoes. The protocols use two pairs of nested COI primers and 1 pair of Cyt *b* primers to amplify short segments of DNA. Resultant sequences are then compared to sequences in GenBank, using the Blastn function, for putative host identification. Vertebrate DNA was amplified from 88% of our sample of wild-caught mosquitoes with blood meals and GenBank BLAST searches putatively identified 98% of the amplified sequences, including: one amphibian, seven mammalian and 14 avian species. Criteria and caveats for putative identification of bloodmeals are discussed in detail.

Key Words: mosquitoes**,** bloodmeal analysis, DNA barcode, *cytochrome b*, *cytochrome oxidase subunit I*, GenBank

INTRODUCTION

Mosquito host choice is recognized as one of the most important factors in understanding mosquito-borne disease transmission and its control (Macdonald 1957). For human-endemic diseases like malaria it is only necessary to determine the anthropophilic index for a region at a given time to calculate vectorial capacity. If the disease has a zoonotic cycle, however, it is important to determine what else the mosquitoes are feeding on to understand their roles as either bridge or amplifying vectors. The importance of mosquito bloodmeal identification is highlighted by the time and effort that has been invested into improving the techniques used to identify mosquito hosts. As precision of host identification increases so does our understanding of mosquito-borne disease cycles.

A variety of tests, with varying levels of specificity, have been developed and employed to identify mosquito bloodmeals. The precipitin test was developed in 1904 and first used in mosquito bloodmeal analysis in 1923 (Washino and Tempelis 1983). In the 1980's enzyme-linked immunosorbent assays (ELISA) became popular for bloodmeal identification. Direct, indirect and sandwich ELISAs (Burkot et al. 1981 and Chow et al. 1993) have been used to detect avian and mammalian antibodies within mosquitoes. However, identification is only specific for those species for which antisera are available and there is the potential for cross reactivity, resulting in false positive errors (Chow et al. 1993). Irby and Apperson (1988) found that only 80% of the collected mosquito bloodmeals reacted with their antisera panels, leaving 20% of the hosts unidentified to any level. Additionally, many of the samples

narrowed down to either avian, mammalian, or amphibian could not be specifically identified.

Over the last decade, DNA-based techniques have grown in popularity as a means to identify the sources of bloodmeals from medically important insects. The most commonly used molecular method is a heteroduplex assay (Apperson et al. 2002, Apperson et al. 2004, Boakye et al. 1999, Bosseno et al. 2006, Lee et al. 2002) with which unknown sequences are identified by analyzing the mobility of heteroduplex products formed from combination of a known probe and the unknown DNA sample. Although highly sensitive to mutation detection and widely used for genetic screening, the resolving power and sensitivity of heteroduplex methods are compromised when applied to bloodmeal analyses. First, as with ELISA, the probe must be from a species closely related to the unknown sample. If the probe is too divergent from the unknown samples, the assay can fail to distinguish different banding patterns for different hosts. Thus, for the identification of unknown bloodmeals, many different probes, from a wide range of potential hosts, may be necessary for species level identification.

Additionally, the mobility profile for every potential host must be known for positive identification, thereby increasing the time, cost and complexity of the procedure. Finally, the sensitivity of the heteroduplex assay is greatly affected by the type of base mismatches and experimental conditions (Nataraj et al 1999). The mobility of heteroduplex products is also sensitive to changes in the gel matrix, gel thickness, buffer solution, and electrophoresis voltage, making it difficult to compare results across studies and preventing creation of a central database of mobility profiles.

Methods for bloodmeal identification using multiplexed polymerase chain reactions (Kent and Norris 2005) and terminal restriction fragment length polymorphism profiles (Meece et al. 2005), while more standardized, also require known standards, limiting host identification to vertebrates for which DNA samples have been obtained and the restriction fragment profiles developed. The major limitation of these molecular methods is the collection of reference samples. In many regions, the number of potential mosquito host species ranges into the hundreds or thousands, and it would be difficult, if not impossible, to sample all of them. This limitation can be overcome by taking advantage of data that are already readily available.

An alternative to either determining which hosts are of interest *apriori*, or generating separate synoptic collections of hosts for each new study, is to outsource the collection of reference samples. The online database GenBank (Wheeler et al. 2000), with over 22,000,000 nucleotide core sequences, provides an increasingly comprehensive and publicly accessible reference collection, making it unnecessary for researchers to independently collect DNA samples for all potential mosquito hosts within a study area. To use this information, vertebrate DNA from specific gene regions is selectively amplified from the bloodmeals of mosquitoes, sequenced, and compared against homologous sequences from identified taxa in GenBank; putative identification is made when a reasonable match is found (see Discussion below on what constitutes “reasonable”).

Several polymerase chain reaction (PCR) assays have been developed to identify the vertebrate bloodmeals of mosquitoes. While various techniques for host

identification have been used, the gene of choice for these molecular analyses, as well as for the molecular analysis of vertebrate phylogeny and phylogeography, has been mitochondrial *cytochrome b* (Cyt *b*). Ngo and Kramer (2005) used eight order-specific primers to narrow down the potential range of hosts in mosquito bloodmeals. Other studies (Molaei et. al. 2006, Molaei and Andreadis 2006 and Molaei et. al. 2007) have used as many as five pairs of primers to amplify vertebrate bloodmeals from mosquitoes. While these methods have wide applicability, the use of multiple primer sets can be cumbersome because each mosquito bloodmeal sample may need to be processed with all of the primer pairs before the putative host can be identified. Additionally, all of the primers used amplify the same region of mtDNA, Cyt *b* limiting the potential for finding a putative match. Unfortunately, there are still broad taxonomic gaps in the published Cyt *b* data available for birds and mammals, and it is unlikely that all potential mosquito hosts in a given study area have been sequenced for this gene.

The addition of a second widely-studied gene for bloodmeal analysis increases the opportunity for host identification. Proponents of "DNA barcoding" (e. g., Hebert et al. 2003) have argued that a 648 bp region of mitochondrial *cytochrome oxidase subunit I* (COI) is an appropriate gene for species identification across all animal life. Identification of mosquito bloodmeals using COI is a logical application for data already being collected from a wide diversity of vertebrates as a part of that initiative. As the COI DNA barcode region from more species is sequenced and submitted to public databases, the capacity for identification of bloodmeals via this marker will become more and more precise.

Attempts to amplify vertebrate DNA from blood-engorged mosquitoes using previously published protocols (Hebert et al. 2004, Malmqvist et al. 2004) resulted in less satisfactory results. The Hebert et al. (2004) barcode primers and protocols amplified multiple fragments from both the known host bloodmeal samples (positive controls) and wild caught samples (Townzen, unpublished data). While it would be possible to identify avian hosts using these primers, additional time would be needed to identify, gel extract, and purify the appropriate band. In contrast, the Cyt *b* primers used by Malmqvist et al. (2004, from Kocher et al. 1989) to amplify vertebrate bloodmeals from blackflies were able to selectively amplify vertebrate DNA in most cases. However, when used with anopheline mosquitoes, the *Anopheles* Cyt *b* gene region was co-amplified along the vertebrate region, preventing identification of the host (Townzen, unpublished data).

The idea of molecular identification of unknown organisms is neither new nor a perfect replacement for morphological identification (Hebert et al. 2003, Meier et al. 2006, Sperling et al. 1994, Tautz et al. 2003, Will et al. 2005). It is, however, a very useful tool for identifying samples that cannot be identified morphologically, for example mosquito bloodmeals, unknown tissues samples in markets or invasive species and biosecurity threats (Armstrong and Ball 2005, Baker and Palumbi 1994, DeSalle and Birstein 1996). For bloodmeal identification, our development of single pairs of primers for Cyt *b* and COI that selectively amplify vertebrate but not invertebrate DNA has greatly streamlined the process, by minimizing the numbers of PCR reactions needed for each sample (Townzen 2005; Townzen et. al. 2007). Here we report the success of simple set of methods to identify specific mammalian and

avian sources of mosquito bloodmeals based on sequences of these gene regions. The amplified vertebrate sequences are compared against identified sequences in GenBank.

MATERIALS AND METHODS

Mosquito samples

Colony-reared mosquitoes engorged with known bloodmeals were used as positive controls for developing and validating the protocols. Eight laboratory reared *Culex tarsalis* fed on chicken blood and eight *Culex pipiens* fed mouse blood were collected and preserved in 90% ethanol, from the Sacramento-Yolo County Mosquito and Vector Control District and the University of California, Davis, Kearney Agricultural Center, respectively. Wild-caught, blood-engorged mosquitoes were subsequently used to test the effectiveness of the protocols. From May through October, 2006, mosquitoes with visible bloodmeals were aspirated off the walls of walk-in red boxes (168 X 80 X 80 cm in dimension) located in urban green spaces in Corvallis, Oregon, and on the Finley National Wildlife Refuge and the E. E. Wilson Wildlife Area located 18.5 km south and 15.3 km north of Corvallis respectively. Additional mosquitoes were collected from the same locations using carbon dioxide-baited and black light traps. Mosquitoes were identified to species, and all samples with visible blood were stored at 4° C in individual microvials of 95% ethanol.

Bloodmeal analysis

Two sets of primers that amplify overlapping regions of COI, COI-Short and COI-Long, and one primer set for *Cyt b* (Table 3.1) were designed by aligning

vertebrate mtDNA sequences obtained from GenBank (Table 3.2), using the BioEdit Sequence Alignment Editor (Hall 1999), and manually comparing them to identify regions of the two genes conserved among vertebrates yet differing from the mosquitoes.

Individual abdomens of engorged mosquitoes were removed, macerated in 1.5 ml microcentrifuge tubes, and whole DNA was extracted using a DNeasy Tissue Kit (Qiagen, Valencia, California USA) following the procedure for purification of total DNA from animal tissues and eluted in 150 µl of Buffer AE. The forceps used to remove the abdomens were sterilized in DNA Away (Molecular BioProducts Inc., San Diego, California USA) between dissections. Each dissection was performed on a clean surface that was wiped down with DNA Away between specimens. The head, thorax, wings and legs of the mosquitoes were returned to 90% ethanol and retained as voucher specimens or for further molecular studies, if needed.

Both gene segments were amplified in 50 µl reactions with 3 µl of DNA, 5 µl of 10x KCl- $MgCl_2$ Buffer (Fermentas, Burlington, Ontario Canada), 5-7 µl of 25 mM $MgCl_2$ (Fermentas, Burlington, Ontario Canada), 1 µl of 10µM primers, 1µl of 10µM dNTPs and 0.3 µl Taq polymerase (Fermentas, Burlington, Ontario Canada). The thermocycling protocol used for both genes was as follows: 95º C for one minute, followed by 35 cycles of 95º C for 30 seconds, 48-52º C for 50 seconds, 72º C for one minute, and finishing with a five minute 72º C extension cycle. Negative controls were run with each extraction and PCR reaction.

If neither the COI-short nor COI-long primer pair amplified enough DNA for sequencing a semi-nested PCR was attempted, using the above thermocycling

protocol. To do this, 3 µl of the PCR product from the reaction using the COI-long primers was re-amplified with the COI-short forward primer and the COI-long reverse primer. PCR products were purified using QIAquick PCR Purification Kit Protocols (Qiagen, California, USA) with a microcentrifuge and eluted in 30 µl of elution buffer. Sequencing was outsourced to a commercial firm (Macrogen, Seoul, South Korea), and generated from both the forward and reverse strands. The entire protocol can be performed in approximately one week for under $15.00 (USD) per sample.

Automated sequence outputs were edited manually in BioEdit (Hall 2005). To infer the identity of the vertebrate blood, each sequence was compared to the database of homologous sequences contained in GenBank using the nucleotide-nucleotide basic alignment search tool (BLAST) (Altschul et al. 1997, http://www.ncbi.nlm.nih.gov/). Unknown bloodmeal sequences are considered putatively identified if they are more than 95% identical (% nucleotide identities) to a sequence in GenBank for either gene. When the bloodmeal sequence had a >95% match to sequences of more than one species in GenBank (ambiguous matches), the geographical range, abundance, and similarity scores for the taxa producing significant alignments were used to aid in selection among alternative potential IDs. Amplified bloodmeal sequences obtained from this study will be annotated and deposited in GenBank.

RESULTS

Amplified DNA from the known bloodmeals of laboratory raised mosquitoes positively matched the host (chicken or mouse) in all 16 cases. A total of 162 wild blood-engorged mosquitoes (nine species) were collected (Table 3.3): *Anopheles*

punctipennis (n=72), *Culex pipiens* (n=30), *Culex tarsalis* (n=20), *Culiseta incidens* (n=11), *Aedes sierrensis* (n=10), *Anopheles freeborni* (n=9), *Culex stigmatosoma* (n=4), *Culex boharti* (n=4), and *Aedes increpitus* (n=2). Eighteen of the wild samples did not amplify using the above protocol. Lowering the PCR annealing temperature by 7º C amplified mosquito DNA from those samples.

A single band of vertebrate DNA was extracted and amplified from 144 samples (Fig. 3.1). Four of these samples showed evidence of polymorphism in the sequence chromatogram suggesting amplification of multiple DNA fragments, and were not identifiable. From the remaining 140 samples, a single, unambiguous vertebrate DNA sequence was amplified.

Putative hosts were identified for the remaining 140 mosquitoes, using COI from 125 of the samples and Cyt *b* from 105 of the samples (Table 3.3). One amphibian, seven mammalian and 14 avian bloodmeal identifications were determined. Three of the bloodmeal matches for Cyt *b* did not have corresponding COI sequences in GenBank (Mule Deer, Pacific Tree Frog and Eastern Cottontail), while one species identified by COI did not have a corresponding matching sequence in GenBank for Cyt *b* (Allen's Hummingbird). Representative sequences for avian, mammalian, and amphibian species putatively identified from the mosquito bloodmeals have been submitted to GenBank (Table 3.3) and are publically available for comparison.

DISCUSSION

Efficacy of the method

Our protocol is a straightforward and effective procedure for identifying the specific vertebrate source of mosquito bloodmeals. Each primer pair amplifies a single segment of vertebrate host DNA from each of the blood engorged mosquito abdomens and there was rarely a need for gel extraction of PCR products (see Figure 3.1). In our trials, vertebrate DNA was amplified and putative host identification determined for 89% of the unknown bloodfed mosquitoes collected. One reason that we were more successful in amplifying COI is that we had three chances to amplify the gene region; once with each of the primer pairs, and then a third time with the semi-nested approach. This was particularly helpful when there was some product from the first reaction, but not enough to sequence.

Four samples showed evidence of signals from two or more DNA segments on their corresponding sequence chromatograms. This could indicate contamination from an external source, contamination from the mosquito, or that the mosquito had fed on more than one vertebrate species. Because mosquito DNA only amplified at extremely low annealing temperatures, it is unlikely to be one of the signals. Absence of amplification of negative controls suggests that the second signal in these samples is not from an external source, such as lab or field personnel**.** Various studies (Apperson et al. 2002, Savage et al. 2007, Molaei et al. 2006 and Molaei et al. 2007) have reported mixed avian and mammalian bloodmeals in 3-9% of mosquitoes, depending on the species. The possibility that the four samples with evidence of multiple signals are mosquitoes that fed on multiple hosts cannot be ruled out. We are

currently developing protocols to distinguish between the multiple signals, not only for samples with mixed avian and mammalian DNA, as can be done by the protocols discussed in the introduction, but also samples where the mosquito had fed on two different species of the same vertebrate class.

What constitutes a "positive ID"?

We are confident that the BLAST searches resulted in bloodmeal identifications for 140 mosquito samples (Table 3.3). However, it is important to keep in mind that search results are only reliable for host identification if the resultant match is nearly identical to the reference sequence. When unknown query sequences are compared against the GenBank database it is rare for the search to return with a sequence that is an exact match of 100% similarity: among our data only 11 COI and three Cyt *b* sequences encountered exact matches in GenBank. Simply using the best match generated in the BLAST search may not always correctly identify the bloodmeal source. As an example, it is extremely unlikely that the mosquito sample EWR6 fed on the species with the best match, *Scomberomorus guttatus* (Indo-pacific Mackerel), which had an 85% similarity to the COI query sequence. For this reason it is necessary to select a minimum similarity cut-off for a bloodmeal to be considered putatively identified.

Setting a similarity cut-off that works across all species is difficult because the amount of intraspecific sequence divergence can vary among different groups of organisms (Funk and Omland 2003, Will and Rubinoff 2004). Nevertheless, examination of the distribution of matches in our data (Figure 3.2), shows that there is a disjunction between best matches that are greater than 95%, and those that are lower

(<90%). We therefore consider matches of 95% or greater to represent a positive identification. Additionally, 96% percent of our sample sequences had matches in GenBank greater than 97%. Those sequences that were between 95 and 97% similar were sequences with ambiguous base pairs. While this pattern could be obscured by additional data from a larger sample, it corresponds well with the Hebert et al. (2004) reported mean intraspecific COI divergence among birds to be < 0.5% (ranging as high as 7%).

There may be instances in which the BLAST search result is ambiguous because there are several species that match the query sequence with greater than 95% similarity. In cases where there are closely-related vertebrate species, any similarity cut-off may result in incorrect identification of the bloodmeal source, as taxa may be undifferentiated, paraphyletic, or share polymorphisms across that gene region (Brower 2006, Funk and Omland, 2003). This is a general problem of single-gene taxonomic endeavours, but it does not mean that such evidence is without empirical utility (Brower et al. 1996). One important supplementary clue to select among closely related sequences is the species' geographical distributions. Four of the five ambiguous results in our analysis can be resolved based on the disjunct geographical distributions of the species in question. It is also important to note that there are sequence identity mistakes in GenBank that can be clarified by examining all of the species generated in the list of sequences producing significant alignments, as is the case for one of our samples. Finally there are some instances where ambiguous results cannot be resolved to the level of a single species, but even these can be narrowed down to a relatively small group of candidates.

Examples of Ambiguous Results

Initial searches using the Cyt *b* sequences from samples BFW 80 and BFW 119 resulted in two species, *Passer domesticus* (House Sparrow) and *Passer hispaniolensis* (Spanish Sparrow), with an equal similarity to the query sequence (99%). The bloodmeals from these samples were putatively identified as *P. domesticus* because it is a common bird in the study site, while *P. hispaniolensis*, a Palearctic species, has never been reported from the Nearctic region. The COI sequences for both samples returned a 100% similarity to *P. domesticus*.

A similar situation occurred with sample FWR 3; putatively identified as *Cervus elaphus* (Elk). In this case, the COI sequence generated an ambiguous result in GenBank with two species meeting the 95% similarity cut-off. The query sequence had a higher percent similarity with *Cervus nippon* (Sika Deer) at 97% than *C. elaphus* (96%). A possible reason for the discrepancy is that *C. elaphus* appears to be paraphyletic. North American and Asian *C. elaphus* populations are more closely related to *C. nippon* than they are to *C. elaphus* from Western Europe, the Middle East and Africa (Ludt et al. 2004 and Wada et al. 2007). GenBank contains Cyt *b* sequences from both groups of *C. elaphus* and *C. nippon*. The Cyt *b* sequence from sample FWR 3 has a 98% similarity with eight *C. elaphus* sequences collected in North America and China.

Sample THR 11, putatively identified as *Poecile atricapillus* (Black-capped Chickadee), returned ambiguous results for both COI and Cyt *b*. The BLAST search for COI returned matches for *P. atricapillus* (99% and 97% similarity) and a 98% similarity for *Baeolophus bicolor* (Tufted Titmouse). However, two other sequences

for *B. bicolor* are also in GenBank and have a 91% similarity with COI from THR11. This could be indicative of a misidentified GenBank sequence. For Cyt *b* the results returned a 98% similarity between the query sequence, one *Poecile montanus* (Willow Tit) sequence and two *P. atricapillus* sequences. *Poecile atricapillus* is a common bird in Oregon (Marshall et al. 2006), and neither *B. bicolor* nor *P. montanus* occur locally, as they range throughout the southeastern United States and Europe, respectively.

The bloodmeals from seven samples were putatively identified as *Aphelocoma californica* (Western Scrub-jay): BFW 21, 52, 72, 129, 133, 135, and 137. For brevity only BFW 21 is discussed, however, the results are similar for the other six samples. Eight COI sequences for *Aphelocoma californica* (Western Scrub-jay) from California, USA, and Washington, USA, matched our query sequence with 99% similarity and an additional *A. californica* sequence from Colorado, USA, with 96% similarity. Two *Aphelocoma insularis* (Island Scrub-jay), a species restricted to Santa Cruz Island off the Coast of California, USA sequences had 98% similarity to the query sequence. The Cyt *b* sequence from BFW 21 has a 98% similarity with one *Aphelocoma coerulescens* (Florida Scrub-jay), and a 95% similarity with one *A. californica* sequence. The "*A. coerulescens*" sequence was obtained from a scrub-jay collected in California (Espinosa de los Monteros and Cracraft 1997) and should probably be considered *A. californica*, as *A. coerulescens* in its more narrow taxonomic circumscription has not been reported from that state.

The last sample returning ambiguous results was BFW91. The Cyt *b* sequence from this sample did not result in a match that met our criteria, the closest match, at 90% similarity, was the non-local *Archilochus colubris* (Ruby-throated

Hummingbird). Eight species of hummingbirds met the 95% similarity cut-off with COI sequenced from BFW91: *Selasphorus sasin* (Allen's hummingbird; 98% similarity), *Selasphorus platycercus* (Broad-tailed Hummingbird; 97% and 96%), *Stellula calliope* (Calliope Hummingbird; 96%), *Selasphorus rufus* (Rufous Hummingbird; 96%), *Calypte costae* (Costa's Hummingbird; 96%) and *Calypte anna* (Anna's Hummingbird; 95%). While we can be reasonably confident that this bloodmeal came from a hummingbird, all of these species occur in Oregon (Marshall et. al. 2006), and the ID must remain ambiguous for now. If a specific identification were needed, a targeted effort could be directed at obtaining samples and sequencing the two gene regions for all of the hummingbirds in the area.

Taxonomic coverage in GenBank for COI and Cyt *b* was found to be comparable, although incomplete, for both of the gene regions used. Of the 22 vertebrate species putatively identified from the mosquito bloodmeals, there were corresponding sequences in GenBank for all but three of the COI sequences and all but one Cyt *b* sequence. Because the coverage in GenBank differed for the two genes, the use of both increased the numbers of matches found. In general, if the BLAST results indicate that the closest matching sequence is more divergent than 95%, then it is likely that the source of the bloodmeal is not yet represented for that gene in GenBank (Fig. 3.2). For example GenBank does not contain the COI gene region for *Odocoileus hemionus* (Mule Deer), the putative host identified in 83 of the mosquitoes collected for this study based on Cyt *b* matches ranging from 95 to 98%. Since both Cyt *b* and COI gene fragments were amplified and sequenced from 49 of these

samples, we can use the good match among them to infer a positive ID for the 25 samples from which we were only able to amplify COI.

Conclusion

The major drawback of prior studies and protocols is that to correctly identify mosquito bloodmeals, they require researchers to obtain, and have on hand, samples from all potential hosts. This is impracticable. Our primers selectively amplify vertebrate COI and Cyt *b* from avian, mammalian and amphibian blood in the guts of a wide range of mosquito species. By using GenBank as a reference database, we were able to putatively identify all of the bloodmeals in our sample. In cases where identifications were ambiguous, the range of potential hosts was at least narrowed to a few closely-related species, making it easier to determine the specific host, if the need arose. As DNA barcode databases continue to grow, it is likely that even these ambiguities will be resolved, at least for COI. Sequences are continuously added to GenBank and their number doubles every 18 months (Benson et al. 2006).

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REFERENCES

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389--3402.

Apperson CS, Harrison BA, Unnasch TR, Hassan H.K, Irby WS, Savage HM, Aspen SE, Watson DW, Rueda LM, Engber BR, Nasci RS. 2002. Host-feeding habits of *Culex* and other mosquitoes (Diptera: Culicidae) in the borough of Queens in New York City, with characters and techniques for identification of *Culex* mosquitoes. *J Med Entomol* 39: 777-785.

Apperson CS, Hassan HK, Harrison BA, Savage HM, Aspen SE, Farajollahi A, Crans WJ, Daniels TJ, Falco RC, Benedict M, Anderson M, McMillen L, Unnasch TR. 2004. Host Feeding Patterns of Established and Potential Mosquito Vectors of West Nile Virus in the Eastern United States. *Vector-Borne and Zoonotic Dis* 4: 71-82.

Armstrong KF, Ball SL. 2005. DNA barcodes for biosecurity: invasive species identification. *Trans R Soc Lond B Biol Sci*, 360: 1813-1823.

Baker CS, Palumbi SR. 1994. Which whales are hunted? A molecular genetic approach to monitoring whaling. *Science* 265: 1538-1539.

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2006. Genbank. *Nucleic Acids Res* 35: D21-D25.

Boakye DA, Tang J, Truc P, Merriweather A, Unasch, TR. 1999. Identification of bloodmeals in haematophagous Diptera by cytochrome B heteroduplex analysis. *Med Vet Entomol* 13: 282--287.

Bosseno MF, García LS, Baunaure F, Gastelúm EM, Gutierrez MS, Kasten FL, Dumonteil E, Breniére S. 2006. Identification in triatomine vectors of feeding sources and *Trypanosoma cruzi* variants by heteroduplex assay and multiplex miniexon polymerase chain reaction. *Am J Trop Med and Hyg* 74: 303-305.

Brower AVZ. 2006. Problems with DNA barcodes for species delimitation: "ten species" of *Astraptes fulgerator* reassessed (Lepidoptera: Hesperiidae). *Sys Bio* 4: 1-6.

Brower AVZ, DeSalle R, Vogler A. 1996. Gene trees, species trees and systematics: a cladistic perspective. *Ann Rev Ecol Sys* 27: 423-450.

Burkot TR, Goodman WG, DeFoliart GR. 1981. Identification of mosquito blood meals by enzyme-linked immunosorbent assay. *Am J Trop Med Hyg* 30: 1336-1341.

CDC. 2003. Epidemic/epizootic West Nile virus in the United States: guidelines for surveillance, prevention and control. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention. http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-aug-2003.pdf , Accessed 10-Jan.-2008.

Chow E, Wirts RA, Scott TW. 1993. Identification of blood meals in *Aedes aegypti* by antibody sandwich enzyme-linked immunosorbent assay. *J Am Mosq Cont Assoc* 9: 196-205.

DeSalle R, Birstein VJ. 1996. PCR identification of black caviar. *Nature* 381: 197-198.

Espinosa de los Monteros A, Crarcraft J. 1997. Intergeneric relationships of the new world jays inferred from cytochrome *b* gene sequences. *The Condor* 99: 490-502.

Finnila S, Lehtonen MS, Majama K. 2001. Phylogenetic network for European mtDNA. *Am J Human Gen* 68: 1475-1484.

Funk DJ, Omland KC. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annu Rev Ecol Syst* 34: 397-423.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 4: 95-98.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identification through DNA barcodes. *Proc R Soc Lond B Biol Sci* 270: 313-321.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM. 2004. Identification of birds through DNA barcodes. *PLoS Bio* 2: e312.

Irby WS, Apperson CS. 1988. Hosts of mosquitoes in the coastal plain of North Carolina. *J Med Entomol* 25: 85-93.

Kent RJ, Norris DE. 2005. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome b. *Am J Trop Med Hyg* 73: 336-342.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC. 1989 Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc Natl Acad Sci U S A* 86: 6196-6200.

Lee JH, Hassan HK, Hill G, Cupp EW, Higazi TB, Mitchell CJ, Godsey MS, Unnasch TR. 2002. Identification of mosquito avian-derived blood meals by polymerase chain reaction-heteroduplex analysis. *Am J Trop Med Hyg* 66: 599-604.

Ludt CJ, Schroeder W, Rottman O, Kuehn R. 2004. Mitochondrial DNA phylogeography of red deer (*Cervus elaphus*). *Mol Phylo Evo* 31:1064-1083.

MacDonald G. 1957. *The Epidemiology and Control of Malaria*. Oxford University Press, London.

Malmqvist B, Strasevicius D, Helgren O, Adler PH, Bensch S. 2004. Vertebrate host specificity of wild-caught blackflies revealed by mitochondrial DNA in blood. *Proc R Soc Lond B* 271: S152-S155.

Marshall DB, Hunter MG, Contreras AL. 2006. *Birds of Oregon*. Oregon State University Press, Corvallis.

Meece JK, Reynolds CE, Stockwell PJ, Jenson TA, Christensen JE, Reed KD. 2005. Identification of mosquito bloodmeal source by terminal restriction fragment length polymorphism profile analysis of the cytochrome b gene. *J Med Entomol* 42**:** 657-667.

Meier R, Shiyang K, Vaidya G, Ng PKL. 2006. DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. *Sys Biol* 55: 715-728.

Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrink CR. 2006. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, Northeastern United States. *Emerg Infect Dise* 12: 468-474.

Molaei G, Andreadis TG. 2006. Identification of avian- and mammalian-derived bloodmeals in *Aedes vexans* and *Culiseta melanura* (Diptera: Culicidae) and its implication for West Nile virus transmission in Connecticut, U.S.A. *J Med Entomol* 43: 1088-1093.

Molaei G, Andreadis TG, Armstrong PM, Bueno Jr. R, Dennett JA, Real SV, Sargent C, Bala A, Randle Y, Guzman H, Travassos da Rosa A, Wuithiranyagool T, Tesh RB. 2007. Host feeding pattern of *Culex*

quinquefasciatus (Diptera: Culicidae) and its role in transmission of West Nile virus in Harris County, Texas. *Am J Trop Med Hyg* 71: 73-81.

Nataraj AJ, Olivos-Glander I, Kusukawa N, Highsmith WE Jr. 1999. Single-strand conformation polymorphism and heteroduplex analysis for gel-based mutation detection. *Electrophoresis* 20: 1177-1186.

Ngo KA, Kramer LD. 2003. Identification of mosquito blood meals using polymerase chain reaction (PCR) with order-specific primers. *J Med Entomol* 40: 215-222.

Sperling FAH, Anderson GS, Hickey DA. 1994. A DNA-base approach to the identification of insect species used for postmortem interval estimation. *J Forensic Sci* 39: 418-427.

Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP. 2003. A plea for DNA taxonomy. *Trends Ecol Evol* 18**:** 70-74.

Townzen JS. 2005, October. Mosquitoes and stormwater management in Corvallis, OR. *44th Ann Conf Northwest Mosq Vector Cont Assoc* Layton, Utah.

Townzen JS, Brower AVZ, Judd DD. 2007, April. Identification of bloodmeals from mosquitoes collected in Corvallis, OR. *Proc 73rd Ann Meeting Am Mosq Cont Assoc*. Orlando, Florida.

Washino RK, Tempelis CH. 1983. Mosquito host bloodmeal identification: methodology and data analysis. *Ann Rev Entomol* 28: 179-201.

Wheeler DL, Chappey C, Lash AE, Leipe DD, Madden TL, Schuler GD, Tatusova TA, Rapp BA 2000. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 28: 10-14.

Will KW, Rubinoff D. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* 20: 47-55.

Will KW, Mishler BD, Wheeler QD. 2005. Perils of DNA barcoding and the need for integrative taxonomy. *Sys Bio* 54: 844-851.

Table 3.1. Primers used to identify mosquito bloodmeals.

Primer Name	Location*	Primer Sequence
COI_long (f)	5934	AACCACAAAGACATTGGCAC
COI_long (r)	6597	AAGAATCAGAATARGTGTTG
COI_short (f)	6267	GCAGGAACAGGWTGAACCG
COI_short (r)	6591	AATCAGAAYAGGTGTTGGTATAG
Cyt b (f)	15150	GAGGMCAAATATCATTCTGAGG
Cyt b (r)	15607	TAGGGCVAGGACTCCTCCTAGT

* Location on human mtDNA (Finnila et al. 2001).

Table 3.2. Sequences used for designing the COI and *Cyt b* primers.

Gene	***Species***	**Accession No.**
COI	*Turdus migratorius*	AF197836
	Gallus gallus	AP003317
	Mus musculus	AB042523
	Ovis aries	AF010406
	Anopheles gambiae	L20934
Cyt b	*Passer domesticus*	AY495393
	Gallus gallus	AP003317
	Mus musculus	EU315229
	Odocoileus hemionus	X56291
	Anopheles gambiae	L20934

Table 3.3. Mosquitoes collected in Corvallis, Oregon, from which vertebrate DNA was sequenced and their corresponding top two matches in GenBank.

* Because the within species sequence variation was largely due to ambiguous basepairs, only a single sequence for each host species was submitted to GenBank.
** Because of the large number of *Homo sapiens* sequences in GenBank a next closest match has not been identified.

Sample	Mosquito Species	Primer Pair	Closest Match	Percent match		Occurs Locally	Next Closest Match	% Match
BFG37	*Anopheles punctipennis*	COI-Long	*Cervus unicolor* (Sambar Deer)	535/602	0.889	No		
		Cyt b	*Odocoileus hemionus* (Mule Deer)	392/397	0.987	Yes	*Odocoileus virginianus* (White-tailed Deer)	0.93
BFG4	*Anopheles freeborni*	COI-Short	*Cervus unicolor* (Sambar Deer)	258/292	0.884	No		
		Cyt b	*O. hemionus* (Mule Deer)	402/406	0.990	Yes	*O. virginianus* (White-tailed Deer)	0.94
BFW14	*Culex tarsalis*	COI-Short	*Corvus brachyrhynchos* (American Crow)	268/269	0.996	Yes	*Corvus abicollis* (White-necked Raven)	0.95
		Cyt b	*C.brachyrhynchos* (American Crow)	387/390	0.992	Yes	*Corvus corone* (Carrion Crow)	0.92
BFW15	*Cx. tarsalis*	COI-Short	*C. unicolor* (Sambar Deer)	256/284	0.900	No		
BFW16	*An. punctipennis*	COI-Short	*Rangifer tarandus* (Caribou)	241/271	0.889	No		
BFW18	*Cx. tarsalis*	COI-Short	*Turdus migratorius* (American Robin)	272/272	1.000	Yes	*Catharus ustulatus* (Swainson's Thrush)	0.91
		Cyt b	*T. migratorius* (American Robin)	384/389	0.987	Yes	*Turdus grayi* (Clay-coloured Robin)	0.92

Table 3.3. Continued.

BFW19	*An. punctipennis*	COI-Short	*Ovis aries* (Sheep)	272/273	0.996	Yes	*Capra hircus* (Domestic Goat)	0.88
BFW20	*Cx. tarsalis*	COI-Long	*C. brachyrhynchos* (American Crow)	610/612	0.997	Yes	*Corvus frugilegus* (Rook)	0.93
		Cyt b	*C. brachyrhynchos* (American Crow)	610/612	0.997	Yes	*C.corone* (Carrion Crow)	0.95
BFW21	*Cx. tarsalis*	COI-Long	*Aphelecoma californica* (Western Scrub-jay)	601/602	0.998	Yes	*Aphelocoma insularis* (Island Scrub-jay)	0.98
		Cyt b	*A. coerulescens* (Florida Scrub-jay)	408/413	0.988	No	*A. californica* (Western Scrub-jay)	0.95
BFW23	*Cx. tarsalis*	COI-Long	*Sturnus vulgaris* (European Starling)	615/616	0.998	Yes	*Acridotheres tristis* (Common Myna)	0.89
		Cyt b	*S. vulgaris* (European Starling)	397/397	1.000	Yes	*Lamprotornis nitens* (Glassy Starling)	0.91
BFW24	*Cx. tarsalis*	COI-Long	*T. migratorius* (American Robin)	621/626	0.992	Yes	*C. ustulatus* (Swainson's Thrush)	0.90
		Cyt b	*T. migratorius* (American Robin)	404/407	0.993	Yes	*Turdus grayi* (Clay-coloured Robin)	0.92
BFW25	*An. freeborni*	COI-Long	*Homo sapiens* (Human)	618/618	1.000	Yes	*	
		Cyt b	*H. sapiens* (Human)	393/395	0.995	Yes	*	
BFW30	*Cx. tarsalis*	COI-Short	*Procyon lotor* (Racoon)	267/268	0.996	Yes	*Tomistoma schlegelii* (False Gharial)	0.82
		Cyt b	*P. lotor* (Racoon)	376/385	0.977	Yes	*Procyon cancrivorus* (Crab-eating Racoon)	0.88
BFW33	*Cx. tarsalis*	COI-Short	*T. migratorius* (American Robin)	275/278	0.989	Yes	*Mimus longicaudatus* (Long-tailed Mockingbird)	0.90

Table 3.3. Continued.

BFW34	*An. punctipennis*	COI-Short	*C. unicolor* (Sambar Deer)	251/283	0.887	No		
		Cyt b	*O. hemionus* (Mule Deer)	392/396	0.990	Yes	*O. virginianus* (White-tailed Deer)	0.93
BFW35	*An. punctipennis*	COI-Short	*C. unicolor* (Sambar Deer)	233/262	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	391/395	0.990	Yes	*O. virginianus* (White-tailed Deer)	0.93
BFW36	*An. punctipennis*	COI-Short	*C. unicolor* (Sambar Deer)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	391/396	0.987	Yes	*O. virginianus* (White-tailed Deer)	0.92
BFW37	*An. freeborni*	COI-Short	*C. unicolor* (Sambar Deer)	*250/283*	0.883	No		
		Cyt b	*O. hemionus* (Mule Deer)	*389/395*	0.985	Yes	*O. virginianus* (White-tailed Deer)	0.92
BFW38	*An. punctipennis*	COI-Short	*Muntiacus reevesi* (Reeves' Muntjac)	246/276	0.891	No		
BFW42	*Culex pipiens*	COI-Short	*Butorides virescens* (Green Heron)	274/278	0.986	Yes	*Butorides striatus* (Striated Heron	0.96
		Cyt b	*B. virescens* (Green Heron)	388/405	0.958	Yes	*Ardea herodias* (Great Blue Heron)	0.86
BFW43	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	390/397	0.982	Yes	*O. virginianus* (White-tailed Deer)	0.92
BFW44	*An. freeborni*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus (*Mule Deer)	244/246	0.992	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW45	*An. punctipennis*	COI-Short	*M. reevesi (*Reeves' Muntjac)	246/277	0.888	No		
		Cyt b	*O. hemionus* (Mule Deer)	392/395	0.992	Yes	*O. virginianus* (White-tailed Deer)	0.93

Table 3.3. Continued.

BFW46	*An. punctipennis*	COI-Long	*R. tarandus* (Caribou)	487/570	0.854	No		
BFW47	*Cx. pipiens*	COI-Short	*B. virescens* (Green Heron)	264/265	0.996	Yes	*B. striatus* (Striated Heron	0.97
		Cyt b	*B. virescens* (Green Heron)	397/399	0.995	Yes	*Egretta caerulea* (Little Blue Heron)	0.90
BFW48	*Cx. pipiens*	COI-Long	*S. vulgaris* (European Starling)	606/606	1.000	Yes	*Leucopsar rothschildi* (Bali Mynah)	0.91
BFW51	*Cx. tarsalis*	COI-Long	*B. virescens* (Green Heron)	330/332	0.994	Yes	*B. striatus* (Striated Heron)	0.97
		Cyt b	*B. virescens* (Green Heron)	382/392	0.974	Yes	*A. herodias* (Great Blue Heron)	0.89
BFW52	*Cx. pipiens*	COI-Long	*A. californica* (Western Scrub-jay)	599/602	0.995	Yes	*A. insularis* (Island Scrub-jay)	0.98
BFW53	*An. punctipennis*	COI-Short	*P. lotor* (Racoon)	280/281	0.996	Yes	*Elops saurus* (Ladyfish)	0.83
BFW54	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	242/272	0.890	No		
		Cyt b	*O. hemionus* (Mule Deer)	376/394	0.954	Yes	*O. virginianus* (White-tailed Deer)	0.88
BFW56	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	242/272	0.890	No		
		Cyt b	*O. hemionus* (Mule Deer)	387/394	0.982	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW57	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	384/394	0.974	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW58	*An. punctipennis*	Cyt b	*O. hemionus* (Mule Deer)	390/397	0.982	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW59	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	254/284	0.894	Yes		
		Cyt b	*O. hemionus* (Mule Deer)	383/393	0.975	Yes	*O. virginianus* (White-tailed Deer)	0.91

Table 3.3. Continued.

BFW60	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	247/277	0.892	No		
		Cyt b	*O. hemionus* (Mule Deer)	388/394	0.985	Yes	*O. virginianus (White-tailed Deer)*	0.91
BFW61	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	247/277	0.892	No		
BFW62	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	389/394	0.987	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW63	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	Yes		
		Cyt b	*O. hemionus* (Mule Deer)	381/398	0.970	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW64	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	388/394	0.985	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW65	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	388/394	0.985	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW66	*An. punctipennis*	Cyt b	*O. hemionus* (Mule Deer)	372/380	0.979	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW67	*An. punctipennis*	COI-Short	*M. reevesi* (Reeves' Muntjac)	246/276	0.891	No		
		Cyt b	*O. hemionus* (Mule Deer)	382/390	0.979	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW68	*An. punctipennis*	Cyt b	*O. hemionus* (Mule Deer)	389/397	0.980	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW69	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	368/378	0.973	Yes	*O. virginianus* (White-tailed Deer)	0.91

Table 3.3. Continued.

BFW71	*Cx. pipiens*	COI-Short	*Bombycilla cedrorum* (Cedar Waxwing)	274/274	1.000	Yes	*Loboparadisea sericea* (Yellow-breasted Bird-of-paradise)	0.92
		Cyt b	*B. cedrorum* (Cedar Waxwing)	388/392	0.990	Yes	*Bombycilla japonica* (Japanese Waxwing)	0.90
BFW72	*Cx. pipiens*	COI-Long	*A. californica* (Western Scrub-jay)	6615/616	0.998	Yes	*A. insularis* (Island Scrub-jay)	0.98
		Cyt b	*A. coerulescens* (Florida Scrub-jay)	389/398	0.977		*A. californica* (Western Scrub-jay)	0.95
BFW78	*An. punctipennis*	COI-Short	*M. reevesi* (Reeves' Muntjac)	258/290	0.890	No		
		Cyt b	*O. hemionus* (Mule Deer)	390/396	0.985	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW80	*Cx. pipiens*	COI-Short	*Passer domesticus* (House Sparrow)	265/265	1.000	Yes	*Passer montanus* (Tree Sparrow)	0.94
		Cyt b	*P. domesticus* (House Sparrow)	376/385	0.977	Yes	*Passer hispaniolensis* (Spanish Sparrow)	0.97
BFW81	*Culex stigmatosoma*	COI-Long	*T. migratorius* (American Robin)	608/613	0.992	Yes	*C. ustulatus* (Swainson's Thrush)	0.90
	Cx. stigmatosoma	Cyt b	*T. migratorius* (American Robin)	389/395	0.985	Yes	*Turdus rufitorques* (Rufous-collared Robin)	0.97
BFW82	*Cx. pipiens*	COI-Short	*B. virescens* (Green Heron)	281/284	0.989	Yes	*B. striatus* (Striated Heron)	0.97
		Cyt b	*B. virescens* (Green Heron)	391/400	0.978	Yes	*A. herodias* (Great Blue Heron)	0.89
BFW83	*Cx. pipiens*	COI-Short	*T. migratorius* (American Robin)	*268/268*	1.000	Yes	*M. longicaudatus* (Long-tailed Mockingbird)	0.91
		Cyt b	*T. migratorius* (American Robin)	404/407	0.993	Yes	*Turdus rufitorques* (Rufous-collared Robin)	0.97

Table 3.3. Continued.

BFW84	*Cx. pipiens*	COI-Short	*B. cedrorum* (Cedar Waxwing)	273/273	1.000	Yes	*L.sericea* (Yellow-breasted Bird-of-paradise)	0.92
		Cyt b	*B. cedrorum (*Cedar Waxwing)	387/392	0.987	Yes	*B. japonica* (Japanese Waxwing)	0.90
BFW85	*An. punctipennis*	Cyt b	*O. hemionus* (Mule Deer)	373/379	0.987	Yes	*O. virginianus* (White-tailed Deer)	0.93
BFW86	*An. punctipennis*	COI-Long	*R. tarandus* (Caribou)	510/572	0.892	No		
		Cyt b	*O. hemionus* (Mule Deer)	389/397	0.980	Yes	*O. virginianus* (White-tailed Deer)	0.92
BFW87	*An. punctipennis*	COI-Long	*M. reevesi* (Reeves' Muntjac)	538/607	0.886	No		
		COI-Short	*R. tarandus* (Caribou)	254/285	0.890	No		
BFW88	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	246/277	0.888	No		
BFW89	*An. punctipennis*	Cyt b	*O. hemionus* (Mule Deer)	406/411	0.988	Yes	O. virginianus (White-tailed Deer)	0.93
BFW90	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	239/271	0.882	No		
BFW91	*Cx. pipiens*	COI-Long	*Selasphorus sasin* (Allen's Hummingbird)	568/578	0.983	No	*Selasphorus platycercus* (Broad-tailed Hummingbird)	0.97
		Cyt b	*Archilochus colubris* (Ruby-throated Hummingbird)	335/382	0.887	No		
BFW92	*Cx. pipiens*	COI-Short	*B. cedrorum* (Cedar Waxwing)	279/279	1.000	Yes	*L.sericea* (Yellow-breasted Bird-of-paradise)	0.92
		Cyt b	*B. cedrorum* (Cedar Waxwing)	393/395	0.995	Yes	*B. japonica* (Japanese Waxwing)	0.90
BFW93	*An. punctipennis*	COI-Long	*R. tarandus* (Caribou)	531/593	0.895	No		
		Cyt b	*O. hemionus* (Mule Deer)	405/410	0.988	Yes	*O. virginianus* (White-tailed Deer)	0.93

Table 3.3. Continued.

BFW95	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	242/272	0.890	No		
		Cyt b	*O. hemionus* (Mule Deer)	393/398	0.987	Yes	*O. virginianus* (White-tailed Deer)	0.93
BFW97	*An. punctipennis*	COI-Short	R. tarandus (Caribou)	247/277	0.892	No		
BFW99	An. punctipennis	COI-Short	*M. reevesi* (Reeves' Muntjac)	236/265	0.891	No		
BFW100	*An. punctipennis*	Cyt b	*O. hemionus* (Mule Deer)	391/402	0.973	Yes	*O. virginianus* (White-tailed Deer)	0.93
BFW101	*Cx. pipiens*	COI-Short	*R. tarandus* (Caribou)	246/276	0.891	No		
		Cyt b	*O. hemionus* (Mule Deer)	391/406	0.963	Yes	*O. virginianus* (White-tailed Deer)	0.92
BFW102	*Cx. pipiens*	COI-Short	*M. reevesi* (Reeves' Muntjac)	258/290	0.890	No		
BFW103	*Cx. tarsalis*	COI-Short	*R. tarandus (Caribou)*	250/281	0.890			
		Cyt b	*O. hemionus* (Mule Deer)	406/410	0.990	Yes	*O. virginianus* (White-tailed Deer)	0.93
BFW104	*Cx. pipiens*	COI-Short	*Carpodacus mexicanus* (House Finch)	271/271	1.000	Yes	*Carduelis pinus* (Pine Siskin)	0.90
		Cyt b	*C. mexicanus* (House Finch)	387/394	0.982	Yes	*Loxia curvirostra* (Common Crossbill)	0.93
BFW105	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	250/280	0.893	No		
BFW106	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	251/281	0.893	No		
		Cyt b	*O. hemionus* (Mule Deer)	389/394	0.987	Yes	*O. virginianus* (White-tailed Deer)	0.92
BFW107	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	257/260	0.989	Yes	*O. virginianus* (White-tailed Deer)	0.90

Table 3.3. Continued.

BFW108	*Cx. tarsalis*	Cyt b	*C. mexicanus* (House Finch)	387/392	0.987	Yes	*L. curvirostra* (Common Crossbill)	0.94
BFW109	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	250/281	0.890	No		
		Cyt b	*O. hemionus* (Mule Deer)	401/404	0.993	Yes	*O. virginianus* (White-tailed Deer)	0.94
BFW110	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	227/259	0.876	No		
BFW111	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	255/285	0.895	No		
		Cyt b	*O. hemionus* (Mule Deer)	406/410	0.990	Yes	*O. virginianus* (White-tailed Deer)	0.93
BFW112	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	378/394	0.959	Yes	*O. virginianus* (White-tailed Deer)	0.90
BFW113	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	246/276	0.891	No		
		Cyt b	*O. hemionus* (Mule Deer)	388/397	0.977	Yes	*O. virginianus* (White-tailed Deer)	0.91
BFW114	*An. punctipennis*	COI-Short	*C. unicolor* (Sambar Deer)	251/284	0.884	No		
BFW115	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
BFW116	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
BFW117	*An. punctipennis*	COI-Long	*R. tarandus* (Caribou)	538/606	0.888	No		
BFW118	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	250/280	0.893	No		
		Cyt b	*O. hemionus* (Mule Deer)	406/410	0.990	Yes	*O. virginianus* (White-tailed Deer)	0.93

Table 3.3. Continued.

BFW119	*Cx. pipiens*	COI-Long	*P. domesticus* (House Sparrow)	607/609	0.997	Yes	*P. montanus* (Tree Sparrow)	0.93
		Cyt b	*P. domesticus* (House Sparrow)	411/412	0.998	Yes	*P. hispaniolensis* (Spanish Sparrow)	0.97
BFW120	*Cx. pipiens*	COI-Short	*B. cedrorum* (Cedar Waxwing)	277/277	1.000	Yes	*L. sericea* (Yellow-breasted Bird-of-paradise)	0.92
		Cyt b	*B. cedrorum* (Cedar Waxwing)	384/389	0.987	Yes	*B. japonica* (Japanese Waxwing)	0.90
BFW121	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	242/272	0.890	No		
BFW122	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/272	0.886	No		
BFW123	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	238/272	0.875	No		
BFW124	*An. punctipennis*	COI-Short	*B. virescens* (Green Heron)	241/246	0.980	Yes	*B. striatus* (Striated Heron)	0.96
BFW125	*Cx. pipiens*	COI-Short	*B. virescens* (Green Heron)	223/233	0.957	Yes	*B. striatus* (Striated Heron)	0.94
BFW126	*Cx. stigmatosoma*	COI-Short	*C. caurinus* (Northwestern Crow)	286/288	0.993	Yes	*C.brachyrhynchos* (American Crow)	0.99
		Cyt b	*C. brachyrhynchos* (American Crow)	396/400	0.990	Yes	*C.corone* (Carrion Crow)	0.95
BFW128	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	247/277	0.892	No		
		Cyt b	*O. hemionus* (Mule Deer)	394/397	0.992	Yes	*O. virginianus* (White-tailed Deer)	0.93
BFW129	*Cx. stigmatosoma*	COI-Long	*A. californica* (Western Scrub-jay)	613/614	0.998	Yes	*A. insularis* (Island Scrub-jay)	0.98
		Cyt b	*A. coerulescens* (Florida Scrub-jay)	393/398	0.987		*A. californica* (Western Scrub-jay)	0.95

Table 3.3. Continued.

BFW130	*Cx. pipiens*	COI-Short	*Zenaida macroura* (Mourning Dove)	279/283	0.986	Yes	*Streptopelia chinensis* (Spotted Dove)	0.90
		Cyt b	*Z. macroura* (Mourning Dove)	393/393	1.000	Yes	*Zenaida graysoni* (Grey Dove)	0.98
BFW131	*Cx. pipiens*	Cyt b	*Carduelis tristis* (American Goldfinch)	383/387	0.990	Yes	*Carduelis lawrencei* (Laurence's Goldfinch)	0.95
BFW132	*Cx. pipiens*	COI-Short	*B. cedrorum* (Cedar Waxwing)	*275/275*	1.000	Yes	*Bombycilla garrulus* (Bohemian Waxwing)	0.92
		Cyt b	*B. cedrorum* (Cedar Waxwing)	392/393	0.998	Yes	*B. japonica* (Japanese Waxwing)	0.91
BFW133	*Cx. pipiens*	COI-Long	*A. californica* (Western Scrub-jay)	596/6-8	0.980	Yes	*A. insularis* (Island Scrub-jay)	0.98
		Cyt b	*A. coerulescens* (Florida Scrub-jay)	403/408	0.988		*A. californica* (Western Scrub-jay)	0.95
BFW135	Cx. stigmatosoma	COI-Long	*A. californica* (Western Scrub-jay)	613/614	0.998	Yes	*A. insularis* (Island Scrub-jay)	0.98
		Cyt b	*A. coerulescens* Florida Scrub-jay)	406/411	0.988		*A. californica* (Western Scrub-jay)	0.95
BFW136	*Cx. pipiens*	COI-Long	*C. ustulatus* (Swainson's Thrush)	608/616	0.987	Yes	*Catharus fuscescens* (Veery)	0.93
		Cyt b	*C. ustulatus* (Swainson's Thrush)	385/394	0.977	Yes	*Catharus minimus* (Grey-cheeked Thrush)	0.92
BFW137	*Cx. pipiens*	COI-Long	*A. californica* (Western Scrub-jay)	611/615	0.993	Yes	*A. insularis* (Island Scrub-jay)	0.98
		Cyt b	*A. coerulescens* (Florida Scrub-jay)	406/411	0.988	No	*A. californica* (Western Scrub-jay)	0.95

Table 3.3. Continued.

EEW2	*An. punctipennis*	COI-Long	*Equus caballus* (Horse)	604/609	0.992	Yes	*Equus asinus* (Ass, Domestic Donkey)	0.90
EWG6	*Culex territans*	COI-Long	*Colostethus nubicola* (Boquete Rocket Frog)	459/549	0.836	No		
		Cyt b	*Pseudacris regilla* (Pacific Tree Frog)	335/339	0.988	Yes	*Hyla chinensis* (Common Chinese Treefrog)	0.82
EWG7	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	393/396	0.992	Yes	*O. virginianus* (White-tailed Deer)	0.92
EWG8	*An. freeborni*	COI-Short	*R. tarandus* (Caribou)	242/272	0.890	No		
EWR2	*Cx. territans*	COI-Long	*C. nubicola* (Boquete Rocket Frog)	426/507	0.840	No		
		Cyt b	*P. regilla* (Pacific Tree Frog)	342/346	0.988	Yes	*H.chinensis* (Common Chinese Treefrog)	0.82
EWR3	*Cx. tarsalis*	COI-Long	*H. sapiens* (Human)	617/618	0.998	Yes		
		Cyt b	*H. sapiens* (Human)	379/384	0.987	Yes		
EWR4	*Cx. territans*	COI-Long	*C. nubicola* (Boquete Rocket Frog)	463/552	0.839	No		
		Cyt b	*P. regilla* (Pacific Tree Frog)	343/348	0.986	Yes	*H. chinensis* (Common Chinese Treefrog)	0.82
EWR5	*An. punctipennis*	Cyt b	*Sylvilagus floridanus* (Eastern Cottontail)	387/396	0.977	Yes	*Sylvilagus transitionalis* (New England Cottontail)	0.89

Table 3.3. Continued.

EWR6	*An. punctipennis*	COI-Short	*Scomberomorus guttatus* (Indo-Pacific Mackerel)	229/269	0.851	No		
		Cyt b	*S. floridanus* (Eastern Cottontail)	389/396	0.982	Yes	*S. transitionalis* (New England Cottontail)	0.89
FIN1	*Cx. tarsalis*	COI-Short	*H. sapiens (Human)*	272/276	0.986	Yes		
FIN2	*Cx. tarsalis*	COI-Short	*T. migratorius* (American Robin)	266/273	0.974	Yes	*C. ustulatus* (Swainson's Thrush)	0.88
FRC1	*Cx. territans*	COI-Long	*C. ustulatus* (Swainson's Thrush)	596/604	0.987	Yes	*C.minimus* (Grey-cheeked Thrush)	0.93
		Cyt b	*C. ustulatus* (Swainson's Thrush)	391/402	0.973	Yes	*Catharus gracilirostris* (Black-billed Nightengale Thrush)	0.92
FRC2	*Cx. tarsalis*	COI-Long	*Melospiza melodia* (Song Sparrow)	615/616	0.994	Yes	*Melospiza lincolnii* (Lincoln's Sparrow)	0.95
		Cyt b	*Atlapetes rufinucha* (Rufous-naped Brush-finch)	357/383	0.932	No		
FWR1	*An. punctipennis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	385/394	0.977	Yes	*O. virginianus* (White-tailed Deer)	0.93
FWR3	*An. freeborni*	COI-Short	*Cervus elaphus* (Elk, Red Deer)	279/287	0.972	No	*Cervus nippon* (Elk, Red Deer)	0.97
		Cyt b	*Cervus elaphus* (Elk, Red Deer)	393/397	0.990	Yes	*C. nippon* (Sika Deer)	0.95
FWR4	*An. freeborni*	COI-Long	*R. tarandus* (Caribou)	526/593	0.887	No		
		Cyt b	*O. hemionus* (Mule Deer)	391/394	0.992	Yes	*O. virginianus* (White-tailed Deer)	0.93

Table 3.3. Continued.

SBCL3	*Cx. pipiens*	COI-Long	*S. vulgaris* (European Starling)	614/614	1.000	Yes	*Leucopsar rothschildi* (Bali Mynah)	0.91
		Cyt b	*S. vulgaris* (European Starling)	385/396	0.972	Yes	*Lamprotornis nitens* (Glassy Starling)	0.88
THR1	*Aedes increpitus*	COI-Long	*C. unicolor* (Sambar Deer)	463/518	0.894	Yes		
		Cyt b	*O. hemionus* (Mule Deer)	393/396	0.992	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR2	*Ae. increpitus*	Cyt b	*S. floridanus* (Eastern Cottontail)	370/383	0.966	Yes	*S. transitionalis* (New England Cottontail)	0.89
THR3	*Aedes sierrensis*	COI-Long	*R. tarandus* (Caribou)	458/515	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	389/394	0.987	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR6	*Ae. sierrensis*	COI-Long	*R. tarandus* (Caribou)	536/602	0.890	No		
		Cyt b	*O. hemionus* (Mule Deer)	391/396	0.987	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR7	*Ae. sierrensis*	Cyt b	*O. hemionus* (Mule Deer)	407/410	0.993	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR10	*Ae. sierrensis*	COI-Short	*R. tarandus* (Caribou)	235/269	0.874	No		
THR11	*Cx. territans*	COI-Long	*Parus atricapillus* (Black-capped Chickadee)	603/604	0.998	Yes	*Baeolophus bicolor* (Tufted Titmouse)	0.95
		Cyt b	*Parus atricapillus* (Black-capped Chickadee)	393/399	0.985	Yes	*Parus montanus* (Willow Tit)	0.98
THR13	*Ae. sierrensis*	COI-Short	*R. tarandus* (Caribou)	247/277	0.892	No		
		Cyt b	*O. hemionus* (Mule Deer)	400/406	0.985	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR14	*Ae. sierrensis*	COI-Short	*M. reevesi* (Reeves' Muntjac)	204/299	0.891	No		

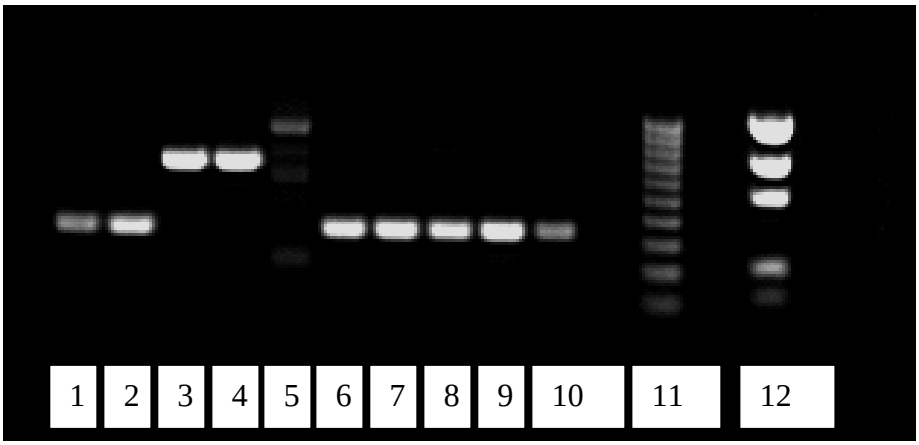
Table 3.3. Continued.

THR15	*Ae. sierrensis*	COI-Short	*R. tarandus* (Caribou)	242/272	0.890	No		
THR47	*Culiseta incidens*	Cyt b	*O. hemionus* (Mule Deer)	391/395	0.990	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR48	*Cs. incidens*	Cyt b	*O. hemionus* (Mule Deer)	406/410	0.990	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR49	*Cs. incidens*	Cyt b	*O. hemionus* (Mule Deer)	406/410	0.990	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR50	*Cs. incidens*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
THR51	*Ae. sierrensis*	Cyt b	*S. floridanus* (Eastern Cottontail)	391/394	0.992	Yes	*S. transitionalis* (New England Cottontail)	0.90
THR52	*Cs. incidens*	COI-Short	*R. tarandus* (Caribou)	240/271	0.886	No		
		Cyt b	*O. hemionus* (Mule Deer)	393/396	0.990	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR54	*Cs. incidens*	COI-Short	*H. sapiens* (Human)	280/280	0.989	Yes	*	
		Cyt b	*H. sapiens* (Human)	363/363	1.000	Yes	*	
THR55	*Ae. sierrensis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	387/394	0.982	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR56	*Ae. sierrensis*	COI-Short	*R. tarandus* (Caribou)	241/271	0.889	No		
		Cyt b	*O. hemionus* (Mule Deer)	391/396	0.987	Yes	*O. virginianus* (White-tailed Deer)	0.93
THR58	*Cs. incidens*	COI-short	*R. tarandus* (Caribou)	254/284	0.894	No		
		Cyt b	*O. hemionus* (Mule Deer)	375/379	0.989	Yes	*O. virginianus* (White-tailed Deer)	0.93
WMP4	*Cs. incidens*	COI-Long	*R. tarandus* (Caribou)	528/593	0.890	No		
		Cyt b	*O. hemionus* (Mule Deer)	386/393	0.982	Yes	*O. virginianus* (White-tailed Deer)	0.91

Table 3.3. Continued.

WMP5	*Cs. incidens*	COI-Long	*R. tarandus* (Caribou)	543/607	0.895	No		
		Cyt b	*O. hemionus* (Mule Deer)	382/387	0.897	Yes	*O. virginianus* (White-tailed Deer)	0.93
WMP6	*Cs. incidens*	COI-Long	*R. tarandus* (Caribou)	530/592	0.895	No		
		Cyt b	*O. hemionus* (Mule Deer)	391/394	0.992	Yes	*O. virginianus* (White-tailed Deer)	0.93

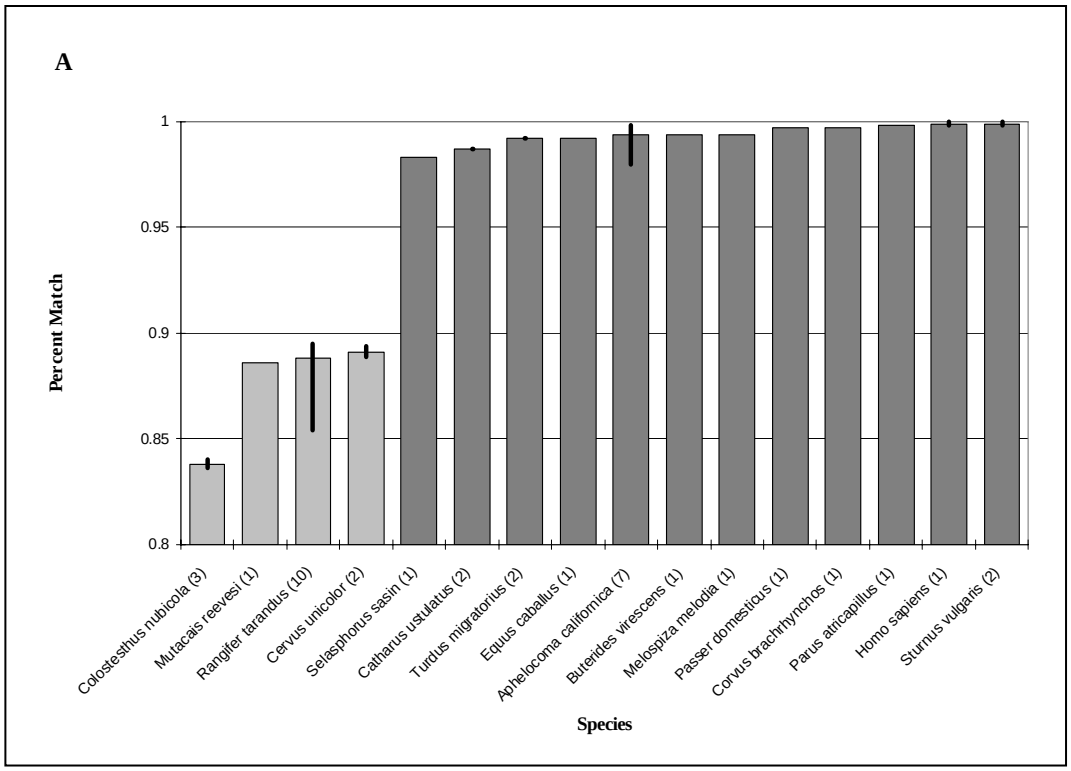
Figure 3.1. Banding pattern of mtDNA amplified with COI_long and COI_short primer pairs.



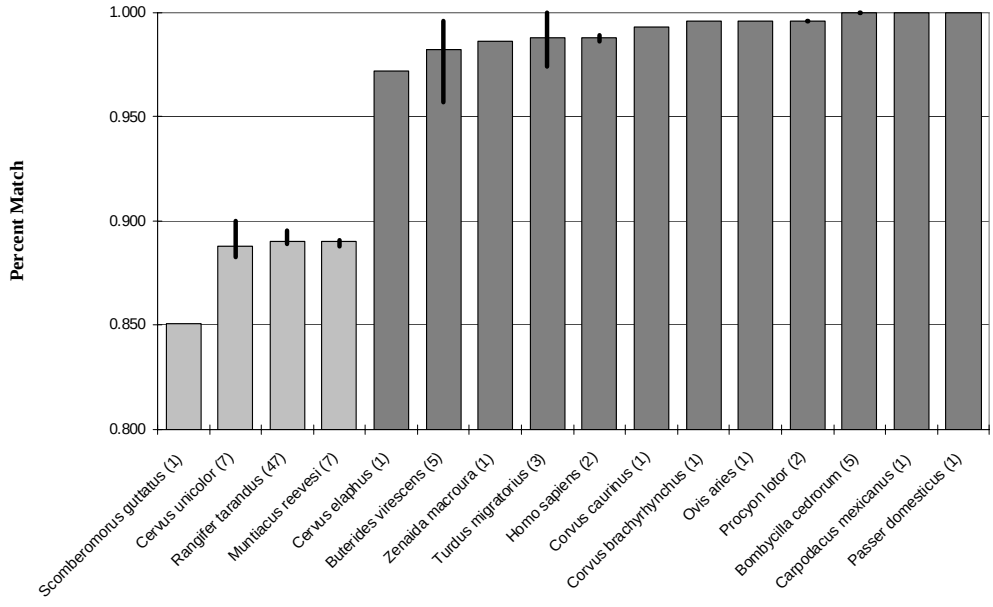
Lane 1 & 2, known mammal fed mosquito amplified with COI_short primer pair; lane 3 &4, known avian fed mosquito amplified with COI_long primer pair; lane five, unfed mosquito amplified with COI_long; lane 6-10, wild caught bloodfed mosquitoes; lane 11, ladder; lane 12, mass standard.

Figure 3.2. Percent sequence identity between bloodmeal and closest GenBank match for each host species identified with COI_long primers (A), COI_short primers (B), and Cyt b primers (C).

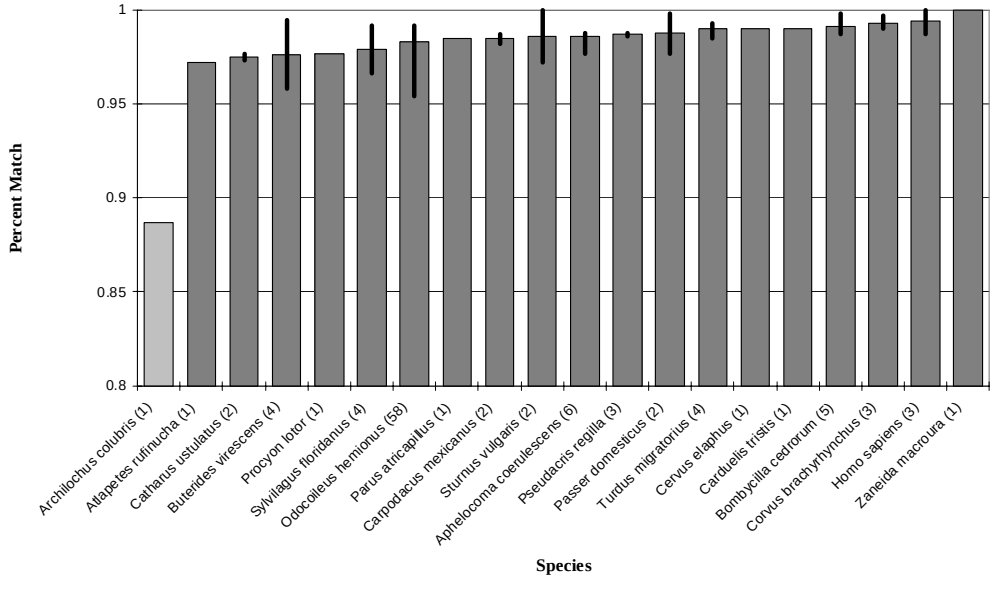
(N) is the number of samples in our data set putatively identified for each species. Wide bars represent averages and narrow bars represent range of variation between the best matching GenBank sequence and multiples sample sequences from mosquito bloodmeals. Light bars represent sequences that are not a good match; dark bars are sequences with a putative match to species.



B


1.000
0.950
0.900
0.850
0.800
Percent Match
Scomberomorus guttatus (1)
Cervus unicolor (7)
Rangifer tarandus (47)
Muntiacus reevesi (7)
Cervus elaphus (1)
Buterides virescens (5)
Zenaida macroura (1)
Turdus migratorius (3)
Homo sapiens (2)
Corvus caurinus (1)
Corvus brachyrhynchus (1)
Ovis aries (1)
Procyon lotor (2)
Bombycilla cedrorum (5)
Carpodacus mexicanus (1)
Passer domesticus (1)
Species

C


1
0.95
0.9
0.85
0.8
Percent Match
Archilochus colubris (1)
Atlapetes rufinucha (1)
Catharus ustulatus (2)
Buterides virescens (4)
Procyon lotor (1)
Sylvilagus floridanus (4)
Odocoileus hemionus (58)
Parus atricapillus (1)
Carpodacus mexicanus (2)
Sturnus vulgaris (2)
Aphelocoma coerulescens (6)
Pseudacris regilla (3)
Passer domesticus (2)
Turdus migratorius (4)
Cervus elaphus (1)
Carduelis tristis (1)
Bombycilla cedrorum (5)
Corvus brachyrhynchus (3)
Homo sapiens (3)
Zaneida macroura (1)
Species

ASSESSMENT OF MOSQUITO BLOODMEAL SOURCES VIA DNA BARCODING: HOST FEEDING PATTERN AND DISEASE TRANSMISSION IN WESTERN OREGON

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ABSTRACT

The host feeding habits of mosquitoes collected in Corvallis, Oregon, were determined by sequencing two regions of vertebrate mtDNA, *cytochrome oxidase subunit I* and *cytochrome b*, from the bloodmeals of engorged mosquitoes. Nine species of blood engorged mosquitoes were collected and 28 vertebrate hosts were identified from the bloodmeals. Five mosquito species fed predominately on mammals: *Aedes increpitus* (100% of identified bloodmeals), *Aedes sierrensis* (100%), *Anopheles freeborni* (92%), *Anopheles punctipennis* (98.5%), and *Culiseta incidens* (99%). *Culex pipiens* (96%) and *Culex stigmatosoma* (100%) were primarily ornithophilic. *Culex tarsalis* readily fed on both avian and mammalian hosts at a ratio of three to one. Collected *Culex territans*, fed solely on Pacific treefrogs. Human bloodmeals were identified in six of the tested mosquito species: *Ae. increpitus*, *Ae. sierrensis*, *An. punctipennis*, *An. freeborni*, *Cx. tarsalis*, and *Cs. incidens*. The role these mosquitoes could play in transmission of West Nile virus is discussed.

Key Words: bridge vector, enzootic vector, *cytochrome b*, *cytochrome oxidase subunit I*, West Nile virus

INTRODUCTION

Identification of host feeding habits in hematophagous insects is essential to understanding the roles played by both vectors and hosts in vector-borne disease transmission cycles. Feeding habit, also called host feeding pattern, describes the taxonomic breadth of feeding on potential hosts as determined by the relative frequency of bloodmeals taken from different organisms for a mosquito population at a defined place and time (Boreham and Garrett-Jones 1973). While basic feeding patterns (e.g. ornithophily) are generally stable within most mosquito species, the feeding habits of mosquitoes on a particular host species are known to vary geographically (Patrican 2007); therefore, the ability of mosquitoes to vector disease may also vary geographically depending on whether they are readily feeding on competent reservoirs.

The transmission cycle for most arboviruses includes an enzootic cycle and an epidemic cycle. Enzootic vectors are responsible for maintenance of the virus in non-human reservoirs of the disease, and bridge vectors transmit the virus from the non-human to human hosts. Enzootic vectors typically have a narrow range of acceptable hosts; for many arboviruses the enzootic hosts are birds. Once large numbers of enzootic reservoirs become infective, a bridge vector may pick up the virus from an infected animal and transmit it to a human the next time it feeds. To better understand the entire transmission cycle and determine appropriate intervention strategies, it is important to know which mosquitoes are involved in both the enzootic and epidemic cycles.

Culex pipiens L. feeds primarily on birds, and is considered the primary enzootic vector of St. Louis encephalitis virus, Western equine encephalitis virus, and West Nile virus (WNV) throughout the U.S. *Culex tarsalis* Coquillette, which readily feeds on both birds and mammals, is thought to be the primary bridge vector in the Western United States. However, these two species may not be solely responsible for maintenance of these diseases in nature. Sixty-two mosquito species have been found naturally infected with WNV (CDC 2007). The potential role a mosquito species or population plays in transmission of pathogens is dependant on that species' vectorial capacity, which incorporates vector abundance and feeding habit, and vector competence or the mosquito's intrinsic ability to transmit a pathogen.

There is little information available on the feeding patterns of Oregon mosquitoes. Only two studies have mentioned the feeding habits of mosquitoes in Oregon. Rush and Tempelis (1967) found that 96% of *Cx. tarsalis* in their study had fed on birds and the remainder on mammals. In Corvallis, OR, the single *Anopheles freeborni* Aitken collected, had fed on sheep and of the 13 *Anopheles punctipennis* (Say) collected eight had fed on cattle and five on sheep (Reeves 1944). This lack of information should be addressed to better understand the transmission cycle and inform community management of diseases, like WNV, in the state. The objectives of this study are to identify the feeding patterns of mosquitoes collected in Corvallis, Oregon, and to assess their potential roles in disease transmission. Mosquito bloodmeals were identified by direct sequencing of mitochondrial gene regions *cytochrome oxidase subunit I* (COI) and *cytochrome b* (Cyt *b*). Host meals were

putatively identified by comparing sequenced gene regions against known published sequences in GenBank.

MATERIALS AND METHODS

Study Site

Mosquitoes were collected, over a two year period (2006-2007), in and around the City of Corvallis, Benton County, Oregon, approximately 90 miles south of Portland. Encompassing 19 square miles, Corvallis is home to 53,000 people. Collection sites were located at two urban greenspaces (Butterfield Wetland and Timberhill), Willamette City Park (Figure 4.1) and a neighboring wildlife area (E.E. Wilson). At each collection site, one or two red boxes (168 X 80 X 80 cm in dimension) were placed in well shaded and vegetated areas, hidden from view by the public. Located in southern Corvallis, Butterfield Wetland is a nine acre greenspace surrounded by single family homes and apartments. A large portion of the wetland retains standing water during the winter and spring months and completely dries out during the summer. The Timberhill greenspace is 22 acres in size and located in the middle of the Timberhill neighborhood on the north side of the city. Several small urban runoff fed creeks run through the greenspace. The E. E. Wilson Wildlife Area is located 18.5 km north of Corvallis; the red box was located in a heavily shaded and dry irrigation canal near the main office. In the first year of collecting, 2006, a red box was also set at Finley National Wildlife Refuge, located 18.5 km south of the city. However, over the course of the season very few mosquitoes were collected there and this site was not used the following year. Instead, in 2007 this red box was moved to

Willamette Park, a 287 acre park, in southern Corvallis, bordered by the Willamette River floodplain to the north and east and single family homes to the west and south. In 2007, a second red box was also erected at the Timberhill greenspace, southwest of the original location.

Mosquito Collection

May through October of each year, the red boxes were checked every other day for mosquitoes. Resting mosquitoes were collected from all surfaces of the boxes using battery powered handheld (Summit Toys, Trussville, AL) and backpack aspirators (John W. Hock, Gainesville, FL). A few blood engorged mosquitoes were collected with dry ice baited EVS traps and UV light traps set near the red box sites. Live mosquitoes were brought back to the lab, frozen, and identified to species. Individual mosquitoes with visible blood were placed in microvials containing 95% ethanol and stored at 4° C.

Bloodmeal analysis

Vertebrate DNA was extracted and amplified from mosquito abdomens following a modified version of Townzen et al. (2008). Individual abdomens of collected mosquitoes were removed, macerated in 1.5 ml microcentrifuge tubes, and whole DNA was extracted using a DNeasy Tissue Kit (Qiagen, Valencia, California USA), following the procedure for purification of total DNA from animal tissues and eluted in 150 µl of Buffer AE (Qiagen, Valencia, California USA). All dissection instruments used during the DNA extraction process were sterilized in DNA Away (Molecular BioProducts Inc., San Diego, California USA) between each dissection. The dissections were performed on a clean hard surface that was wiped down with

DNA Away between specimens. The head, thorax, wings and legs of the mosquitoes were retained as voucher specimens for further molecular studies and placed back into microvials containing 95% ethanol and stored at 4° C.

Three pairs of primers (Table 4.1) were used to amplify the two mitochondrial gene segments in 50 µl reactions with 3 µl of DNA, 5 µl of 10x KCl or $(NH_2)_4SO_4$ Buffer, 5-7 µl of 25 mM $MgCl_2$, 1 µl of 10µM primers, 1µl of 10µM dNTPs, and 0.3 µl Taq polymerase (Fermentas, Burlington, Ontario Canada). The thermocycling protocol used for both genes was as follows: 95º C for one minute, followed by 35 cycles of 95º C for 30 seconds, 48-52º C for 50 seconds, 72º C for one minute, and finishing with a five minute 72º C extension cycle. Negative controls were run with each extraction and PCR reaction.

If neither the COI-short nor COI-long primer pair amplified enough DNA for sequencing a semi-nested reaction was performed; PCR following the above thermocycling protocol using the COI-short forward and COI-long reverse primers and substituting 1.5 µl of the PCR product from an initial PCR reaction using COI-long primers in place of the whole genomic DNA. PCR products were purified using QIAquick PCR Purification Kits and protocols (Qiagen, California, USA) using a microcentrifuge and eluted in 30 µl of elution buffer. Sequencing of PCR products was outsourced to a commercial firm (Macrogen, Seoul, South Korea). Initially both directions of the gene regions were sequenced. Subsequently, as no additional information was gained by sequencing both forward and reverse, only the forward direction of the COI segment and the reverse of the Cyt *b* segment were sequenced.

Bloodmeal identification

ABI sequencing files were checked and edited manually in BioEdit (Hall 2005). To infer sequence identity, each sequence was compared to the database of homologous sequences contained in GenBank using the nucleotide-nucleotide basic alignment search tool (BLAST) (Altschul et al. 1997, http://www.ncbi.nlm.nih.gov/). Unknown bloodmeal sequences are considered putatively identified if they are greater than 95% identical (percent nucleotide identities) to a sequence in GenBank for either gene region (Townzen et al. 2008). Putatively identified sequences will be deposited in GenBank.

RESULTS

A total of 425 blood-engorged mosquitoes were collected over the course of the 2006 and 2007 mosquito seasons. Nine species from four genera were collected from all the collection sites (Table 4.2): *Aedes increpitus* Dyar (n=5), *Aedes sierrensis* (Ludlow) (34), *Anopheles freeborni* (13), *Anopheles punctipennis* (150), *Culiseta incidens* (Thomson) (75), *Culex pipiens* (70), *Culex stigmatosoma* Dyar (18), and *Culex tarsalis* (41) and *Culex territans* Walker (19). A total of 29 vertebrate species (see Table 4.3 for scientific names of vertebrate hosts) were identified from the bloodmeals of the collected mosquitoes.

A variety of species were collected from each of the collection sites but not all species were collected at each site (Figure 4.2). The majority, 74%, of the blood engorged *An. punctipennis*, 91% of the *Cx. pipiens*, 100% of *Cx. stigmatosoma*, and 65% of *Cx. tarsalis* were collected at Butterfield Wetland. The Timberhill site

produced 94% of the *Ae*. *sierrensis* and 96% of the *Cs*. *incidens*. Eighty-four percent of the *Cx*. *territans* were collected at E. E. Wilson. A single *Cx*. *pipiens* was collected with a blacklight trap from a stormwater catch basin located in southern Corvallis and a single blood-engorged *Ae*. *sierrensis* was collected with a CO_2 trap at a residence in southern Corvallis. A single *Cx*. *pipiens* was collected in a southern Corvallis catch basin with a black light trap, and a singe *Ae*. *sierrensis* was collected with a CO_2 trap at a residence in southern Corvallis.

Five mosquito species fed primarily on mammals (Table 4.4): *Ae*. *increpitus*, *Ae*. *sierrensis*, *An*. *freeborni*, *An*. *punctipennis*, and *Cs*. *incidens*. Vertebrate DNA was amplified from 261 (94%) of the 277 mammal feeding mosquitoes collected. All but one was identified to host species. Nine mammalian hosts were identified. A single mosquito contained mammalian DNA that could not be matched to a known sequence in GenBank with COI, the only segment that was amplified for this sample. The closest match was the brown hare (*Lepus europaeus*) at 83% similarity. Mule deer accounted for 73% of the mammalian blood taken for all mosquito species. Seven individual mosquitoes, from six species, were determined to have fed on humans. Human DNA was identified from the bloodmeals of six species: *Ae*. *increpitus*, *Ae*. *sierrensis*, *An*. *freeborni*, *An*. *punctipennis*, *Cx*. *tarsalis*, and *Cs*. *incidens*. Laboratory contamination is unlikely because all extractions and amplifications were conducted in batches, and none of the samples with human were extracted or amplified at the same time. There were three different sequences for Cyt *b* and two for COI, for a total of four human haplotypes identified from mosquito bloodmeals.

Culex pipiens, *Cx. stigmatosoma*, and *Cx. tarsalis* fed primarily on birds (Table 4.5). Eighteen species of birds were identified from the bloodmeals of these mosquitoes. American crow was the dominant avian species identified in bloodmeals from *Cx. pipiens*, *Cx. stigmatosoma*, and *Cx. tarsalis*, accounting for 41%, 72%, and 24% of their identifiable bloodmeals respectively. The next most frequently identified bloodmeals for *Cx. pipiens* and *Cx. stigmatasoma* was California scrub-jay, 10% and 22% respectively. The four of these bloodmeals from the *Cx. pipiens* were identified as deer; two were collected in August of 2006, and two in August of 2007. For *Cx. tarsalis*, two species, deer and American robin were the most frequently identified bloodmeals, with each host species identified in 13% of the identified bloodmeals. Three species of mammals were identified from the bloodmeals of *Cx. tarsalis*: deer (n=9), racoon (2), and human (2). *Culex territans* fed solely on Pacific treefrog. In addition to avian derived bloodmeals, mammalian DNA was identified in 6%of the *Cx. pipiens* and 20% of *Cx. tarsalis*.

DISCUSSION

Feeding Patterns of Mosquitoes

Of the nine basic mosquito feeding patterns described by Tempelis (1970), four can be attributed to the mosquitoes collected in Corvallis: 1) mosquitoes that feed almost exclusively on mammals, 2) mosquitoes that feed almost exclusively on birds, 3) mosquitoes that feed readily on both mammals and birds and 4) mosquitoes that feed almost exclusively on amphibians. Each of these basic feeding patterns has possible significance in transmission of disease. Mosquitoes that feed primarily on

birds may be involved in enzootic transmission of arboviruses, while mosquitoes that feed on both birds and mammals may be the vector that serves a bridge from birds to mammals. Mosquitoes feeding primarily on mammals may also play a limited role as a bridge vector if they even infrequently feed on competent avian reservoirs. Finally, mosquitoes that feed on amphibians, particularly frogs, may vector filarial worms (Crans 1969) and trypanosomes (Desser 1973) to their hosts.

Several methods have been developed over the years to identify the vertebrate hosts of mosquitoes by analyzing their bloodmeals. Identification of mosquito feeding habit can be as simple as direct observations of mosquitoes feeding in the wild or at baited traps. However, these types of observations yield little information relative to the amount of effort, and provide little information about the relative frequencies with which different host species are fed upon in nature. An efficient method of bloodmeal identification requires sensitivity and specificity (Weitz 1956), both of which are lacking in casual observations. Host specificity becomes increasingly important when only a narrow range of hosts are involved in maintenance of a disease.

One of the earliest methods used to identify hosts from mosquito bloodmeals was the direct examination of erythrocytes under the microscope, allowing differentiation between avian, mammalian and reptilian bloodmeals (Weitz 1956). Over time the precipitin, passive hemagglutination inhibition and ELISA were developed and applied to bloodmeal identification (Washino and Tempelis 1983, Chow 1993). While sensitivity increased with each of these tests, specificity was still lacking. Many of the early feeding pattern studies did not resolve mosquito hosts past class or in some cases order. Modern molecular techniques, including sequencing of

mtDNA segments, can be used to identify mosquito bloodmeals to host species. However, these techniques have only recently been used in for identifying mosquito feeding habits and there are many species and populations of mosquitoes for which only the basic feeding patterns are known. Recent studies using molecular methods have been based in the Northeastern U.S. and Tennessee. All of the available information in the Western U.S. is based on older and less specific methodologies (Reeves 1944, Reeves et al. 1994, Tempelis 1970, Tempelis and Washino 1967, Washino and Tempelis 1983).

Culex territans is a small brown mosquito that can be confused with *Cx. pipiens*. Both larvae and adults of the two species are commonly found in association with each other in Corvallis. The adults of the two species can be distinguished from each other by the banding pattern on the dorsal surface of the abdomen; in *Cx. pipiens* the light bands are located basally and in *Cx. territans* the bands are apical. The basic feeding pattern of *Cx. territans* falls into category four, mosquitoes that feed almost exclusively on amphibians. Crans (1970) tested 315 *Cx. territans* and was able to identify 306 of the bloodmeals to order; amphibians were identified as the host in 88.5% of the samples. While species specific host identifications based on bloodmeals were not made in this study, *Cx. territans* was observed feeding on spring peeper, *Hyla crucifer*, southern leopard frog, *Rana sphenocephala*, carpenter frog, *Rana virgatipes*, bullfrog, *Rana catesbeiana*, and green frog, *Rana clamitans* (Crans 1970). All of the *Cx. territans* collected in Corvallis had fed on Pacific treefrog, which were also observed resting in the red boxes at Butterfield Wetland and the E. E. Wilson Wildlife Area.

Two species, *Cx. pipiens* and *Cx. stigmatosoma*, displayed the basic pattern of feeding almost exclusively on birds in Corvallis. Not much information is available regarding the feeding habit of *Cx. stigmatosoma* elsewhere, other than a basic pattern of feeding on birds. In the Sacramento Valley 96% of the *Cx. stigmatosoma* collected had fed on avians, 84% of those bloodmeals were from passeriforms (Tempelis and Washino 1967). This pattern held for the *Cx. stigmatosoma* collected in Corvallis, as well, with this mosquito feeding solely on passeriform birds, 72% of which were American crow.

In recent studies, the proportion of *Cx. pipiens* feeding on birds has varied from 34% in New Jersey (Apperson et al. 2004), 71% to 81% in Tennessee (Apperson et al. 2004, Savage et. al. 2007), 85% to 95% in New York (Apperson et al. 2002, Apperson et al. 2004) and 95% in Massachusetts (Molaei et al. 2006). Several studies found that *Cx. pipiens* predominantly fed on American robin (Savage et al. 2007, Molaei et al. 2006). Kilpatrick et al. (2006) claimed this mosquito preferred to feed on American robins over other hosts. However, other birds have also been identified as the predominant avian bloodmeal for *Cx. pipiens*, including tufted titmouse (*Baeolophus bicolor*) northern mockingbird (*Mimus polyglottos*) (Apperson et al. 2004) and northern cardinal (*Cardinalis cardinalis*) (Patrican 2007).

In Corvallis, the host feeding pattern of *Cx. pipiens* changed from one year to the next, possibly based on host availability. In 2006, *Cx. pipiens* most frequently fed on green heron and cedar waxwing; each represented 20% of the identified bloodmeals in 2006. In 2007, they primarily fed upon American crow, comprising 62% of identified bloodmeals. During the same time period the numbers of American

crows counted per hour during the Audubon Society's annual Christmas Bird Count continually increased from December 2005 to December 2007 with a four-fold increase between 2006 and 2007 (Audubon 2008). The numbers of cedar waxwings also increased from 2005 to 2007; however, there was a decrease in the numbers counted per hour during the 2006 count (Audubon 2008).

In Corvallis, *Cx. tarsalis* fed readily on both birds and mammals. 24.4% of the *Cx. tarsalis* had fed on mammals and 75.6% on avian hosts. Similar results were obtained in Kern and Sutter Counties, California, with 17% and 27% of *Cx. tarsalis*, respectively, feeding on mammals (Reeves et al. 1963, Wekesa et al. 1997). Out of concern for transmission of disease to humans, as opposed to determining avian reservoirs of disease, specific identification of hosts has focused on identification of mammalian hosts over identification of avian hosts. Specific mammalian hosts identified from *Cx. tarsalis* have included sheep, deer, cattle, black-tailed jackrabbit (*Lepus californicus*), and Audubon's cottontail (*Sylvilagus audubonii*) (Washino and Tempelis 1983, Wekesa et al. 1997). This result differed from the mammalian hosts identified from the bloodmeals of *Cx. tarsalis* in Corvallis, which had fed on deer, raccoon, and human. Only Wekesa (1997) attempted to classify *Cx. tarsalis* avian hosts past Class. Similar to the Wekesa (1997) findings, *Cx. tarsalis*, in Corvallis, fed predominantly on Passeriformes.

The remaining five species of mosquitoes collected had a basic feeding pattern that was almost exclusively mammalian. Two species of mammal feeding *Aedes* were collected during the course of this study: *Ae. increpitus* and *Ae. sierrensis*. Few studies have reported on the feeding habit of *Ae. sierrensis* and no reference for the feeding

habit of *Ae*. *increpitus* could be found, as neither species is considered to be an important vector of disease to humans. In California, *Ae*. *sierrensis* was found to feed on large mammals including cattle and horses (Tempelis and Washino 1967, Nielsen et al. 2008). In the present study, both species fed solely on mammals, though not only restricted to large mammals (Table 4.4). A pattern was identifiable for the *Ae*. *sierrensis*, with 85% of the 34 identifiable bloodmeals identified as deer. Five *Ae*. *increpitus* were collected in Corvallis and four hosts were identified from their bloodmeals: deer, eastern cottontail, domestic cat, and human. Due to the insufficient number of *Ae*. *increpitus* collected, no pattern could be identified for this species. The one difference in hosts between the two *Aedes* spp. was that domestic dog but not domestic cat was identified from an *Ae*. *sierrensis* bloodmeal.

Anopheles freeborni and *An*. *punctipennis* fed almost exclusively on mammals. In California, both species have a habit of feeding on mammals including cattle, horses and, in the case of *An*. *freeborni*, rabbits (Reeves and Hammon 1944, Reeves 1944, Washino and Tempelis 1967, Wekesa et al. 1997). Reeves and Hammon (1944) found only 3% of the *An*. *freeborni* and 3.8% of the *An*. *punctipennis* from California, Oregon and Washington had fed on humans. More recently, in Tennessee and New York, the majority of *An*. *punctipennis* collected had fed on deer (Apperson et al. 2004, Savage et al. 2007). Deer was also the most frequently identified bloodmeal for both *An*. *freeborni* (54% of identifiable bloodmeals) and *An*. *punctipennis* (68%). While other studies did not mention location-dependent feeding patterns, there was a noticeable difference in hosts among collecting sites in Corvallis. Even though *An*. *punctipennis* predominantly fed on deer overall in Corvallis, at Willamette Park,

which was approximately 120m from a small residential sheep pen, 60% of the *An. punctipennis* bloodmeals were identified as sheep and none of the bloodmeals were identified as deer.

Collected *Cs. incidens* displayed a primarily mammalian feeding pattern with 98% of those collected having fed on mammals. Of those, 97 % had fed on deer. The *Cs. incidens* from Corvallis had also fed on sheep, human, and crow. Other studies have found that *Cs. incidens* primarily feeds on mammals including cattle, domestic horse and domestic dog (Reeves and Hammon 1944, Tempelis and Washino 1967, Reisen et al. 1990).

Disease Transmission

The mosquito species collected in this study have the potential to transmit diseases to humans and non-humans. *Anopheles punctipennis* and *An. freeborni* are both competent vectors of human malaria (Bohart and Washino 1978), which at one time was endemic to much of Oregon (Boyd 1975 and Gjullin and Eddy 1972). *Culiseta incidens* and *Ae. sierrensis* are implicated in transmission of canine heartworm, *Dirofilaria immitis* (Leidy), to dogs and other mammals (Walters and Lavoipierre 1982, Scoles et al. 1993 and Theis et al. 2000). *Culex territans* may vector both filarial worms (Crans 1969) and trypanosomes (Desser 1973) to frogs. Mosquitoes, including *Cx. pipiens* and *Cx. tarsalis,* also transmit a variety of viral encephalitides, including West Nile virus (WNV) to birds and humans.

West Nile virus is currently the most important mosquito-borne encephalitis in the United States. The first human cases of the disease in the United States occurred in 1999 in New York. Since that time 27,598 human cases, 1086 of which were fatal,

have been reported to the Centers for Disease Control and Prevention (CDC 2008). The mosquito-borne disease quickly spread outside New York and was detected in Oregon in 2004. In the three years that WNV has been reported in Oregon, 113 human and 97 equine cases have been reported (DeBess 2008). The extent to which a species of mosquito is involved in transmission of WNV depends, in part, on whether or not the mosquito feeds on the hosts involved in maintaining the pathogen and the competence of the mosquito at transmitting the pathogen.

West Nile virus is a zoonotic disease of birds; humans and other mammals are considered incidental hosts. Across the United States, WNV has been detected in 317 species of birds (CDC 2008). However, it is unlikely that all of these birds are involved in maintaining the natural avian cycle of the disease. Reservoir competence studies have indicated infected passerine birds produce the highest viremic titers (Komar et al. 2003 and Reisen et al. 2005) and are likely candidates for maintenance of the virus. Within the passerines, American crow, California scrubjay, House Finch and House sparrow, are all competent reservoirs of WNV and produce high enough titer levels for feeding mosquitoes to become infected (Komar et al. 2003 and Reisen et al. 2005). American crow was the most frequently identified avian bloodmeal, comprising 45% of all avian-derived bloodmeals from the Corvallis mosquitoes. Six mosquito species fed on American crow: *Cx. pipiens*, *Cx. stigmatosoma*, *Cx. tarsalis*, *An. freeborni*, *An. punctipennis*, and *Cs. incidens*.

Another factor in transmission of disease by mosquitoes is vector competence. In California, *Cx. pipiens*, *Cx. stigmatosoma* and *Cx. tarsalis* are competent vectors of WNV (Goddard et al. 2002). Although these three species are able to transmit the

virus under laboratory conditions, their ability to transmit the virus varies greatly. After 14 days, 71% of wild caught *Cx. pipiens* and 19% of *Cx. stigmatosoma* orally infected with WNV transmitted the virus. The transmission rate for *Cx. tarsalis* ranged from 60 to 100% after 14 days, depending on the population it was from (Goddard et al. 2002). Due to their general feeding patterns *Cx. stigmatosoma* and *Cx. pipiens* are considered moderate and important zoonotic vectors, respectively, while *Cx. tarsalis* is considered an important bridge vector (Turell et al. 2005).

Three other mammalian-feeding mosquito species collected in Corvallis, *An. punctipennis*, *An. freeborni*, and *Cs. incidens*, could play a role in WNV transmission cycle. In addition to feeding on mammals, including humans, these species also fed on American crow, indicating that they could become infected with WNV if the virus was present in local crow populations. Both *An. punctipennis* and *An. freeborni* have been found naturally infected with WNV (CDC 2007 and Kulaskera et al. 2001). Unfortunately, neither species has been studied for WNV vector competence. *Culiseta incidens* have also been found naturally infected with WNV (CDC 2007). Under laboratory conditions, *Cs. incidens* had a low rate (ranging from 13 to 33%) of infection and a high rate of transmission (43 to 80%) (Reisen et al. 2006).

Since the disease was first detected in Oregon four years ago, there has been very little WNV activity in Benton County. Only three infected birds, collected as a part of the dead bird surveillance program, and a single non-locally acquired human WNV case have been reported (ODHS 2008). Even though there has been little recorded activity to date, if a large enough population of infected birds were to migrate into the area, all of the pieces are in place for WNV transmission to occur in Corvallis.

The mosquitoes commonly collected in Corvallis are competent vectors of WNV. They also commonly feed on competent avian reservoirs of the disease, and several species feed on both the avian reservoirs and human hosts and thus could act as bridge vector.

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LITERATURE CITED

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389--3402.

Apperson CS, Harrison BA, Unnasch TR, Hassan HK, Irby WS, Savage HM, Aspen SE, Watson DW, Rueda LM, Engber BR, Nasci RS. 2002. Host-feeding of *Culex* and other mosquitoes (Diptera: Culicidae) in the borough of Queens in New York City, with characters and techniques for identification of *Culex* mosquitoes. *J Med Entomol* 39: 777-785.

Apperson CS, Hassan HK, Harrison BA, Savage HM, Aspen SE, Farajollahi A, Crans WJ, Daniels TJ, Falco RC, Benedict M, Anderson M, McMillen L, Unnasch TR. 2004. Host Feeding Patterns of Established and Potential Mosquito Vectors of West Nile Virus in the Eastern United States. *Vector-Borne and Zoonotic Dis* 4: 71-82.

Audubon. 2008. Annual Christmas bird count: current year results. [Internet]Available from the National Audubon Society, New York. [Accessed May 12, 2008] http://cbc.audubon.org/cbccurrent/current_table.html.

Bohart RM, Washino RK. 1978. Mosquitoes of California. Division of Agricultural Science, University of California, Berkeley, California.

Boreham PFL, Garrett-Jones C. 1973. Prevalence of mixed blob meals and double feeding in a malaria vector. *Bull Wld Hlth Org* 48: 605-614.

Boyd RT. 1975. Another look at the “fever and ague” of Western Oregon. *Ethnohistory* 22: 135-154.

CDC 2007. West Nile virus: vertebrate ecology. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Ft. Collins, Colorado. [Accessed May 12, 2008] http://www.cdc.gov/ncidod/ dvbid /westnile/birdspecies.htm.

CDC 2007. West Nile virus: entomology. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Ft. Collins, Colorado. [Accessed May 12, 2008] http://www.cdc.gov/ncidod/ dvbid/ westnile/mosquitoSpecies.htm.

CDC. 2008. West Nile virus: statistics, surveillance, and control. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Ft. Collins, Colorado. [Accessed May 12, 2008] http:// www.cdc.gov/ncidod/dvbid/westnile/Mapsactivity/surv&control08Maps.htm.

Crans WJ. 1969. Preliminary observations of frog filariasis in New Jersey. *Proc Ann Conf Bull Wildlife Disease Assoc* 5: 342-347.

Crans WJ. 1970. The blood feeding habits of *Culex territans* Walker. *Mosq News* 30: 445-448.

DeBess E. 2008. 2007 West Nile virus summary report for Oregon. [Internet] Available from Oregon Department of Human Services, Acute and Communicable Disease Prevention, Portland, Oregon. [Accessed May 22, 2008] http://www.oregon.gov/DHS/ph/acd/diseases/wnile/wnv2007rpt.pdf.

Desser SS, McIver SB, Ryckman A. 1973. *Culex territans* as a potential vector of *Trypanosoma rotatorium*. I. development of the flagellate in the mosquito. *J Parasitol* 59: 353-358.

Finnila, S., Lehtonen, M.S., & Majama, K. (2001) Phylogenetic network for European mtDNA. *Am J Human Gen* 68: 1475-1484.

Gjullin CM, Eddy GW. 1972. *The Mosquitoes of the Northwestern United States*. Technical Bulletin No. 1447. United States Department of Agriculture, Washington D.C.

Goddard LB, Roth AE, Reisen WK, Scott TW. 2002. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis* 8: 1385-1391.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 4: 95-98.

Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD. 2006. Host heterogeneity dominates West Nile virus transmission. *Proc R Soc B* 273: 2327-2333.

Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Bunning M. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis* 9: 311-322.

Kulasekera VL, Kramer L, Nasci RS, Mostashari F, Cherry B, Trock SC, Glaser C, Miller JR. 2001. West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000. *Emerg Infect Dis* 7: 722-725.

Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrink CR. 2006. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, Northeastern United States. *Emerg Infect Dis* 12: 468-474.

Nielsen CF, Reisen WK, Armijos MV, MacLachlan NJ, Scott TW. 2008. High subclinical West Nile virus incidence among nonvaccinated horses in Northern

California associates with low vector abundance and infection. *Am J Trop Med Hyg* 78: 45-52.

ODHS. 2008. West Nile virus. [Internet] Available from the Oregon Department of Human Services, Acute and Communicable Disease Prevention, Portland, Oregon. [Accessed May 22, 2008]http://www.oregon.gov/DHS/ph/acd/ diseases/ wnile/wnile.shtml.

Patrican LA, Hackett LE, Briggs JE, McGowan JW, Unnasch TR, Lee JH. 2007. Host-feeding patterns of *Culex* mosquitoes in relation to trap habitat. *Emerg Infect Dis* 13: 1921-1923.

Reeves WC. 1944. Preliminary studies on the feeding habits of Pacific Coast Anopheles. *Natl Malaria Soc J* 3: 325-331.

Reeves WC, Hammon WM. 1944. Feeding habits of the proven and possible mosquito vectors of Western Equine and St. Louis encephalitis in the Yakima Valley, Washington. *Am J Trop Med* 24: 131-134.

Reeves WC, Tempelis CH, Bellamy RE, Lofy MF. 1963. Observations on the feeding habits of *Culex tarsalis* in Kern County, California, using precipitating antisera produced in birds. *Am J Trop Med Hyg* 12: 929-935.

Reisen WK, Meyer RP, Tempelis CH, Spoehel JJ. 1990. Mosquito abundance and bionomics in residential communities in Orange and Los Angeles Counties, California. *J Med Entomol* 27: 358-367.

Reisen WK, Fang Y, Martinez VM. 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. *J Med Entomol* 42: 367-375.

Reisen WK, Fang Y, Martinez VM. 2006. Vector competence of *Culiseta incidens* and *Culex thriambus* for West Nile virus. *J Am Mosq Cont Assoc* 22: 662-665.

Rush WA, Tempelis CH. 1967. Biology of *Culex tarsalis* during the spring season in Oregon in relation to western encephalitis virus. *Mosq News* 27: 307-315.

Savage HM, Aggarwal D, Apperson CS, Katholi CR, Gordon E, Hassan HK, Anderson M, Charnetzky D, McMillen L, Unnasch EA, Unnasch TR. 2007. Host Choice and Wet Nile virus infection rates in blood-fed mosquitoes, including members of the *Culex pipiens* complex, from Memphis, Tennessee, 2002-2003. *Vector Borne Zoonotic Dis* 7: 365-396.

Scoles GA, Dickson SL, Blackmore MS. 1993. Assessment of *Aedes sierrensis* and a vector of canine heartworm in Utah using a new technique for determining the

infectivity rate. *J Am Mosq Cont Assoc* 9: 88-90.

Tempelis CH. 1970. Host Preferences of Mosquitoes. *Proc Calif Mosq Cont Assoc 1970* 38: 25-8.

Tempelis CH, Washino RK. 1967. Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley, California, with notes on other species. *J Med Entomol* 3: 315-318.

Theis JH, Kovaltchouk JG, Fujioka KK, Saviskas B. 2000. Vector competence of two species of mosquitoes (Diptera: Culicidae) from Southern California *Dirofilaria immitis* (Filariidae: Onchoceridae). *J Med Entomol* 37: 295-297.

Townzen JS, Brower AVZ, Judd DD. In Press. Utility of identifying the sources of mosquito bloodmeals using mitochondrial *cytochrome oxidase subunit I* and *cytochrome b* sequences. *Med Vet Ent*.

Turell MJ, Dohm DJ, Sardelis MR, O'Guinn ML, Andreadis TG, Blow JA. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *J Med Entomol* 42: 57-62.

Walters LL, Lavoipierre MMJ. 1984. Landscape epidemiology of mosquito-borne canine heartworm (*Dirofilaria immitis*) in northern California, US: 1. Community-based surveys of domestic dogs in three landscapes. *J Med Entomol* 21: 1-16.

Washino RK, Tempelis CH. 1983. Mosquito host bloodmeal identification: methodology and data analysis. *Ann Rev Entomol* 28: 179-201.

Weitz B. 1956. Identification of bloodmeals of blood-sucking arthropods. *Bull Wld Hlth Org* 15: 473-490.

Wekesa JW, Yuval B, Washino RK, de Vasquez AM. Blood feeding patterns of *Anopheles freeborni* and *Culex tarsalis* (Diptera: Culicidae): effects of habitat and host abundance. *Bull Entomol Res* 87: 633-641.

Figure 4.1. Locations of the four red box sites in the City of Corvallis, Oregon, where blood fed mosquitoes were collected.

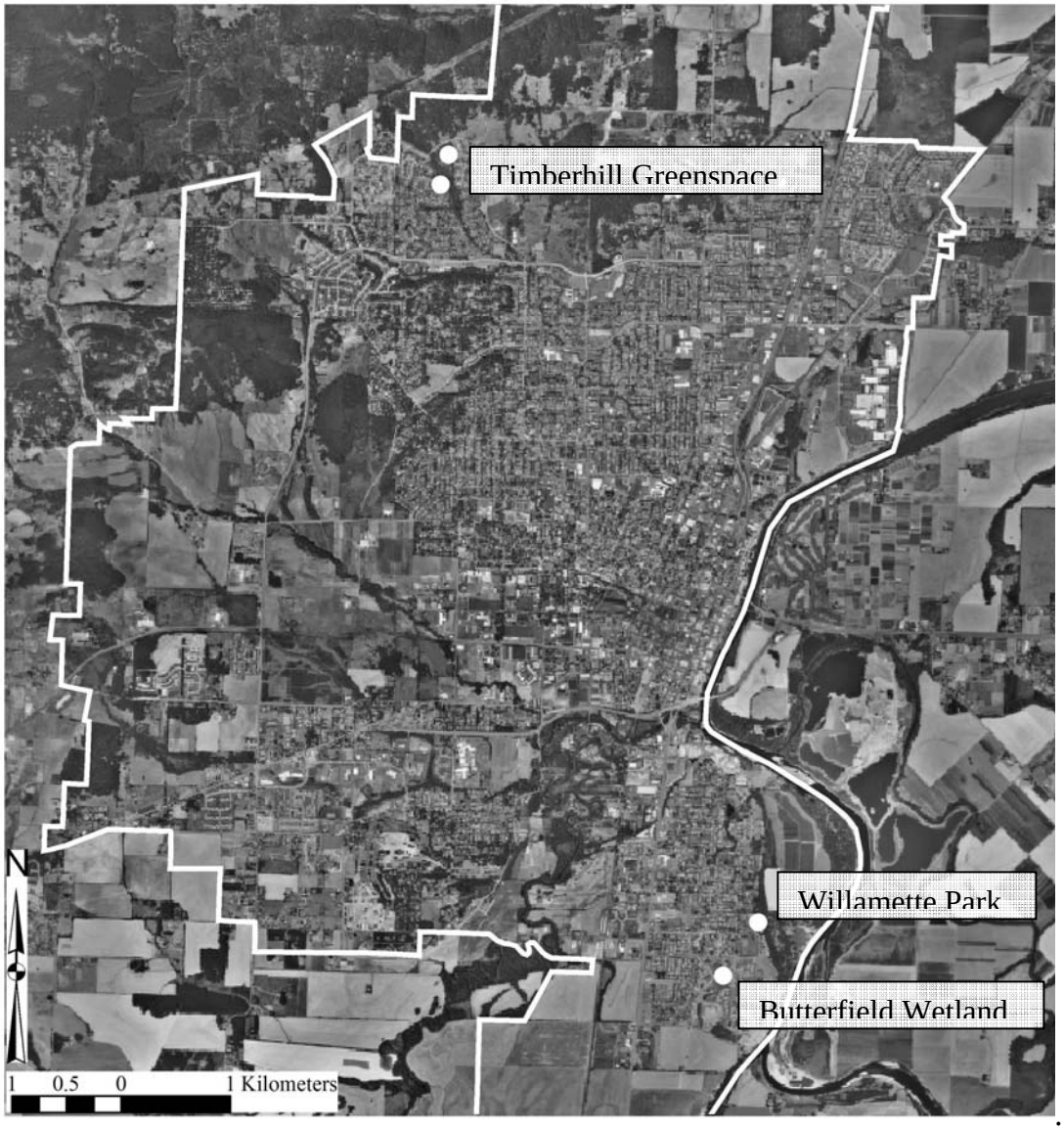

Table 4.1. Primers used to amplify vertebrate DNA from mosquito bloodmeals.

Primer Name	**Location***	**Primer Sequence**
COI_long (f)	5934	AACCACAAAGACATTGGCAC
COI_long (r)	6597	AAGAATCAGAATARGTGTTG
COI_short (f)	6267	GCAGGAACAGGWTGAACCG
COI_short (r)	6591	AATCAGAAYAGGTGTTGGTATAG
Cyt b (f)	15150	GAGGMCAAATATCATTCTGAGG
Cyt b (r)	15607	TAGGGCVAGGACTCCTCCTAGT
* Location on human mtDNA (Finnila et al. 2001).		

Table 4.2. Corvallis, Oregon, collecting locations and the numbers of blood-engorged mosquitoes and species collected at each site.

	Aedes increpitus	*Aedes sierrensis*	*Anopheles freeborni*	*Anopheles punctipennis*	*Culex pipiens*	*Culex stigmatosoma*	*Culex tarsalis*	*Culex territans*	*Culiseta incidens*
Timber Hills Neighborhood	2	32		3	2		4		72
Butterfield Wetland	3		8	111	64	18	26	3	
Willamette City Park		1	1	25					3
E.E. Wilson Wildlife Area			2	10	3		5	16	
William L. Finley National Wildlife Refuge			2	1			6		
Other		1			1				
Total	5	34	13	150	70	18	41	19	75

Table 4.3. Scientific names for vertebrates identified from the bloodmeals of mosquitoes collected in Corvallis, Oregon.

	Common Name	**Latin Name**	**Order**	**Family**
Avian	Green heron	*Butorides virescens*	Ciconiiformes	Areidae
	Mourning dove	*Zenaida macroura*	Columbiformes	Columbidae
	European starling	*Sturnus vulgaris*	Passeriformes	Sturnidae
	Western scrub-jay	*Aphelocoma californica*	Passeriformes	Corvidae
	American crow	*Corvus brachyrhynchos*	Passeriformes	Corvidae
	Cedar waxwing	*Bombycilla cedrorum*	Passeriformes	Bombycillidae
	American robin	*Turdus migratorius*	Passeriformes	Turdidae
	House finch	*Carpodacus mexicanus*	Passeriformes	Fringillidae
	House sparrow	*Passer domesticus*	Passeriformes	Passeridae
	Allen's hummingbird	*Selasphorus sasin*	Apodiformes	Trochilidae
	Swainson's thrush	*Catharus ustulatus*	Passeriformes	Turdidae
	Cooper’s hawk	*Accipiter cooperii*	Falconiformes	Accipitridae
	Brewer's blackbird	*Euphagus cyanocephalus*	Passeriformes	Icteridae
	Bewick's wren	*Thryomanes bewickii*	Passeriformes	Troglodytidae
	Blackheaded grosbeak	*Pheucticus melanocephalus*	Passeriformes	Cardinalidae
	Blackcapped chickadee	*Parus atricapillus*	Passeriformes	Paridae
	Song sparrow	*Melospiza melodia*	Passeriformes	Emberizidae
Non-avian	Pacific treefrog	*Pseudacris regilla*	Anura	Hylidae
	Mule deer	*Odocoileus hemionus*	Artiodactyla	Cervidae
	Elk	*Cervus canadensis*	Artiodactyla	Cervidae
	Domestic goat	*Capra hircus*	Artiodactyla	Bovidae
	Domestic sheep	*Ovis aries*	Artiodactyla	Bovidae
	Domestic horse	*Equus caballus*	Perissodactyla	Equidae
	Raccoon	*Procyon lotor*	Carnivora	Procyonidae
	Domestic dog	*Canis lupus*	Carnivora	Canidae
	Domestic cat	*Felis catus*	Carnivora	Felidae
	Human	*Homo sapiens*	Primates	Hominidae
	Eastern cottontail	*Sylvilagus florindus*	Lagomorpha	Leporidae

Table 4.4. Identification of bloodmeals from mosquitoes primarily feeding on mammals.

Mosquito Species	No. amplified / No. collected (%)	No. (%) feeding on					
		Deer	Cottontail rabbit	Domestic cat	Human	Domestic dog	Elk
Ae. increpitus	5/5 (100)	2 (40)	1 (20)	1 (20)	1 (20)		
Ae. sierrensis	34/34 (100)	29 (85.3)	3 (8.8)		1 (3)	1 (3)	
An. freeborni	13/13 (100)	7 (53.8)	1 (7.7)		1 (7.7)	2 (15.4)	1 (7.7)
An. punctipennis	135/150 (90)	92 (68.1)	3 (2.2)	5 (3.7)	1 (0.7)	6 (4.4)	
Cs. incidens	74/75 (99)	71 (96)			1 (1.4)		

Mosquito Species	No. (%) feeding on (Cont'd)						
	Domestic horse	Domestic sheep	Raccoon	Domestic goat	Unident. mammal	Green heron	American crow
Ae. increpitus							
Ae. sierrensis							
An. freeborni							1 (7.7)
An. punctipennis	1 (0.7)	16 (11.9)	6 (4.4)	2 (1.5)	1 (0.7)	1 (0.7)	1 (0.7)
Cs. incidens		1 (1.4)					1 (1.4)

Table 4.5. Identification of bloodmeals from *Culex* spp. collected in Covallis, OR.

Mosquito Species	No. amplified / No. collected (%)	Mammal to Avian Ratio	No. (%) feeding on				
			Deer	Human	Raccoon	Pacific treefrog	Green heron
Cx. territans	19/19 (100)	N/A				19 (100)	
Cx. pipiens	67/70 (96)	4:63	4 (6)				5 (7.5)
Cx. stigmatosoma	18/18 (100)	0:18					
Cx. tarsalis	39/42 (93)	8:31	5 (12.8)	1 (2.6)	2 (5.1)		1 (2.6)

Mosquito Species	No. (%) feeding on (cont'd)						
	European starling	California scrubjay	American crow	Cedar waxwing	American robin	House finch	House sparrow
Cx. territans							
Cx. pipiens	2 (3)	7 (10.4)	26 (38.8)	6 (9)	3 (4.5)	3 (4.5)	2 (3)
Cx. stigmatosoma		4 (22.2)	13 (72.2)		1 (5.6)		
Cx. tarsalis	1 (2.6)	2 (5.1)	9 (23.1)		5 (12.8)	1 (2.6)	2 (5.1)

Table 4.5. (continued)

Mosquito Species	No. (%) feeding on (cont'd)					
	Allen's humming-bird	Swainson's thrush	Cooper's hawk	Brewers blackbird	Bewick's wren	Black-headed grosbeak
Cx. territans						
Cx. pipiens	1 (1.5)	1 (1.5)	2 (3)	1 (1.5)	2 (3)	1 (1.5)
Cx. stigmatosoma						
Cx. tarsalis		1 (2.6)	1 (2.6)			1 (2.6)

Mosquito Species	No. (%) feeding on (cont'd)			
	Mourning dove	Black-capped chickadee	Song sparrow	Domestic chicken
Cx. territans				
Cx. pipiens	1 (1.5)			
Cx. stigmatosoma				
Cx. tarsalis	1 (2.6)	1 (2.6)	1 (2.6)	1 (2.6)

URBAN MOSQUITOES OF CORVALLIS, OREGON: CATCH BASINS AND ADULT MOSQUITO EMERGENCE

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ABSTRACT

Floating emergence traps were designed to collect adult mosquitoes from 40 catch basins randomly selected from two areas in the City of Corvallis, Oregon. Three mosquito species were collected emerging from basins: *Culex pipiens*, *Culex stigmatosoma*, and *Culiseta incidens*. Over a 22 day period in August 2006, a total of 18,461 mosquitoes were collected from 20 catch basins in the southern area of Corvallis. In September 2006, over a 20 day period, a total of 14,978 emerging adult mosquitoes were collected from the 324 catch basins in northern Corvallis. During the two sampling periods, an estimated 138,348 female *Cx. pipiens* emerged from all 324 catch basins in the entire southern area and 84,432 emerged from the 500 catch basins in the entire northern area of Corvallis. The timing of mosquito production in catch basins and the emergence of large numbers of mosquitoes from the catch basins coincides with the height of the West Nile virus transmission season.

Key Words: Emergence traps, adult population estimate, stormwater, West Nile, sampling

INTRODUCTION

Stormwater catch basins are an integral part of stormwater management. Many catch basins are designed with a sump to temporarily hold stormwater and allow particulate pollutants to settle out of the water column. In addition to their role in water quality, catch basins have long been recognized as potential mosquito habitat. Several recent studies have been conducted examining the population dynamics of immature mosquitoes in sumped stormwater catch basins (Kronenwetter-Koepel et al. 2005, Gingrich et al. 2006, Rey et al. 2006, Butler et al. 2007, Kwan et al. 2008, Metzger et al. 2008, Townzen et al. in review) and the efficacy of control measures (Geery and Holub 1989**,** Knepper et al. 1992, McCarry 1996, Pfuntner 1978, and Siegel and Novak 1999). All of these studies relied on sampling larval mosquitoes using methodologies that do not readily equate to total numbers of adults emerging. This is the first published study collecting adult mosquitoes as they emerge from catch basins.

In Corvallis, Oregon, only *Culex pipiens* L., *Culex stigmatosoma* Dyar, and *Culiseta incidens* (Thomson) readily use catch basins for immature habitat (Townzen et al. in review). All three species have been found naturally infected with West Nile virus (WNV) (CDC 2008, DeBess 2008) and are competent vectors of the virus (Goddard et al. 2002, Reisen et al. 2006, Turell et al. 2005). While laboratory studies indicate a similar vector competence for the three species, the feeding habits of the two *Culex* spp. and *Culiseta incidens* differed according to field studies in Corvallis. Both *Cx. pipiens* and *Cx. stigmatosoma* fed primarily on passerine birds and *Cs. incidens* fed on mammals (Chapter 4). These feeding patterns suggest that in

Corvallis, *Cx*. *pipiens* and *Cx*. *stigmatosoma* could be involved in enzootic transmission of WNV and *Cs*. *incidens* in epidemic transmission of the disease. Due to their potential importance in disease transmission further study of these mosquitoes and their aquatic urban habitat, including catch basins, in urban sources is warranted.

At the time of the study there were just over 6500 sumped catch basins in the City of Corvallis and approximately 85% of those held water during the summer months (Townzen unpublished). Two catch basin designs are used in Corvallis (Figure 5.1) and floating emergence traps were designed to fit snuggly into each one and collect mosquitoes as they emerged. Female mosquitoes were counted to determine the total numbers emerging from catch basins in each area and to determine if equal numbers were emerging from the two catch basin designs. From this, the total population of mosquitoes emerging from catch basins in the areas sampled was estimated.

MATERIALS AND METHODS

Corvallis, Oregon, is located about 90 miles south of Portland, in Benton County. The city has a population of nearly 53,000 people, encompasses 19 square miles, and there is no organized mosquito control. There are two different catch basins designs used in Corvallis (Figure 5.1). One design features a small grate built into the horizontal curb of the sidewalk, through which water drains into a concrete lined vault built below the sidewalk. The sump of the catch basin is accessible from above via a metal panel set into the sidewalk. The vault has a water surface area of 4475 cm^2 (Figure 5.1B) The second type is smaller with only 1836 cm^2 of water

surface area and is set directly in the street with water draining into it via a larger, removable, open grate (Figure 5.1A). Depth from the roadway to the surface of the water and water depth is variable and not standardized.

The catch basins used for sampling emerging mosquitoes were the same as those chosen from the southern and northern areas of the city for larval monitoring in a previous study (Townzen et al. 2008) with a few exceptions. Four of the catch basins from the first study were too deep for safe insertion and retrieval of emergence traps and the nearest catch basin of the same type, which could safely be monitored, was used. In brief, the catch basins sampled were randomly chosen, using the RANDBETWEEN function in Excel 2003 (Microsoft 2002), from a list of catch basins in the area. At the time of the study the southern area of Corvallis had 324 sumped catch basins, 163 are the smaller basin and 161 are the larger basin. The northern area of the city had 500 catch basins; 120 were the smaller catch basin type and 380 the larger. The southern area is bordered by the Willamette River to the east and north, and Hwy 99 to the west. The north area is bordered by a forested area to the north and west, NW Circle Blvd to the south and NW 29th street to the west

Floating emergence traps, modified from Aubin et al. (1973), were designed to fit snugly into the catch basins and cover all the surface water (Figure 5.3). Traps were pyramid shaped and 31.5 cm long, 49 cm wide and 27 cm tall. The trap frame was constructed out of 1 x 2 inch cedar and screened with vinyl window screen held in place with heavy duty staples and high temperature hot glue. The wooden frame was coated with marine varnish to protect the wood from water logging traps and to further seal the screen to the wood. The top of the pyramid consisted of a flat wood square,

parallel to the trap base, with a hole in the middle in which the mouth of a 1 pint mason jar could be inserted. When the trap was in place, eclosing mosquitoes would be funneled up the inside of the screened in pyramid and collected into the mason jar at the top of the trap. The mouth of the jar was fitted with an inverted cone of window screen to keep mosquitoes from flying back out of the jar (Figure 5.3.A). Wacky Noodle foam noodles (Lifoam, Hunt Valley, Maryland) were fitted to the base of each traps and used to float the traps on the surface of the water. The placement and size of the foam was altered to create a foam seal between the catch basin walls and trap base, thereby minimizing surface water outside of the trap. A single trap was placed in the smaller catch basins and two were placed in the larger (Figure 5.3).

Traps were placed in the catch basins in the southern end of the city on August 8th, 2006 and remained in place until August 30th. On August 30th the traps were removed from the southern catch basins and any damage, such as loose foam, was repaired. That afternoon the traps were placed in the catch basins in the northern area. The traps remained in the northern catch basins until September 19th. The length of time the traps remained in the catch basins was based on the estimate time, 22 days, it would take for 95% of the *Cx. pipiens* to mature from eggs to adults. In both study areas jars were removed and replaced every other day. Once removed, the lids were attached and the jars were transported back to the laboratory where the mosquitoes were sorted by species, sex, and then counted.

All data were analyzed using S-Plus 6.1 (Insightful 2002) and Excel (Microsoft 2002). The numbers of female mosquitoes using the two catch basin styles were analyzed using two-sample t-tests. Because all of the mosquitoes emerging from each

of the catch basins sampled were collected, estimation of the population from the sampling area is a straightforward calculation of the total number of catch basins in the area multiplied by the average number of mosquitoes collected. Information on the numbers and locations of catch basins was determined from geographical information system (GIS) data available at the City of Corvallis GIS ftp site (ftp://ftp.ci.corvallis.or.us/pw/gis) and was analyzed using ArcMap 8.2 (ESRI 2002).

RESULTS

A total of 33,439 emerging mosquitoes were collected from the 40 catch basins sampled in Corvallis, representing three species: *Culex pipiens*, *Culex stigmatosoma*, and *Culiseta incidens*. *Culex pipiens* was the dominant species collected, representing 91.4% of the mosquitoes from the southern Corvallis catch basins in August and 84.6% of the mosquitoes collected from the northern Corvallis catch basins in September (Table 5.1). *Culiseta incidens* was the second most frequently collected mosquito, representing 6.7% of the August collection and 15% of the September collection.

In August, 18,461 mosquitoes were collected (Figure 5.4) from the catch basins in the southern sampling area. Emerging mosquitoes were collected from all but two of the catch basins. 91% of the mosquitoes collected (n=16,816) were *Cx. pipiens* and 50.9% (n=8557) of those were female. Female *Cs. incidens* (n=570) and *Cx. stigmatosoma* (n=228) were each collected from 8 of the 20 catch basins in the southern sampling area. Too few *Cs. incidens* and *Cx. stigmatosoma* were collected for further evaluation. The average number of female *Cx. pipiens* collected from the

smaller catch basins was 585 (95% CI -53.5 to 769.9), and from the larger catch basins the average number was 497 (95% CI 86.8 to 908.2). There was no difference in the total numbers collected in the two catch basin types (p=0.59, 18 df, t = -0.5429). The average number of mosquitoes collected in all of the catch basins was 427 (95% CI 163 to 691). Using the average for all catch basins, an estimated 138,348 (SE ± 40,824) female *Cx. pipiens* emerged from the catch basins in the southern area during the 22-day sampling period.

In September, a total of 14,978 emerging adult mosquitoes were collected from the catch basins in the northern sampling area (Figure 5.5). Mosquitoes were collected from 16 of the 20 catch basins sampled in the northern area. The majority of the mosquitoes collected were *Cx. pipiens* (n=12,668). Of those 45.6% (n=5781) were female. Female *Cs. incidens* (n=1099) were collected from half of the catch basins and female *Cx. stigmatosoma* (n=10) were only collected in two of the northern catch basins. *Culiseta incidens* and *Cx. stigmatosoma* were not evaluated firther. The average number of female *Cx. pipiens* that emerged from the smaller catch basin during the sampling period was 109 (95% CI 43 to 174) and 469 (95% CI -175 to 1114) for the larger catch basins. However, one of the larger catch basins collected significantly more mosquitoes than any of the others with a total of 3002 collected. With that outlier removed the average number of *Cx. pipiens* collected from the larger catch basins was 188 (95% CI 69 to 306). However, there was no measurable difference between the two catch basin types with the outlier included (p=0.22, 18 df, t=-1.26) or without the outlier excluded (p=0.19, 18 df, t=-1.37). The

conservative estimate, with the outlier removed, for the number of mosquitoes emerging from the northern catch basin is 84,432 (SE ± 22,860) female *Cx. pipiens*.

DISCUSSION

Emergence traps have been widely used for collecting aquatic insects and come in a variety of designs that can either be submerged or float on the waters surface (Service 1976). Like most trapping methods, there are some biases that can be introduced when using emergence traps. For instance some species may be differentially attracted to or repelled by the shadows cast by the trap (Service 1976). Additionally, changes to the size or depth of the water body being sampled or the height of emergent vegetation may affect trap results (Service 1976, Workman and Walton 2000). Most of these potential biases are mitigated when traps completely cover the emergence area, such as covering treehole openings and catch basins tops. Corbet (1965) suggested that some mosquitoes may stay in the traps, as apposed to entering the collection jars as soon as they emerge; however, this would not significantly affect the numbers of mosquitoes collected over time, as they will eventually enter the collection jars. When the traps for this study were briefly removed for minor repairs, very few dead adult mosquitoes were found on the surface of the water.

While the numbers of immature mosquitoes collected during an earlier study (Townzen et al. 2008) could not be used to estimate mosquito populations, the percentages of each mosquito species collected were similar between the two studies. *Culex pipiens* dominated the collections of both studies; 87% of the emerging adults

and 79% of larvae collected in the northern and southern areas were *Cx pipiens*. The numbers of *Cs. incidens* (11% of adults, 14% of larvae) and *Cx. stigmatosoma* (1% of adults and 6% of larvae) collected were much lower. The biggest difference between the two studies was the percentage of *Cx. stigmatosoma* collected in the southern area of Corvallis. In 2004 twice as many *Cx. stigmatosoma* (8% of the collection) were collected in comparison to *Cs. incidens* (4%).

Estimating the population outside of the sampling area is a purely speculative, but interesting, exercise. Within the city limits of Corvallis at the time of the study, in 2006, there were approximately 6,500 catch basins (Olsen personal communication), with 85% of those holding water throughout the summer (Townzen, unpublished). Based on the numbers of female *Cx. pipiens* collected in southern Corvallis in August the roughly 2,360,000 of these mosquitoes emerged from all of the southern catch basins during the collection period and roughly 800,000 emerged from all of the catch basins in the northern area during the September Collection period, There are currently over 7,500 sumped catch basins in Corvallis, as the numbers of catch basins increases in the city it is likely that, without control, the numbers of mosquitoes emerging from the catch basins will also increase.

When thinking about this study in terms of designing a control program, the key is the numbers of catch basins that were positive for mosquitoes. Immature mosquitoes were collected from 92% of the catch basins sampled (Townzen et al. 2008). Emerging adults were collected from 90% of the catch basins sampled in the southern area and 80% of the catch basins sampled in the northern area. One of the reasons for the differences in the number of catch basins positive for emerging

mosquitoes could be the timing and not necessarily the difference in location. Fewer immature mosquitoes were collected in all areas of Corvallis in September in 2004.

In 2004 immature mosquito populations in the catch basins peaked in the late summer and dips in the sampling numbers were associated with rainfall events (Townzen 2008). In 2006 there was very little rainfall in August, with only one recorded rainfall event of less than 0.13 cm on August 31st. There was an expected gradual decrease in the numbers of mosquitoes collected through the course of the August sampling, but not the drastic drop in numbers associated with major rainfall. A decrease in the numbers of mosquitoes emerging from the catch basins (Figure 5.4 and 5.5) was expected because the emergence traps were designed to fit snugly in the catch basins, thereby excluding ovipositioning mosquitoes and a constant influx of eggs. In September, rainfall events began on the 15th and continued until the 19th when the traps were removed. In that time 1.3 cm of rain fell in Corvallis (OCS 2008), enough water to fill many of the catch basins to the outfall pipe and wash immature mosquitoes out of the basins and could account for the significant reduction in emerging mosquitoes to zero at the end of the September sampling period.

In North America, most WNV activity occurs from July through October (Hayes et al. 2005) and is consistent with peaks in the numbers of immature mosquitoes collected from the catch basins in Corvallis. All three of mosquito species collected from the catch basins could play a part in transmission of WNV in Corvallis (Chapter 4) and the numbers of mosquitoes emerging from the catch basins is not insignificant. Control of immature mosquitoes in small enclosed areas, such as catch basins, is relatively straightforward (Geery and Holub 1989**,** Knepper et al. 1992,

McCarry 1996, Pfuntner 1978, and Siegel and Novak 1999). While the cost of control may be significant, between $3.00 and $4.00 per catch basin, depending on product and formulation (Candito personal communication), it should be considered as a management tool, if WNV becomes endemic in Corvallis.

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LITERATURE CITED

Aubin A, Bourassa JP, Pellissier M. 1973. An effective trap for the capture of mosquitoes. *Mosq News* 33: 251-252.

Butler M, Ginsberg HS, LeBrun RA, Gettman AD, Pollnak F. 2007. Natural communities in catch basins in southern Rhode Island. *Northeastern Naturalist* 14: 235-250.

Candito, Dennis. Adapco Inc.. Personal communication. 14 July 2008.

CDC. 2008. West Nile virus: statistics, surveillance, and control. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Ft. Collins, Colorado. [Accessed May 12, 2008] http:// www.cdc.gov/ncidod/dvbid/westnile/Mapsactivity/surv&control08Maps.htm.

Corbet PS. 1965. Insect emergence trap for quantitative studies in shallow ponds. *Can Entomol* 97: 845-848.

DeBess E. 2008. 2007 West Nile virus summary report for Oregon. [Internet] Available from Oregon Department of Human Services, Acute and Communicable Disease Prevention, Portland, Oregon. [Accessed May 22, 2008] http://www.oregon.gov/DHS/ph/acd/diseases/wnile/wnv2007rpt.pdf.

Gingrich JB, Anderson RD, Williams GM, O'Connor L, Harkins K. 2006. Stormwater ponds, constructed wetlands, and other best management practices as potential breeding sites for West Nile virus vectors in Delaware during 2004. *J Am Mosq Control* Assoc. 22: 282-291.

Goddard LB, Roth AE, Reisen WK, Scott TW. 2002. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis* 8: 1385-1391.

Greery PR, Holub RE. 1989. Seasonal abundance and control of *Culex* spp. in catch basins in Illinois. *J Am Mosq Control Assoc*. 5: 537-540.

Hayes, E.B., Komar, N., Nasci, R.S., Montgomery, S.P., O'Leary, D. R., & Campbell, G. L. (2005). Epidemiology and transmission dynamics of West Nile virus disease. *Emerging Infectious Disease*, 11, 1167-1173.

Knepper RG, Leclair AD, Strickler JD, Walker ED. 1992. Evaluation of Methoprene (Altosid ® XR) sustained release briquets for control of *Culex* mosquitoes in urban catch basins. *J Am Mosq Control Assoc* 8: 228-230.

Kronenwetter-Koepel TA, Meece JK, Miller CA, Reed KD. 2005. Surveillance of above- and below-ground mosquito breeding habitats in a rural Midwestern

community: baseline data for larvicidal control measures against West Nile virus vectors. *Clin Med & Res* 1: 3-12.

Kwan, J.A., Riggs-Nagy, J.M., Fritz, C.L., Shindelbower, M., Castro, P.A., Kramer, V.L., Metzger M.E. (2008). Mosquito Production in Stormwater Treatment Devices in the Lake Tahoe Basin, California. *J Am Mosq Control Assoc* 24: 82-89.

Metzger ME, Myers CM, Kramer VL. 2003. The "dark side" of stormwater runoff management: Vectors associated with BMPs. *Proc Calif Mosq Vector Control Assoc* 70: 2-10.

Olsen J. City of Corvallis, Personal Communication. 26 March 2008.

OCS. 2008. Oregon Climate Service, Hyslop Station, Corvallis. [Internet] Available from Oregon Climate Service, Corvallis, Oregon. [Accessed July 13, 2008] http://www.ocs.oregonstate.edu/index.html.

Pfunter AR. 1978. The development and control of *Culex quinquefasciatus* Say and *Culex peus* Speiser in urban catch basins. *Proc & Pap 46th Annul Conf Calif Mosq Control Assoc* 46: 126-129.

Reisen WK, Fang Y, Martinez VM. 2006. Vector competence of *Culiseta incidens* and *Culex thriambus* for West Nile virus. *J Am Mosq Cont Assoc* 22: 662-665.

Rey JR, O'Meara GF, O'Connell SM, Cutwa-Francis MM. 2006. Factors affecting mosquito production from stormwater drains and catch basins in two Florida cities. *J Vector Ecol* 3: 334-343.

Service MW. 1976. *Mosquito Ecology: Field Sampling Methods*. Applied Science Publishers, Essex, England.

Siegel JP, Novak RJ. 1999. Duration of activity of the microbial larvicide Vectolex CG® (*Bacillus sphaericus*) in Illinois catch basins and waste tires. *J Am Mosq Cont Assoc* 15: 366-370.

Townzen JS, Judd DD, Rossignol PA. 2008 in review. Characterization of stormwater catch basins as habitat for immature mosquitoes in Corvallis, Oregon. *J Env Health*.

Turell MJ, Dohm DJ, Sardelis MR, O'Guinn ML, Andreadis TG, Blow JA. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *J Med Entomol* 42: 57-62.

Workman PD, Walton WE. 2000. Emergence patterns of *Culex* mosquitoes at an experimental constructed treatment wetland in Southern California. *J Am Mosq Cont Assoc* 16: 124-130.

Figure 5.1. Photographs of the small (A) and large (B) catch basin designs in Corvallis, OR.

A.

B.

Figure 5.2. Map of Corvallis, OR, showing the locations of the study catch basins. The circles and triangles represent the larger and smaller catch basins respectively.

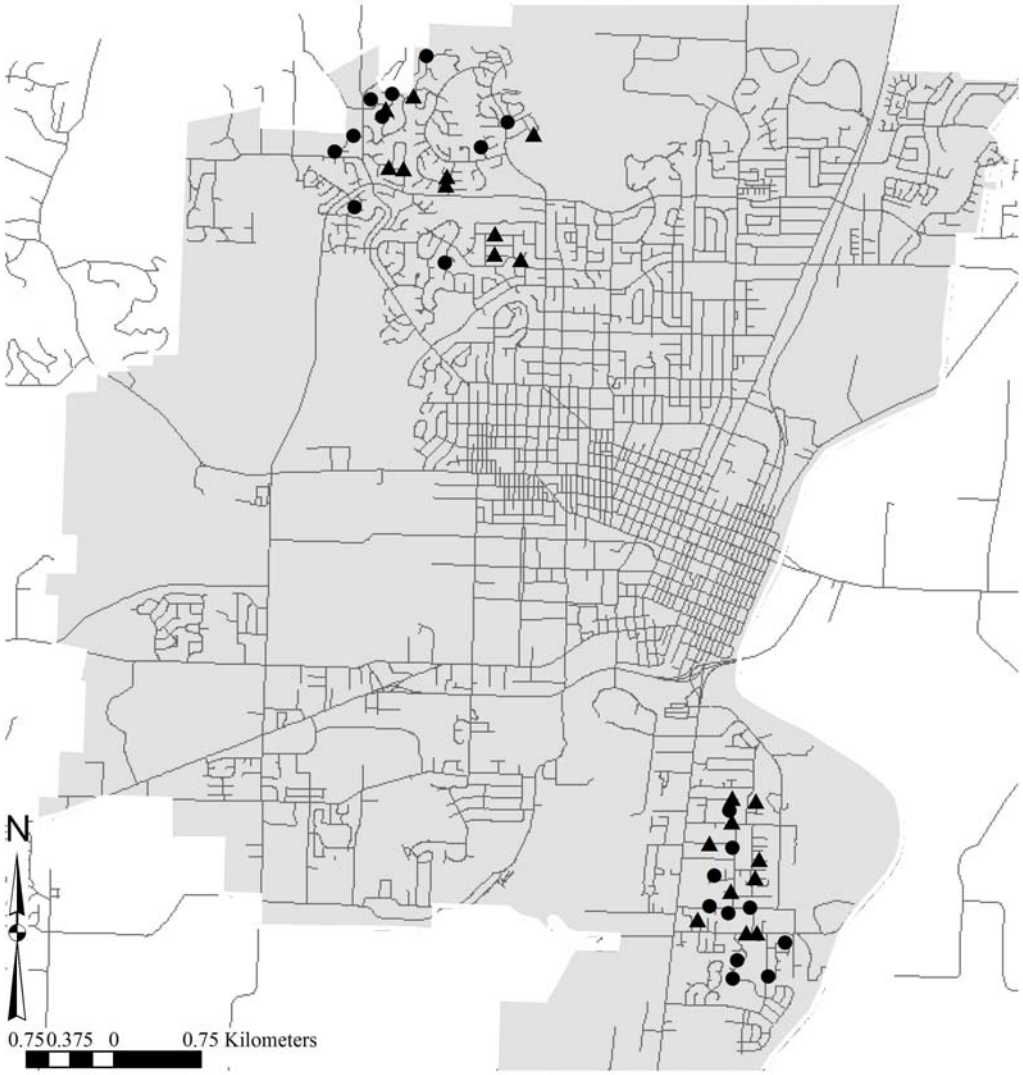


Figure 5.3. Catch basins with traps.

A.

B.

C.

Table 5.1. Total numbers of each mosquito species collected by area.

	Culex pipiens		***Culex stigmatosoma***		***Culiseta incidens***	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Southern Area	8259	8557	181	228	666	570
Northern Area	6887	5781	46	10	1155	1099

Figure 5.4. Numbers of mosquitoes collected in emergence traps in the southern area of Corvallis, OR.

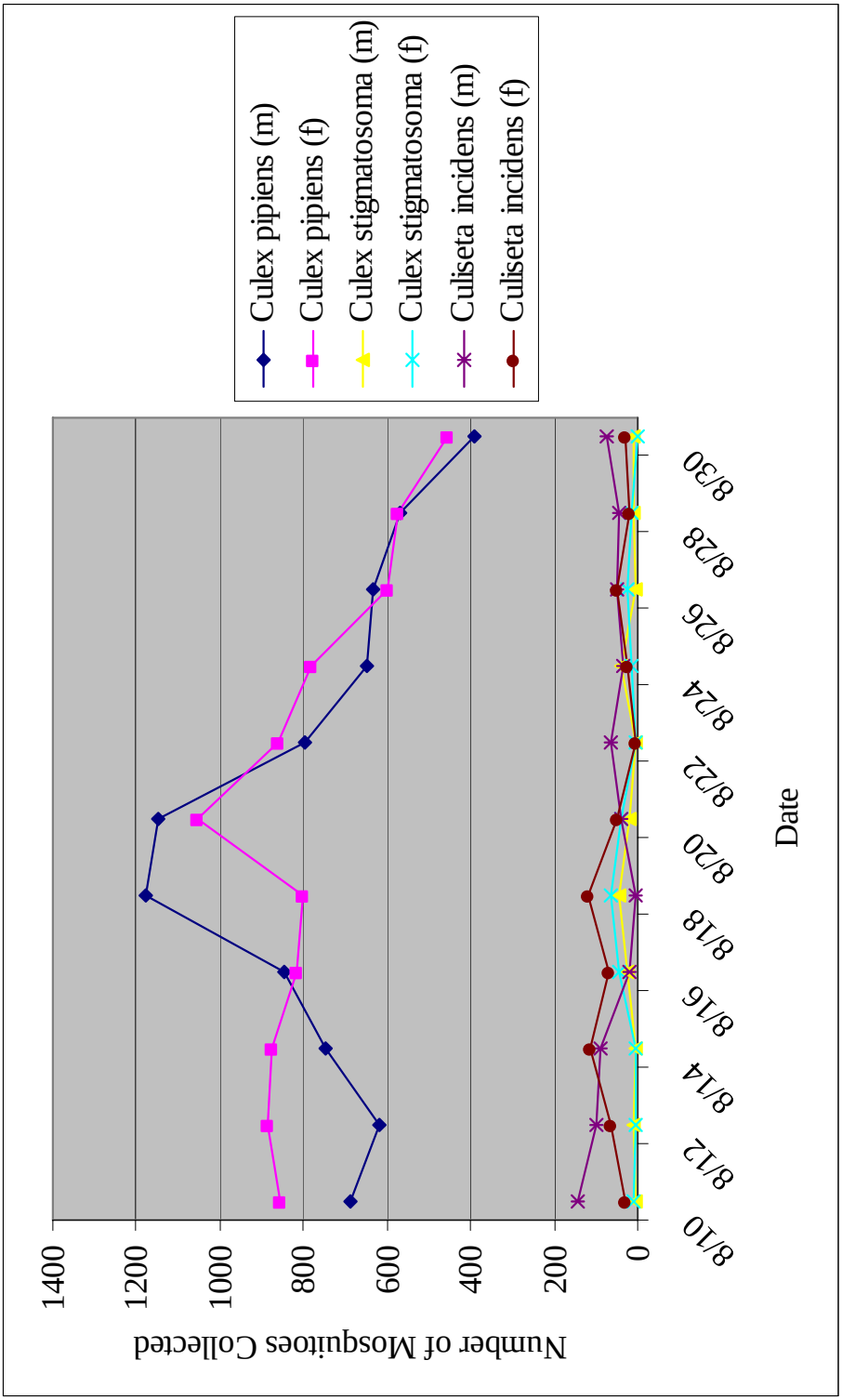
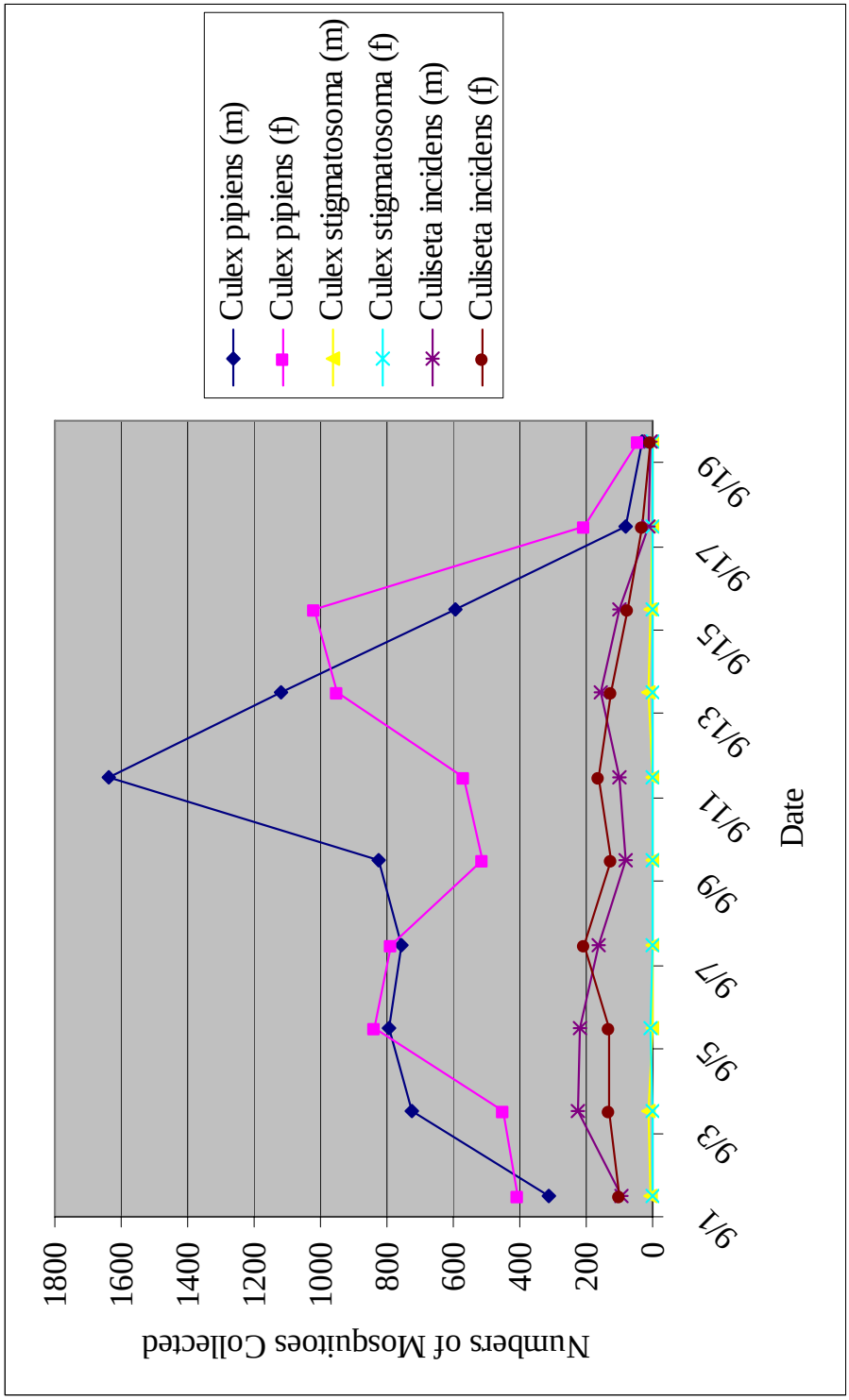


Figure. 5.5. Numbers of mosquitoes collected in emergence traps in the northern area of Corvallis over the 20 day sampling period in September.



CHAPTER 6. CONCLUSION

OVERVIEW OF RESULTS

This research examined two of the parameters of vectorial capacity for mosquitoes associated with catch basins in Corvallis, Oregon. The parameters of interest were: 1) abundance of mosquitoes associated with the catch basins and 2) feeding patterns of local mosquito species. Three species of mosquitoes were collected from Corvallis catch basins: *Culex pipiens* L., *Culex stigmatosoma* Dyar, and *Culiseta incidens* (Thomson). Over 32 weeks of sampling from the 60 catch basins, in 2004, a total of 1,920 catch basin visits were made and 79,760 immature mosquitoes were collected. Emerging mosquitoes were collected from 20 catch basins in southern Corvallis for 22 days in August 2006 and from 20 catch basins in northern Corvallis for 20 days in September 2006. Based on the numbers of mosquitoes collected from the 20 catch basins sampled, an estimated 138,348 female *Cx. pipiens* emerged from the 324 of the catch basins in the southern area and 84,432 emerged from the 500 northern catch basins. Molecular analysis of the blood meals from *Cx. pipiens*, *Cx. stigmatosoma*, and *Cs. incidens* collected in Corvallis parks and greenspaces found that the two *Culex* spp. fed primarily on avian hosts and *Cs. incidens* primarily on mammalian hosts.

THE CATCH BASIN STUDIES

Corvallis, Oregon, located about 90 miles south of Portland, in the Willamette Valley, was the ideal place to study mosquitoes associated with catch basins because

they do not currently conduct mosquito control in the city. The city has a population of nearly 53,000 people and encompasses 14 square miles. At the start of this research, in 2004, there were just over 6,000 sumped catch basins within the city limits. By the end of the project, in 2008, there were approximately 7,500 catch basins in the city (J. Olsen, personal communication). This increase in catch basin construction highlights the importance of better understanding the role these sites play as mosquito habitat and the potential role that the mosquitoes utilizing these sites might play in disease transmission.

Catch basins are the street drains found throughout much of the United States. There are two styles of catch basins in Corvallis, one is smaller with a more open grate design and the other is larger, covered by a large metal cover and has only a small grate (Figure 6.1). Both catch basin styles are designed with the outflow pipe located approximately 45 cm off the bottom of the basin that creates a sump. Water is held so that particulate matter washed off streets during rain events can settle out. Because they hold water, catch basins also provide habitat for ovipositing and immature mosquitoes.

Immature mosquitoes and emerging adult mosquitoes were collected from the catch basins in two different studies, Chapter 2 and Chapter 5 respectively. Immature mosquitoes were collected by taking 350 ml samples of water using a long handled dipper. Dipping for mosquitoes is an easy and efficient way to sample immature mosquitoes and the resulting samples can be used to determine the species present and their relative abundance. Dipping can be used to compare the numbers of

mosquitoes collected per dip in one site with those collected in another; however, this type of larval dipping cannot be used to estimate the entire population of a site.

Three species of mosquitoes were routinely collected from catch basins in Corvallis: *Culex pipiens* L., *Culex stigmatosoma* Dyar, and *Culiseta incidens* (Thomson). Immature mosquitoes were collected weekly from 60 catch basins for 32 weeks. In all, 1,920 samples were collected, containing a total of 79,760 mosquitoes (Figure 6.2). On two dates, July 16th 2004 and August 15th 2004, the numbers of late instar larvae (third and fourth) and pupae collected were analyzed to determine if there was a difference in the numbers sampled in the three areas of the city and the two catch basin styles. On July 16th, 57 of the 60 (95%) catch basins contained water and immature mosquitoes were found in 45 (79%) of the wet catch basins. An average of 23, 3rd and 4th instar larvae and pupae (95% CI 14 to 32), were sampled per catch basin. Additionally, there was only a suggestive difference in the numbers of late instar larvae (third and fourth) and pupae found in the three areas of the city (f-stat = 4.06, df = 2, 57, P = 0.0225) and no measurable difference in the two catch basin styles (t-stat=0.62, df = 58, P = 0.535). On August 15th, 57 of 60 catch basin contained water and mosquitoes were collected from 45 (79%) of the wet basins. An average of 34.9 late instar larvae and pupae (95% CI 19 to 51) was collected per catch basin on this date and no evidence of a difference in numbers by either catch basin design (t-stat = 1.30, df = 58, P = 0.1995) or the area in which it was located (F-stat = 0.077, df = 2, 57, P = 0.9262) was found.

Emerging adult mosquitoes were collected from the catch basins using modified emergence traps (Aubin et al. 1973) designed to fit snugly into the catch

basins and collect all of the adults emerging over a given period of time. Once the emerging mosquitoes were counted, the entire population of mosquitoes emerging from the catch basins in the sampling area was estimated for the time period sampled. Emerging mosquitoes were collected from 20 catch basins in the southern area of Corvallis in August and 20 catch basins in the northern area in September. The catch basins were sampled every other day for 22 and 20 days respectively and a total of 33,439 mosquitoes were collected. The dominant mosquito collected emerging from the catch basins was *Cx. pipiens*. Based on the numbers of females collected during the August sampling period (n = 8,557), an estimated 138,348 (SE ± 40,824) female *Cx. pipiens* emerged from the catch basins in the southern area during the 22 day sampling period. The estimate for the number of female *Cx. pipiens* emerging from the northern catch basin is 84,432 (SE ± 22,860).

IDENTIFYING MOSQUITO BLOODMEALS

One of the earliest methods used to identify hosts from mosquito bloodmeals was the direct examination of erythrocytes under the microscope, allowing differentiation between avian, mammalian and reptilian bloodmeals (Weitz 1956). Over time the precipitin, passive hemagglutination inhibition and ELISA were developed and applied to bloodmeal identification (Washino and Tempelis 1983, Chow et al. 1993). While sensitivity increased with each of theses tests, specificity was still lacking. Many of the early feeding pattern studies did not resolve mosquito hosts past class or in some cases order. Modern molecular techniques, including

sequencing of mtDNA segments, can be used to identify mosquito bloodmeals to host species.

At the time that this study was initiated sequencing vertebrate DNA from mosquito bloodmeals was not commonly used as a methodology for identifying mosquito host feeding patterns. A molecular protocol was developed (Chapter 3) to meet the need for a quick and easy, while still sensitive and specific, way to identify the hosts of mosquitoes collected in Corvallis. Pairs of primers were designed to amplify vertebrate *cytochrome oxidase subunit 1* (COI) and *cytochrome b* (Cyt *b*) mtDNA from the abdomens of engorged mosquitoes. Sequencing of the resulting mtDNA fragments was then outsourced to a commercial firm (Macrogen, Seoul, South Korea). Putative identification of bloodmeals was made by comparing our sequences to the known sequences in GenBank using the BLASTN (Altschul et al. 1997, http://www.ncbi.nlm. nih.gov/) function. Sequences were considered a match if they had a greater than 95% similarity to a sequence in GenBank. By using both COI and Cyt *b* we were able to identify the vertebrate host of 140 samples.

Once the protocols were developed the next step was to identify the feeding patterns of the local mosquitoes. Over the course of a two year sampling period 425 blood-engorged mosquitoes were collected. The collection consisted of nine species from four genera: *Aedes increpitus* Dyar (n=5), *Aedes sierrensis* (Ludlow) (34), *Anopheles freeborni* (13), *Anopheles punctipennis* (150), *Culiseta incidens* (75), *Culex pipiens* (70), *Culex stigmatosoma* (18), and *Culex tarsalis* (41) and *Culex territans* Walker (19). A total of 29 vertebrate species were identified as hosts (Figure 6.3).

All three of the species associated with catch basins were collected and analyzed for host feeding pattern. The two *Culex* spp. collected in Corvallis fed predominately on birds. *Culex stigmatosoma* fed solely on passerine birds, 72% of which were American crow. 94% of the *Cx. pipiens* fed on 15 different species of birds while the remaining 6% had fed on deer. The species specific host feeding pattern of *Cx. pipiens* changed from one year to the next. In 2006, *Cx. pipiens* most frequently fed on green heron and cedar waxwing; each represented 20% of the identified bloodmeals and in 2007 they primarily fed upon American crow, representing 62% of identified bloodmeals. In contrast, *Cs. incidens* displayed a primarily mammalian feeding pattern with 98% of those collected having fed on mammals. Of those, 97 % had fed on deer. The remainder of the *Cs. incidens* had fed on sheep, human, and crow.

TYING IT ALL TOGETHER

Mosquitoes are the vectors of a variety of diseases humans and animals. Anopheline mosquitoes transmit malaria. *Culiseta incidens* and *Ae. sierrensis* are implicated in transmission of canine heartworm, *Dirofilaria immitis* (Leidy), to dogs and other mammals (Walters and Lavoipierre 1982, Scoles et al. 1993 and Theis et al. 2000). A wide variety of mosquitoes are involved in transmission of encephalitides, including West Nile virus (WNV), to humans and birds. West Nile virus is currently the most important mosquito-borne disease in the United States. The first human cases in the U. S. occurred in 1999 in New York. Since that time 27,598 human cases, 1086 of which were fatal, have been reported to the Centers for Disease Control and

Prevention (CDC 2008). In the three years since WNV was first detected in Oregon, 113 human and 97 equine cases have been reported (DeBess 2008). So far only three infected birds, collected as a part of the dead bird surveillance program, and a single non-locally acquired human WNV case have been reported in Benton County, where Corvallis is located (DeBess 2008). Even though there has been little recorded activity to date, if a large enough population of infected birds were to migrate into the area, all of the pieces are in place for WNV transmission to occur in Corvallis.

Implicating a mosquito in disease transmission is not as simple as finding a mosquito infected with a pathogen. Vectorial capacity and vector competence are both involved in determining whether or not a species, or population, of mosquitoes is involved in transmission of disease. Vector competence is the mosquito's intrinsic ability to transmit a pathogen and includes the mosquito's susceptibility to infection and the ability of the pathogen to replicate within the mosquito. Competency is determined through laboratory studies in which mosquitoes are fed infected blood, or directly injected with a pathogen, and then monitored to see if they develop an infection which is then transmitted during subsequent feedings. The study of vector competence was outside the scope of this project. However, other studies have found that all three species of mosquitoes, *Cx. pipiens*, *Cx. stigmatosoma*, and *Cs. incidens*, collected from the catch basins in Corvallis are competent vectors of WNV (Goddard et al. 2002, Turell et al. 2005, Reisen et al. 2006)

Vectorial capacity incorporates the extrinsic factors involved in mosquito-borne disease transmission. The model for vectorial capacity includes longevity of the mosquito, the intrinsic incubation period of the pathogen and the two parameters

studied for this research: abundance and feeding habit of the mosquito. Knowledge of the feeding pattern of a mosquito is essential in determining its role, if any, in disease transmission. If a mosquito does not feed on competent vertebrate hosts of a pathogen then it would be unlikely to come in contact with that pathogen. For a disease like malaria, which cycles between humans and mosquitoes, the mosquitoes involved in transmission will be those that feed primarily on humans. For mosquito-borne encephalitides, such as WNV, a mosquito involved in transmission of the disease to humans must feed on both birds, the enzootic reservoir of the disease, and humans.

Across the United States, WNV has been detected in 317 species of birds (CDC 2008). However, passerine birds, especially American crow, California scrubjay, house finch and house sparrow, produce the highest viremic titers (Komar et al. 2003 and Reisen et al. 2005) and are likely candidates for maintenance of the virus. American crow was identified as the host for 38% of the *Cx. pipiens* bloodmeals, 72% of *Cx. stigmatosoma* and a single *Cs. incidens* collected in Corvallis. *Culex stigmatosoma* fed solely on passerine birds in Corvallis and are a good candidate for enzootic transmission. *Culex pipiens* fed primarily on birds, implicating this species as a zoonotic vector as well; however, they also fed on mammals and could play a role in epidemic transmission too. *Culiseta incidens* fed primarily on mammals, including a human host. A single *Cs. incidens* also fed on American crow; this species could be involved in epidemic transmission of WNV.

Abundance is one of the two parameters of vectorial capacity that can be manipulated, through managing mosquitoes, by man. The other, longevity, can be managed through adult mosquito control, while abundance can be lowered through

both larval and adult mosquito control. Mosquitoes must feed to become infected and then feed again once infective to transmit a pathogen, therefore the goal of shortening the longevity of mosquitoes is to prevent them from living long enough to feed multiple times. Minimizing mosquito abundance prevents the disease from building in the host population, preferably before it becomes an epidemic.

Larviciding is an effective way to minimize mosquito abundance and multiple studies have been conducted using a variety of larvicides in catch basins (Geery and Holub 1989**,** Knepper et al. 1992, McCarry 1996, Pfuntner 1978, and Siegel and Novak 1999). There are currently over 7,500 catch basins in the City of Corvallis (Olsen personal communication) and approximately 85% of those hold water (Townzen unpublished). The estimated cost for managing mosquitoes in the Corvallis catch basins is about $22,500 (Candito personal communication). While WNV may not be a serious health issue in Corvallis at this time that may not always be the case. If the virus becomes established in the city, as it has elsewhere, controlling mosquitoes in catch basins would eliminate a significant source of mosquitoes in close association with humans.

LITERATURE CITED

Aubin A, Bourassa JP, Pellissier M. 1973. An effective trap for the capture of mosquitoes. *Mosq News* 33: 251-252.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389--3402.

Candito, Dennis. Adapco Inc.. Personal communication. 14 July 2008.

CDC 2007. West Nile virus: entomology. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Ft. Collins, Colorado. [Accessed May 12, 2008] http://www.cdc.gov/ncidod/ dvbid/ westnile/mosquitoSpecies.htm.

CDC. 2008. West Nile virus: statistics, surveillance, and control. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Ft. Collins, Colorado. [Accessed May 12, 2008] http:// www.cdc.gov/ncidod/dvbid/westnile/Mapsactivity/surv&control08Maps.htm.

Chow E, Wirts RA, Scott TW. 1993. Identification of blood meals in *Aedes aegypti* by antibody sandwich enzyme-linked immunosorbent assay. *Journal of the Am Contr Assoc* 10: 196-205.

DeBess E. 2008. 2007 West Nile virus summary report for Oregon. [Internet] Available from Oregon Department of Human Services, Acute and Communicable Disease Prevention, Portland, Oregon. [Accessed May 22, 2008] http://www.oregon.gov/DHS/ph/acd/diseases/wnile/wnv2007rpt.pdf.

Goddard LB, Roth AE, Reisen WK, Scott TW. 2002. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis* 8: 1385-1391.

Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Bunning M. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis* 9: 311-322.

Olsen J. City of Corvallis, Personal Communication. 26 March 2008.

Reisen WK, Fang Y, Martinez VM. 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. *J Med Entomol* 42: 367-375.

Reisen WK, Fang Y, Martinez VM. 2006. Vector competence of *Culiseta incidens* and *Culex thriambus* for West Nile virus. *J Am Mosq Cont Assoc* 22: 662-665.

Scoles GA, Dickson SL, Blackmore MS. 1993. Assesment of *Aedes sierrensis* and a vector of canine heartworm in Utah using a new technique for determining the infectivity rate. *J Am Mosq Cont Assoc* 9: 88-90.

Theis JH, Kovaltchouk JG, Fujioka KK, Saviskas B. 2000. Vector competence of two species of mosquitoes (Diptera: Culicidae) from Southern California *Dirofilaria immitis* (Filariidae: Onchoceridae). *J Med Entomol* 37: 295-297.

Turell MJ, Dohm DJ, Sardelis MR, O'Guinn ML, Andreadis TG, Blow JA. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *J Med Entomol* 42: 57-62.

Walters LL, Lavoipierre MMJ. 1984. Landscape epidemiology of mosquito-borne canine heartworm (*Dirofilaria immitis*) in northern California, US: 1. Community-based surveys of domestic dogs in three landscapes. *J Med Entomol* 21: 1-16.

Washino RK, Tempelis CH. 1983. Mosquito host bloodmeal identification: methodology and data analysis. *Ann Rev Entomol* 28: 179-201.

Weitz B. 1956. Identification of bloodmeals of blood-sucking arthropods. *Bull Wld Hlth Org* 15: 473-490.

Figure 6.1. Photographs of the small (A) and large (B) catch basin designs found in Corvallis, OR.

A.

B.

Figure 6.2. Numbers of mosquitoes collected by date and species in Corvallis, OR.

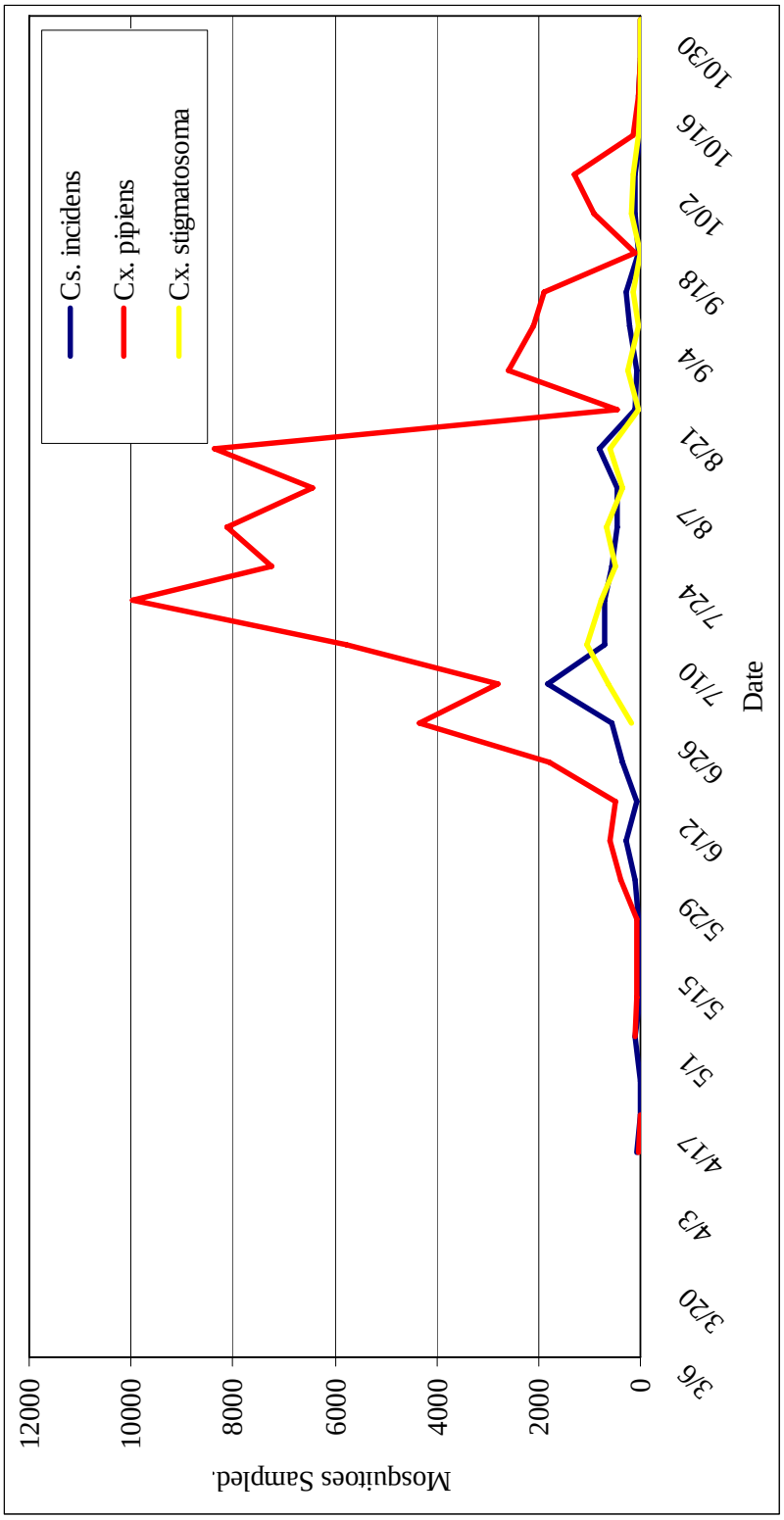


Table 6.1. Identification of bloodmeals from mosquitoes primarily feeding on mammals.

Mosquito Species	No. amplified / No. collected (%)	No. (%) feeding on					
		Deer	Cottontail rabbit	Domestic cat	Human	Domestic dog	Elk
Ae. increpitus	5/5 (100)	2 (40)	1 (20)	1 (20)	1 (20)		
Ae. sierrensis	34/34 (100)	29 (85.3)	3 (8.8)		1 (3)	1 (3)	
An. freeborni	13/13 (100)	7 (53.8)	1 (7.7)		1 (7.7)	2 (15.4)	1 (7.7)
An. punctipennis	135/150 (90)	92 (68.1)	3 (2.2)	5 (3.7)	1 (0.7)	6 (4.4)	
Cs. incidens	74/75 (99)	71 (96)			1 (1.4)		

Mosquito Species	No. (%) feeding on (Cont'd)						
	Domestic horse	Domestic sheep	Raccoon	Domestic goat	Unident. mammal	Green heron	American crow
Ae. increpitus							
Ae. sierrensis							
An. freeborni							1 (7.7)
An. punctipennis	1 (0.7)	16 (11.9)	6 (4.4)	2 (1.5)	1 (0.7)	1 (0.7)	1 (0.7)
Cs. incidens		1 (1.4)					1 (1.4)

Table 6.2. Identification of bloodmeals from *Culex* spp. collected in Corvallis, OR.

Mosquito Species	No. amplified / No. collected (%)	Mammal to Avian Ratio	No. (%) feeding on				
			Deer	Human	Raccoon	Pacific treefrog	Green heron
Cx. territans	19/19 (100)	N/A				19 (100)	
Cx. pipiens	67/70 (96)	4:63	4 (6)				5 (7.5)
Cx. stigmatosoma	18/18 (100)	0:18					
Cx. tarsalis	39/42 (93)	8:31	5 (12.8)	1 (2.6)	2 (5.1)		1 (2.6)

Mosquito Species	No. (%) feeding on (cont'd)						
	European starling	California scrubjay	American crow	Cedar waxwing	American robin	House finch	House sparrow
Cx. territans							
Cx. pipiens	2 (3)	7 (10.4)	26 (38.8)	6 (9)	3 (4.5)	3 (4.5)	2 (3)
Cx. stigmatosoma		4 (22.2)	13 (72.2)		1 (5.6)		
Cx. tarsalis	1 (2.6)	2 (5.1)	9 (23.1)		5 (12.8)	1 (2.6)	2 (5.1)

Table 6.2. (continued)

Mosquito Species	No. (%) feeding on (cont'd)					
	Allen's humming-bird	Swainson's thrush	Cooper's hawk	Brewers blackbird	Bewick's wren	Black-headed grosbeak
Cx. territans						
Cx. pipiens	1 (1.5)	1 (1.5)	2 (3)	1 (1.5)	2 (3)	1 (1.5)
Cx. stigmatosoma						
Cx. tarsalis		1 (2.6)	1 (2.6)			1 (2.6)

Mosquito Species	No. (%) feeding on (cont'd)			
	Mourning dove	Black-capped chickadee	Song sparrow	Domestic chicken
Cx. territans				
Cx. pipiens	1 (1.5)			
Cx. stigmatosoma				
Cx. tarsalis	1 (2.6)	1 (2.6)	1 (2.6)	1 (2.6)

BIBLIOGRAPHY

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389--3402.

Apperson CS, Harrison BA, Unnasch TR, Hassan H.K, Irby WS, Savage HM, Aspen SE, Watson DW, Rueda LM, Engber BR, Nasci RS. 2002. Host-feeding habits of *Culex* and other mosquitoes (Diptera: Culicidae) in the borough of Queens in New York City, with characters and techniques for identification of *Culex* mosquitoes. *J Med Entomol* 39: 777-785.

Apperson CS, Hassan H.K, Harrison BA, Savage HM, Aspen SE, Farajollahi A, Crans WJ, Daniels TJ, Falco RC, Benedict M, Anderson M, McMillen L, Unnasch TR. 2004. Host Feeding Patterns of Established and Potential Mosquito Vectors of West Nile Virus in the Eastern United States. *Vector-Borne and Zoonotic Dis* 4: 71-82.

Armstrong KF, Ball SL. 2005. DNA barcodes for biosecurity: invasive species identification. *Philosophical Transactions of the Royal Society B*, 360: 1813-1823.

Aubin A, Bourassa JP, Pellissier M. 1973. An effective trap for the capture of mosquitoes. *Mosq News* 33: 251-252.

Audubon. 2008. Annual Christmas bird count: current year results. [Internet]Available from the National Audubon Society, New York. [Accessed May 12, 2008] http://cbc.audubon.org/cbccurrent/current_table.html.

Bailey NTJ. 1982. *The Biomathematics of Malaria*. Oxford University Press, New York, NY.

Baker CS, Palumbi SR. 1994. Which whales are hunted? A molecular genetic approach to monitoring whaling. *Science* 265: 1538-1539.

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2006. GenBank. *Nucleic Acids Res* 35: D21-D25.

Boakye DA, Tang J, Truc P. 1999. Identification of bloodmeals in haematophagous Diptera by cytochrome B heteroduplex analysis. *Med Vet Entomol* 13: 282-287.

Bohart RM, Washino RK. 1978. Mosquitoes of California. Division of Agricultural Science, University of California, Berkeley, California.

Boreham PFL, Garrett-Jones C. 1973. Prevalence of mixed blob meals and double feeding in a malaria vector. *Bull Wld Hlth Org* 48: 605-614.

Bosseno MF, García LS, Baunaure F, Gastelúm EM, Gutierrez MS, Kasten FL, Dumonteil E, Breniére S. 2006. Identification in triatomine vectors of feeding sources and *Trypanosoma cruzi* variants by heteroduplex assay and multiplex miniexon polymerase chain reaction. *Am J Trop Med and Hyg* 74: 303-305.

Boyd RT. 1975. Another look at the "fever and ague" of Western Oregon. *Ethnohistory* 22: 135-154.

Brower AVZ. 2006. Problems with DNA barcodes for species delimitation: "ten species" of *Astraptes fulgerator* reassessed (Lepidoptera: Hesperiidae). *Sys Bio* 4: 1-6.

Brower AVZ, DeSalle R, Vogler A. 1996. Gene trees, species trees and systematics: a cladistic perspective. *Ann Rev Ecol Sys* 27: 423-450.

Bruce-Chwatt LJ. 1976. Mathematical models in the epidemiology and control of malaria. *Trop Geogr Med* 28: 1-8.

Burkot TR, Goodman WG, DeFoliart GR. 1981. Identification of mosquito blood meals by enzyme-linked immunosorbent assay. *Am J Trop Med Hyg* 30: 1336-1341.

Butler M, Ginsberg HS, LeBrun RA, Gettman AD, Pollnak F. 2007. Natural communities in catch basins in southern Rhode Island. *Northeastern Naturalist* 14: 235-250.

Candito, Dennis. Adapco Inc.. Personal communication. 14 July 2008.

CDC. 2003. Epidemic/epizootic West Nile virus in the United States: guidelines for surveillance, prevention and control. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention. http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-aug-2003.pdf , Accessed 10-Jan.-2008.

CDC, 2004. West Nile virus homepage. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector Borne Infectious Disease, Ft. Collins, CO. [Accessed April 2008] http://www.cdc.gov/ ncidod/dvbid/westnile/index.htm. Accessed 2000 – 2004.

CDC 2007. West Nile virus: vertebrate ecology. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Ft. Collins, Colorado. [Accessed May 12, 2008] http://www.cdc.gov/ncidod/

dvbid /westnile/birdspecies.htm.

CDC 2007. West Nile virus: entomology. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Ft. Collins, Colorado. [Accessed May 12, 2008] http://www.cdc.gov/ncidod/ dvbid/ westnile/mosquitoSpecies.htm.

CDC. 2008. West Nile virus: statistics, surveillance, and control. [Internet] Available from Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Ft. Collins, Colorado. [Accessed May 12, 2008] http:// www.cdc.gov/ncidod/dvbid/westnile/Mapsactivity/surv&control08Maps.htm.

CDHS. 2001. A preliminary assessment of vectors associated with stormwater management structures in the United States. California Department of Health Services, Vector Borne-Disease Section, Sacramento, CA.

CDHS. 2002. A three year assessment of mosquito production in structural best management practices in Southern California. California Department of Health Services, Vector Borne-Disease Section, Sacramento, CA.

CDHS. 2004. California West Nile virus homepage. [Internet] Available from California Department of Health Services, Sacramento, CA. [Accessed November 2004] http://www.westnile.ca.gov/.

Chow E, Wirtz RA, Scott TW. 1993. Identification of blood meals in *Aedes aegypti* by antibody sandwich enzyme-linked immunosorbent assay. *JAm Mosq Control Assoc* 9: 196-205.

Copeland C. 2006. Water Quality: Implementing the Clean Water Act. CSR Repot for Congress. [Internet] Available from National Council for Science and the Environment, Washington, D.C. [accessed March 26, 2008] www.cnie.org/ NLE/CRSreports/ 06Nov/RL33466.pdf .

Corbet PS. 1965. Insect emergence trap for quantitative studies in shallow ponds. *Can Entomol* 97: 845-848.

Corvallis, 2004. City of Corvallis GIS data. [Internet] Available from Corvallis GIS ftp site. [Accessed November 2004] ftp://ftp.ci.corvallis.or.us/pw/gis/.

Crans WJ. 1969. Preliminary observations of frog filariasis in New Jersey. *Proc Ann Conf Bull Wildlife Disease Assoc* 5: 342-347.

Crans WJ. 1970. The blood feeding habits of *Culex territans* Walker. *Mosq News* 30: 445-448.

Dambacher JM, Levins R, Rossignol PA. 2005. Life expectancy change in perturbed communities: derivation and qualitative analysis. *Math Biosci* 197: 1-14.

DeBess E. 2008. 2007 West Nile virus summary report for Oregon. [Internet] Available from Oregon Department of Human Services, Acute and Communicable Disease Prevention, Portland, Oregon. [Accessed May 22, 2008] http://www.oregon.gov/DHS/ph/acd/diseases/wnile/wnv2007rpt.pdf.

DeSalle R, Birstein VJ. 1996. PCR identification of black caviar. *Nature* 381: 197-198.

Desser SS, McIver SB, Ryckman A. 1973. *Culex territans* as a potential vector of *Trypanosoma rotatorium*. I. development of the flagellate in the mosquito. *J Parasitol* 59: 353-358.

Edman JD, and Taylor DJ. 1968. *Culex nigripalpus*: seasonal shift in the bird-mammal feeding ratio in a mosquito vector of human encephalitis. *Science*. 161: 67-68.

Espinosa de los Monteros A, Crarcraft J. 1997. Intergeneric relationships of the new world jays inferred from cytochrome *b* gene sequences. *The Condor* 99: 490-502.

Finnila, S., Lehtonen, M.S., & Majama, K. (2001) Phylogenetic network for European mtDNA. *Am J Human Gen* 68: 1475-1484.

Fonseca DM, Keyghobadi N, Malcolm CA. 2004. Emerging vectors in the *Culex pipiens* complex. *Science*. 303: 1535-1538.

Funk, D. J. & Omland, K. C. (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annu Rev Ecol Syst* 34: 397-423.

Garrett-Jones C. 1964. The human blood index of malaria vector in relation to epidemiological assessment. *Bull World Health Organ* 30: 241-261.

Gingrich JB, Anderson RD, Williams GM, O'Connor L, Harkins K. 2006. Stormwater ponds, constructed wetlands, and other best management practices as potential breeding sites for West Nile virus vectors in Delaware during 2004. *J Am Mosq Control* Assoc. 22: 282-291.

Gjullin CM, Eddy GW. 1972. *The Mosquitoes of the Northwestern United States*. Technical Bulletin No. 1447. United States Department of Agriculture, Washington D.C.

Goddard LB, Roth AE, Reisen WK, Scott TW. 2002. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis* 8: 1385-1391.

Greery PR, Holub RE. 1989. Seasonal abundance and control of *Culex* spp. in catch basins in Illinois. *J Am Mosq Control Assoc*. 5: 537-540.

Hamer, GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, Walker ED. 2008. *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. *J Med Ent* 45: 125-128.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 4: 95-98.

Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL. 2005. Epidemiology and transmission dynamics of West Nile virus disease *Emerg Infect Dis* 11: 1167-1173.

Hebert PDN, Cywinska A, Ball SL and deWard JR. 2003. Biological identifications through DNA barcodes. *Proc R Soc London B Biol Sci* 270: 313-321.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM. 2004. Identification of birds through DNA barcodes. *PLoS Bio* 2: e312.

Irby WS, Apperson CS. 1988. Hosts of mosquitoes in the coastal plain of North Carolina. *J Med Entomol* 25: 85-93.

Kent RJ, Norris DE. 2005. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome b. *Am J Trop Med Hyg* 73: 336-342.

Kilpatrick AM., Kramer LD, Campbell SR, Alleyne EO, Dobson AP and Daszak P. 2005.West Nile virus risk assessment and the bridge vector paradigm. *Emerg Infec Dis* 11: 425- 429.

Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD. 2006. Host heterogeneity dominates West Nile virus transmission. *Proc R Soc B* 273: 2327-2333.

Klee AL, Maldin B, Edwin B, Poshni I, Mostashari F, Fine A, Layton M, and Nash D. 2004. Long-term prognosis for clinical West Nile virus infection. *Emerg Infec Dis* 10: 1405-1411.

Knepper RG, Leclair AD, Strickler JD, Walker ED. 1992. Evaluation of Methoprene (Altosid ® XR) sustained release briquets for control of *Culex* mosquitoes in urban catch basins. *J Am Mosq Control Assoc* 8: 228-230.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC. 1989 Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc Natl Acad Sci U S A* 86: 6196-6200.

Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Bunning M. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis* 9: 311-322.

Kronenwetter-Koepel TA, Meece JK, Miller CA, Reed KD. 2005. Surveillance of above- and below-ground mosquito breeding habitats in a rural Midwestern community: baseline data for larvicidal control measures against West Nile virus vectors. *Clin Med & Res* 1: 3-12.

Kulasekera VL, Kramer L, Nasci RS, Mostashari F, Cherry B, Trock SC, Glaser C, Miller JR. 2001. West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000. *Emerg Infect Dis* 7: 722-725.

Kwan JA, Riggs-Nagy JM, Fritz CL, Shindelbower M, Castro PA, Kramer VL, Metzger ME. 2008. Mosquito Production in Stormwater Treatment Devices in the Lake Tahoe Basin, California. *J Am Mosq Control Assoc* 24: 82-89.

Laird, M. 1988 *The Natural History of Larval Mosquito Habitats*. Academic Press, San Diego, CA.

Lee JH, Hassan HK, Hill G, Cupp EW, Higazi TB, Mitchell CJ, Godsey MS, Unnasch TR. 2002. Identification of mosquito avian-derived blood meals by polymerase chain reaction-heteroduplex analysis. *Am J Trop Med Hyg* 66: 599-604.

Ludt CJ, Schroeder W, Rottman O, Kuehn R. 2004. Mitochondrial DNA phylogeography of red deer (*Cervus elaphus*). *Mol Phylo Evo* 31:1064-1083.

MacDonald G. 1957. *The Epidemiology and Control of Malaria*. Oxford University Press, London.

Malmqvist B, Strasevicius D, Helgren O, Adler PH, Bensch S. 2004. Vertebrate host specificity of wild-caught blackflies revealed by mitochondrial DNA in blood. *Proc R Soc Lond B* 271: S152-S155.

Marshall DB, Hunter MG, Contreras AL. 2006. *Birds of Oregon*. Oregon State University Press, Corvallis.

McCarry MJ. 1996. Efficacy and persistence of Altosid ® Pellets Against *Culex* species in catch basins in Michigan. *J Am Mosq Control Assoc*. 12: 144-146.

Meece JK, Reynolds CE, Stockwell PJ, Jenson TA, Christensen JE, Reed KD. 2005. Identification of mosquito bloodmeal source by terminal restriction fragment length polymorphism profile analysis of the cytochrome b gene. *J Med Entomol* 42**:** 657-667.

Meier R, Shiyang K, Vaidya G, Ng PKL. 2006. DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. *Sys Biol* 55: 715-728.

Metzger ME, Myers CM, Kramer VL. 2003. The "dark side" of stormwater runoff management: Vectors associated with BMPs. *Proc Calif Mosq Vector Control Assoc* 70: 2-10.

Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrink CR. 2006. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, Northeastern United States. *Emerg Infect Dise* 12: 468-474.

Molaei G, Andreadis TG. 2006. Identification of avian- and mammalian-derived bloodmeals in *Aedes vexans* and *Culiseta melanura* (Diptera: Culicidae) and its implication for West Nile virus transmission in Connecticut, U.S.A. *J Med Entomol* 43: 1088-1093.

Molaei G, Andreadis TG, Armstrong PM, Bueno Jr. R, Dennett JA, Real SV, Sargent C, Bala A, Randle Y, Guzman H, Travassos da Rosa A, Wuithiranyagool T, Tesh RB. 2007. Host feeding pattern of *Culex quinquefasciatus* (Diptera: Culicidae) and its role in transmission of West Nile virus in Harris County, Texas. *Am J Trop Med Hyg* 71: 73-81.

Nataraj AJ, Olivos-Glander I, Kusukawa N, Highsmith WE Jr. 1999. Single-strand conformation polymorphism and heteroduplex analysis for gel-based mutation detection. *Electrophoresis* 20: 1177-1186.

Nedelman, J., 1985. Some new thought about some old malaria models. *Math Biosci* 73: 159-182.Niebylski, M. L. and C. L. Meek. 1992. Blood-feeding of *Culex* mosquitoes in an urban environment. *J Am Mosq Control Assoc* 8: 173-177.

Ngo KA, Kramer LD. 2003. Identification of mosquito blood meals using polymerase chain reaction (PCR) with order-specific primers. *J Med Entomol* 40: 215-222.

Nielsen CF, Reisen WK, Armijos MV, MacLachlan NJ, Scott TW. 2008. High subclinical West Nile virus incidence among nonvaccinated horses in Northern California associates with low vector abundance and infection. *Am J Trop Med Hyg* 78: 45-52.

ODHS. 2004. West Nile virus page. [Internet] Available from Oregon Department of Health Services, Acute and Communicable Disease Prevention [Accessed April 2008] http://www.dhs.state.or.us/publichealth/acd/wnile/index.cfm.

Olsen J. City of Corvallis, Personal Communication. 26 March 2008.

Patrican LA, Hackett LE, Briggs JE, McGowan JW, Unnasch TR, Lee JH. 2007. Host-feeding patterns of *Culex* mosquitoes in relation to trap habitat. *Emerg Infect Dis* 13: 1921-1923.

Pfunter AR. 1978. The development and control of *Culex quinquefasciatus* Say and *Culex peus* Speiser in urban catch basins. *Proc & Pap 46th Annul Conf Calif Mosq Control Assoc* 46: 126-129.

Pitt R, Lilburn M, Durrans SR, Burian S, Nix S, Voorhees J, Martinson J. 1999. Guidance Manual for Integrated Wet Weather Flow (WWF) Collection and Treatment Systems for Newly Urbanized Areas (New WWF Systems. [Internet accessed March 26, 2008] Available from http://rpitt.eng.ua.edu/Publications/ BooksandReports/Design%20for%20the%20Future/DesignforFuture.htm.

Reeves WC. 1944. Preliminary studies on the feeding habits of Pacific Coast Anopheles. *Natl Malaria Soc J* 3: 325-331.

Reeves WC, Hammon WM. 1944. Feeding habits of the proven and possible mosquito vectors of Western Equine and St. Louis encephalitis in the Yakima Valley, Washington. *Am J Trop Med* 24: 131-134.

Reeves WC, Tempelis CH, Bellamy RE, Lofy MF. 1963. Observations on the feeding habits of *Culex tarsalis* in Kern County, California, using precipitating antisera produced in birds. *Am J Trop Med Hyg* 12: 929-935.

Reisen WK, Meyer RP, Tempelis CH, Spoehel JJ. 1990. Mosquito abundance and bionomics in residential communities in Orange and Los Angeles Counties, California. *J Med Entomol* 27: 358-367.

Reisen WK, Fang Y, Martinez VM. 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. *J Med Entomol* 42: 367-375.

Reisen WK, Fang Y, Martinez VM. 2006. Vector competence of *Culiseta incidens* and *Culex thriambus* for West Nile virus. *J Am Mosq Cont Assoc* 22: 662-665.

Rey JR, O'Meara GF, O'Connell SM, Cutwa-Francis MM. 2006. Factors affecting mosquito production from stormwater drains and catch basins in two Florida cities. *J Vector Ecol* 3: 334-343.

Ruiz MO, Tedesco C, McTighe TJ, Austin C, and Kitron U. 2004. Environmental and social determinants of human risk during a West Nile virus outbreak in the Greater Chicago area, 2002. *Int J Health Geogr* [Internet] Available at http:// www.ijhealthgeographics.com/ content/3/1/8.

Rush WA, Tempelis CH. 1967. Biology of *Culex tarsalis* during the spring season in Oregon in relation to western encephalitis virus. *Mosq News* 27: 307-315.

Savage HM, Aggarwal D, Apperson CS, Katholi CR, Gordon E, Hassan HK, Anderson M, Charnetzky D, McMillen L, Unnasch EA, Unnasch TR. 2007. Host Choice and Wet Nile virus infection rates in blood-fed mosquitoes, including members of the *Culex pipiens* complex, from Memphis, Tennessee, 2002-2003. *Vector Borne Zoonotic Dis* 7: 365-396.

Scoles GA, Dickson SL, Blackmore MS. 1993. Assesment of *Aedes sierrensis* and a vector of canine heartworm in Utah using a new technique for determining the infectivity rate. *J Am Mosq Cont Assoc* 9: 88-90.

Service MW. 1976. *Mosquito Ecology: Field Sampling Methods*. Applied Science Publishers, Essex, England.

Siegel JP, Novak RJ. 1999. Duration of activity of the microbial larvicide Vectolex CG® (*Bacillus sphaericus*) in Illinois catch basins and waste tires. *J Am Mosq Cont Assoc* 15: 366-370.

Smith CM, Shisler JK. 1981. An assessment of storm water drainage facilities as sources of mosquito breeding. *Mosq News* 41: 226-230.

Smith DL and McKenzie FE. 2004. Statics and dynamics of malaria infection in Anopheles mosquitoes. *Malar J* [Internet] Available at www.malariajournal. com/content/3/1/13.

Smith JL and Fonseca DM. 2004. Rapid assays for identification of members of the *Culex* (Culex) *pipiens* complex, their hybrids and other sibling species (Diptera: Culicidae). *Am J Trop Med Hyg* 70: 339-345.

Sperling FAH, Anderson GS, Hickey DA. 1994. A DNA-base approach to the identification of insect species used for postmortem interval estimation. *J*

Forensic Sci 39: 418-427.

Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP. 2003. A plea for DNA taxonomy. *Trends Ecol Evol* 18**:** 70-74.

Tempelis, C. H., 1975. Host-feeding patterns of mosquitoes with a review of advances in analysis of bloodmeals by serology. *J Med Entomol* 11: 635-653.

Tempelis CH and Reeves WC. 1964. Feeding habits of one Anopheline and three Culicine mosquitoes by the precipitin test. *J Med Entomol* 1: 148-151.

Tempelis, C. H. and R. K. Washino. 1967. Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley Callifornia, with notes on other species. *J Med Entomol* 4: 315-318.

Tempelis CH, Wasino RK. 1967. Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley, California, with notes on other species. *J Med Entomol* 3: 315-318.

Terry J. 2004. Oregon State Public Health Laboratory, Virology/Immunology Section. Personal Communication June 15, 2004.

Theis JH, Kovaltchouk JG, Fujioka KK, Saviskas B. 2000. Vector competence of two species of mosquitoes (Diptera: Culicidae) from Southern California *Dirofilaria immitis* (Filariidae: Onchoceridae). *J Med Entomol* 37: 295-297.

Townzen KR. California Department of Health Services, Vector-Borne Disease Section. Personal communication June 2005.

Townzen JS. 2005, October. Mosquitoes and stormwater management in Corvallis, OR. *44th Ann Conf Northwest Mosq Vector Cont Assoc* Layton, Utah.

Townzen JS, Brower AVZ, Judd DD. 2007, April. Identification of bloodmeals from mosquitoes collected in Corvallis, OR. *Proc 73rd Ann Meeting Am Mosq Cont Assoc*. Orlando, Florida.

Townzen JS, Brower AVZ, Judd DD. In Press. Utility of identifying the sources of mosquito bloodmeals using mitochondrial *cytochrome oxidase subunit I* and *cytochrome b* sequences. *Med Vet Ent* in press.

Turell MJ, Dohm DJ, Sardelis MR, O'Guinn ML, Andreadis TG, Blow JA. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *J Med Entomol* 42: 57-62.

USGS. 2004. West Nile virus maps. [Internet] Available from United States Geological Survey [Accessed November 2004]http://westnilemaps.usgs.gov/.

Vinogradova EB. 2000. *Culex pipiens pipiens Mosquitoes: Taxonomy, Distribution, Ecology, Physiology, Genetics, Applied Importance and Control*. Pensoft Publishing. Sofia, Moscow.

Walters LL, Lavoipierre MMJ. 1984. Landscape epidemiology of mosquito-borne canine heartworm (*Dirofilaria immitis*) in northern California, US: 1. Community-based surveys of domestic dogs in three landscapes. *J Med Entomol* 21: 1-16.

Washino RK and Tempelis CH. 1983. Mosquito host bloodmeal identification: methodology and data analysis. *Annu Rev Entomol* 28: 179:201.

Weitz B. 1956. Identification of bloodmeals of blood-sucking arthropods. *Bull Wld Hlth Org* 15: 473-490.

Wekesa JW, Yuval B, Washino RK, de Vasquez AM. Blood feeding patterns of *Anopheles freeborni* and *Culex tarsalis* (Diptera: Culicidae): effects of habitat and host abundance. *Bull Entomol Res* 87: 633-641.

Wheeler DL, Chappey C, Lash AE, Leipe DD, Madden TL, Schuler GD, Tatusova TA, Rapp BA 2000. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 28: 10-14.

Workman PD, Walton WE. 2000. Emergence patterns of *Culex* mosquitoes at an experimental constructed treatment wetland in Southern California. *J Am Mosq Cont Assoc* 16: 124-130.

WRBU. 2005. 2001 Systematics Catalog of the Culicidae [Internet] Available from Walter Reed Biosystematics Unit [Accessed November 2005] http://www.mosquitocatalog.org/main.asp.

WHO. 2004. Integrated Vector Management. World Health Organization Regional Office for the Eastern Mediterranean. Cairo.

Will KW, Rubinoff D. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* 20: 47-55.

Will KW, Mishler BD, Wheeler QD. 2005. Perils of DNA barcoding and the need for integrative taxonomy. *Sys Bio* 54: 844-8.

Zavaleta JO and Rossignol PA. 2004. Community level analysis of risk of vector-borne disease. *Trans R Soc Trop Med Hyg* 98: 610-618.

Zohrabian A, Meltzer MI, Ratard R, Kaafee B, Molinari NA, Kakoli R, Scott II RD, Peterson L. 2004. West Nile virus economic impact, Louisiana, 2002. *Emerg Infec Dis* 10: 1736-1744.