PNWD-3520

Analysis of Conditions: Potential for Changing Mosquito Population Dynamics at the Willapa Bay Restoration Project Site in Light of West Nile Virus

S.L. Sargeant
L.K. O'Rourke
M.L. Blanton

Battelle Marine Sciences Laboratory
Sequim, Washington

May 2005

Prepared for
U.S.D.A Natural Resources Conservation Service
Under U.S. Army Corps of Engineers (Seattle District)
Contract DACW57-00-D-009
Battelle Contract 46613

Battelle, Pacific Northwest Division
of Battelle Memorial Institute

Battelle
The Business of Innovation

LEGAL NOTICE

This report was prepared by Battelle as an account of sponsored research activities. Neither Client nor Battelle nor any person acting on behalf of either:

MAKES ANY WARRANTY OR REPRESENTATION, EXPRESS OR IMPLIED, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, process, or composition disclosed in this report may not infringe privately owned rights; or

Assumes any liabilities with respect to the use of, or for damages resulting from the use of, any information, apparatus, process, or composition disclosed in this report.

Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by Battelle. The views and opinions of authors expressed herein do not necessarily state or reflect those of Battelle.

This document was printed on recycled paper.

PNWD-3520

ANALYSIS OF CONDITIONS: POTENTIAL FOR CHANGING MOSQUITO POPULATION DYNAMICS AT THE WILLAPA BAY RESTORATION PROJECT SITE IN LIGHT OF WEST NILE VIRUS

Susan L. Sargeant
Lohna K. O'Rourke
Michael L. Blanton

Battelle Marine Sciences Laboratory
Sequim, Washington

May 2005

Prepared for
U.S.D.A. Natural Resources Conservation Service
Under U.S. Army Corps of Engineers (Seattle District)
Contract DACW57-00-D-009
Battelle Contract 46613

Battelle Memorial Institute
Pacific Northwest Division
Richland, Washington 99352

EXECUTIVE SUMMARY

Full tidal restoration is proposed for a 300-acre estuarine marsh through removal of 10,000 linear feet of existing dike along the Willapa River. Additionally, enhancement of 120 acres of freshwater wetlands is proposed through creation of waterfowl and amphibian ponds. The City of South Bend, Washington, situated approximately 1.5 miles upstream from the proposed restoration site, already experiences nuisance mosquito problems. The city's proximity to many natural mosquito production areas that are enhanced by tidal inundation and rainfall means that residents of the City of South Bend will continue to experience high numbers of mosquitoes regardless of the proposed restoration project. This study details an investigation of how alteration of restoration site hydrology could change mosquito species composition, distribution, and abundance in the region, with an emphasis on the potential for increased exposure to West Nile virus.

Relatively low ground surface elevations of the estuarine portion of the site mean that most of the area should be inundated daily. This factor, in combination with proposed management actions of filling low areas and incorporating drainage through excavation of remnant slough channels, will remove almost 300 acres from mosquito production while it transitions from pasture to salt marsh habitat. As the salt marsh matures, mosquito species and numbers are expected to be similar to what exist currently within the diked area. Current designs for shallow, seasonal freshwater wetlands are likely to increase freshwater mosquito production at the site. *Culex pipiens*, a confirmed West Nile virus vector in other regions of the county, comprised 35% of mosquitoes trapped at existing freshwater habitats on the restoration property. Overall, the baseline probability that a resident of the City of South Bend would contract WN virus is very small (there was less than a 0.4% chance of becoming infected with the virus in the states with human cases reported during 2002 [O'Leary et al. 2004]). The expected increase in freshwater mosquitoes as a result of the restoration project could increase the potential vector population by a small amount (relative to the large existing mosquito population already found in the City of South Bend), and thereby incrementally increase the chance for human/WN virus-vector encounters if these mosquitoes travel to South Bend. However, the overall impact of the restoration project will be extremely small and would most likely represent an insignificant increase in potential risk. The extent of the increase in mosquitoes and the subsequent potential to impact residents of the City of South Bend depends, in large part, on how water is managed in the freshwater wetland ponds.

ACKNOWLEDGMENTS

Many thanks are in order for the help that was provided us related to this project. Karlee Rust assisted with the field work during her internship. Information on where to find (and even the provision of) relevant mosquito keys for Washington was given by Karen Ripley, Forest Entomologist, Washington Department of Natural Resources, and Dr. Eric LaGasa, State Entomologist, Washington State Department of Agriculture. Dr. Ron Stecker, Sequim, Washington, gave a hands-on mosquito anatomy lesson and provided advice on proper mounting techniques for specimens. Terry Whitworth, of Whitworth Pest Control, provided local information on mosquito species and current control methods in the City of South Bend, Washington. Dr. William Walton provided invaluable advice on the methods of mosquito sampling appropriate for the study site and project goals, authored a great deal of literature relevant to the subject of created wetlands and mosquito production, keyed our voucher specimens, and graciously reviewed the report. Additional reviewers included Dr. Ron Thom and Dr. Chris May, Battelle Northwest Division. Lee Miller (Battelle) created the maps and the wind rose. And finally, appreciation to our colleagues at Battelle who allowed us to raise mosquitoes under their noses, without complaint. Thanks for the confidence in our abilities to keep them under control!

ACRONYMS

CDC	Centers for Disease Control
CE	California encephalitis
CHS	Canadian Hydrographic Service
Corps	U.S. Army Corps of Engineers
CPUE	catch per unit effort
DU	Ducks Unlimited, Inc.
FW	Freshwater
GPS	global positioning system
ID	Inside Dike
JBE	Japanese B encephalitis
MF	Mudflat
MLLW	mean lower low water
mph	miles per hour
NEPA	National Environmental Policy Act
NOAA	National Oceanic and Atmospheric Administration
NRCS	U.S. Department of Agriculture Natural Resources Conservation Service
OD	Outside Dike
ppt	part per thousand
RNA	ribonucleic acid
SLE	St. Louis Encephalitis
USFWS	U.S. Department of Interior Fish and Wildlife Service
UTMs	Universal Transverse Mercator units
WDFW	Washington Department of Fish and Wildlife
WDOH	Washington State Department of Health
WEE	Western Equine Encephalitis
WN	West Nile
WRCC	Western Regional Climate Center
WRP	Wetland Reserve Program
WSDOT	Washington State Department of Transportation

Referenced Species of Vegetation

Scientific Name	Common Name
Agrostis spp.	pasture grass
Atriplex patula	saltweed
Carex lyngbyei	Lyngby sedge
Cirsium spp.	thistle
Convolvulus spp.	morning glory
Deschampsia cespitosa	tufted hairgrass
Distichlis spicata	saltgrass
Elocharis spp.	spike rush
Epilobium angustifolium	fireweed
Juncus effusus	soft rush
Lemna minor	duckweed
Lotus corniculatus	bird's foot trefoil
Phalaris arundinacea	reed canarygrass
Potentilla pacifica	Pacific silverweed
Pteridium aquilinum	bracken fern
Rubus spp.	blackberry
Salicornia virginica	pickleweed
Scirpus maritimus	saltmarsh bulrush
Solanum dulcamaria	bittersweet nightshade
Spartina alterniflora	salt marsh grass
Triglochin maritmum	seaside arrowgrass
Typha latifolia	common cattail
Zostera japonica	seagrass (non-native)

CONTENTS

Executive Summary i

Acknowledgements ii

Acronyms iii

Referenced Species of Vegetation iv

1.0 Introduction 1

2.0 Materials and Methods 4

2.1 Characterization of Existing Mosquito Conditions in and around the City of South Bend 4
2.2 Identification of Mosquito Species and Associated Habitat 4
2.2.1 Site Selection and Characteristics 4
2.2.2 Mosquito Sampling and Identification Techniques 26
2.2.3 Timing 27
2.2.4 Species Identification 27
2.2.5 Supplemental Data Collection 28
2.3 Predicting Mosquito Population Dynamics and the Potential to Alter Exposure of South Bend Residents to the West Nile Virus Following Restoration 29

3.0 Results 30

3.1 Characterization of the Existing Conditions for Mosquito Breeding in and around the City of South Bend 30
3.2 Identification of Mosquito Species and Associated Habitat 34
3.2.1 Trapped Mosquitoes 35
3.2.2 Larval Dip Samples 39
3.2.3 Net Samples 39
3.2.4 Timing 39
3.3 Summary of Results by Site 41
3.3.1 Sampling Sites Associated with the City of South Bend 41
3.3.2 Willapa River Restoration Site Sampling Stations 42
3.3.3 Natural Salt Marsh Reference Site and Previously Restored Salt Marsh Sampling Stations 44
3.4 Supplemental Data 46
3.4.1 Precipitation 46
3.4.2 Tidal Cycles 48
3.4.3 Temperature 49
3.4.4 Wind Speed and Direction 51

4.0 Discussion and Recommendations 52

4.1 West Nile Virus and Potential Mosquito Vectors in Pacific County, Washington 52
4.1.1 West Nile Virus 52
4.1.2 Potential Mosquito Vectors in Pacific County, Washington 55
4.2 Predictions of Mosquito Population Dynamics and the Potential to Alter Exposure of South Bend Residents to the West Nile Virus Following Restoration 63
4.2.1 Impacts of Estuarine Habitat Restoration 63
4.2.2 Impacts of Freshwater Habitat Restoration 69

4.2.3 Combined Project Impact on Mosquito Species and Abundance in the City of South Bend 72
4.3 Final Recommendations 73
5.0 References 75

FIGURES

1. Vicinity map showing the City of South Bend, Washington, and the proposed Willapa River estuarine restoration project site 2
2. Detailed view of the proposed Willapa River estuarine restoration project site. 3
3. Mosquito trapping and larval dipping locations at the proposed Willapa River estuarine restoration site and near the City of South Bend, Washington, 2004. 5
4. Detailed map indicating locations of mosquito trap and larval dip sampling stations at the Willapa River restoration site and at Potter Slough, 2004. 6
5. Overview of all trapping and larval dipping locations in Pacific and Grays Harbor Counties, Washington, 2004. 7
6. Wastewater Treatment Plant site at high water (~ +10 ft MLLW). 8
7. Wastewater larval dip areas. 9
8. Wastewater Treatment Plant trap. 9
9. South Bend Alder House trap. 10
10. Pond sampled for mosquitoes near the Alder House facility. 11
11. Outside Dike trap. 12
12. Outside Dike larval dip location relative to the trap. 12
13. Mudflat trap. 13
14. Inside Dike trap. 15
15. Inside Dike-Dike larval dip sampling location. 16
16. Inside Dike-East borrow ditch: larval dip sampling location. 17
17. Inside Dike-West borrow ditch: larval dip sampling location. 17
18. Freshwater trap. 18
19. Freshwater-East borrow ditch: larval dip sampling location. 19
20. Freshwater-West borrow ditch: larval dip sampling location. 19
21. Potter Slough trap. 21
22. Potter Slough larval dip sampling site (foreground), and slough channels (background). 22
23. North River restoration site. 22
24. North River trap. 23
25. North River larval dip areas. 23

26. Elk River trap. 24
27. Low-lying larval dip areas near the Elk River trap. 25
28. Elk River site covered with water at high tide (+10 ft MLLW). 25
29. Trapped adult mosquitoes. 27
30. Abandoned tires and other debris located behind the local high school stadium, capable of providing breeding habitat for mosquito species that utilize artificial containers. 31
31. Period of Record Precipitation Summary (1980 to 2004), Raymond, Washington 32
32. Maximum Tide Elevations (Feet Mean Lower Low Water) on the Willapa River, South Bend. 33
33. Summary of wind direction for the period of May-October, 2004, measured at Hoquiam, Washington. 33
34. Overall percentage of composition of mosquito genera captured using CDC traps. 35
35. Number of mosquitoes at a trap site per hour the trap was operational. 36
36. Average mosquitoes captured per hour, by species, at each trap site per hour the trap was operational 36
37. Site-wise comparison of trapped mosquito genera; percentage of composition. 37
38. Number of mosquitoes during a sampling trip per hour the trap was operational, all sites combined. 37
39. Mosquitoes captured per operational trap hour during each site visit and at each sampling site 38
40. Mosquito larvae and pupae in a roadside pool at the Wastewater Treatment Plant. 41
41. Dry pool at North River that contained dead mosquito larvae and threespine sticklebacks. 45
42. A comparison of monthly average precipitation for Year 2004 and period of record (1980-2004) for Raymond, Washington. 47
43. A comparison of daily precipitation for Year 2004 and daily averages for the period of record (1980 – 2004) for Raymond, Washington. 47
44. Maximum (high) and minimum (low) tides and lunar phase for the Willapa River at South Bend during mosquito sampling period. 48
45. Maximium (high) and minimum (low) tides for the Willapa River at South Bend duringYear 2004. 49
46. A comparison of monthly average temperatures for Year 2004 and for the period of record (1980 to 2004) for Raymond, Washington. 50
47. A comparison of daily average temperatures for the Year 2004 sampling period and for the period of record (1980-2004) for Raymond, Washington. 51
48. Mosquito species found during the sampling period or considered likely West Nile virus vectors in Western Washington organized into four functional groups based on four biological characteristics. 57
49. Comparative tidal inundation of Potter Slough at +10 ft MLLW and at approximately +3.0 ft MLLW, 2004. 68

TABLES

1. Mosquito sampling by site and date (2004) 29

2. Habitat types sampled during this study with their associated mosquito species, and the relative abundance and reported potential of these species to carry and transmit West Nile virus. 34

3. Positively identified mosquito species (or genera) according to site and sampling method..... 40

4. Maximum and minimum air temperatures recorded at Raymond, Washington, during mosquito collection dates in 2004..... 50

5. Wind speed summary for Year 2004 measured at Hoquiam, Washington. 52

6. Biological data on mosquito species found during sampling period or considered likely West Nile virus vectors in Western Washington. 56

7. Mosquito Species Documented in Pacific County by the Washington State Department of Health and from this Study 58

1.0 INTRODUCTION

During the late 1960s and early 1970s, the disposal of dredged material in diked intertidal mudflat and wetland habitat adjacent to the Federal Willapa River Navigation Channel by the U.S. Army Corps of Engineers (Corps) resulted in the loss of several hundred acres of productive estuarine habitat. To recover this lost habitat, the U.S. Department of Agriculture Natural Resources Conservation Service (NRCS), the Washington Department of Fish and Wildlife (WDFW), and other agencies are currently working together to restore estuarine habitat and function along the lower Willapa River[1]. The proposed estuarine restoration project site is located on the left (south) bank of the Willapa River approximately 1.5 miles (2.4 km) downstream of the City of South Bend in Pacific County, Washington (Figure 1). The U.S. Department of Agriculture Natural Resources Conservation Service (NRCS) obtained Warranty Easement Deeds from five landowners through the Wetland Reserve Program[2]. Following the establishment of the wetland easements, all landowners sold their fee title to WDFW. Through this process, the WDFW and NRCS acquired approximately 400 acres of diked lands and approximately 35 acres of adjoining undiked tidelands. The Corps was also involved with project development under their authority pursuant to Section 1135 of the Water Resources Development Act, which is a habitat restoration authority. The Corps acted as the lead Federal agency under NEPA and prepared an Environmental Assessment and Finding of No Significant Impact (FONSI) (February 2005). Due to national budget constraints, the Corps was unable to continue their partnership on the project beyond February 2005. However, all work provided by the Corps, including engineering documents and environmental reports, remain part of the project record.

Approximately 10,000 linear feet of existing dike along the Willapa River would be removed to achieve the project restoration goals. Inlets to five primary slough channels on the riverside of the existing dike would be excavated. On the landward side of the existing dike, most of the existing borrow ditches would be filled, and the five primary slough channels and three secondary channels would be restored through excavation to allow for natural tidal hydrology and fish access to the interior of the site. Completion of the project as proposed would result in the inundation of approximately 300 acres of land on the northeast (river) side of U.S. 101. (Figure 2, U.S. Army Corps of Engineers Project Area).

In addition, the project would enhance approximately 120 acres of freshwater habitat through grading to create three freshwater wetland cells and six amphibian ponds on the southwest (landward) side of U.S. 101 and through enhancement of waterfowl habitat on property located immediately downstream of the WDFW property on the northeast side U.S. 101 (Figure 2, Ducks Unlimited Project Areas). For the purposes of this report, wetland enhancement is defined as the modification or rehabilitation of an existing or degraded wetland, where specific functions and/or values are improved for the purpose of meeting specific project objectives. Some functions may remain unchanged while others may be degraded (NRCS 2005).

Cooperating agencies include the NRCS, the Washington State Department of Transportation (WSDOT), the U.S. Department of Interior Fish and Wildlife Service (USFWS), and Ducks Unlimited, Inc. (DU). The project is described in detail in the *Willapa River Section 1135 Estuarine Habitat Restoration Project, Pacific County, Washington: Final Environmental Assessment* (USACE 2005).

---

[1] The U.S. Army Corps of Engineers was a design partner for the proposed project, but could not secure funding in 2005 to continue to support project construction. The Corps' lack of participation does not change the design or intent of the project.

[2] http://www.nrcs.usda.gov/programs/wrp/

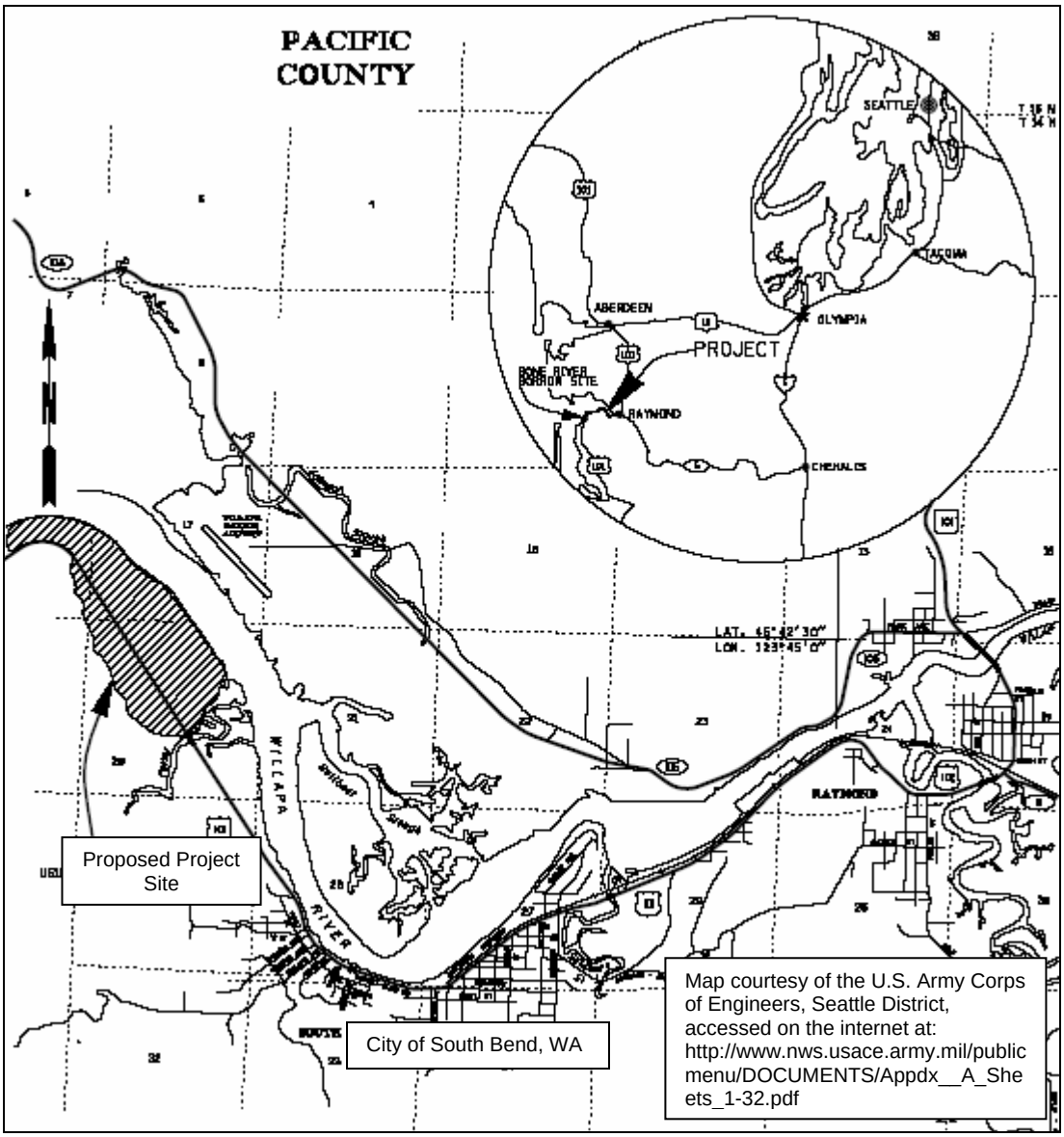
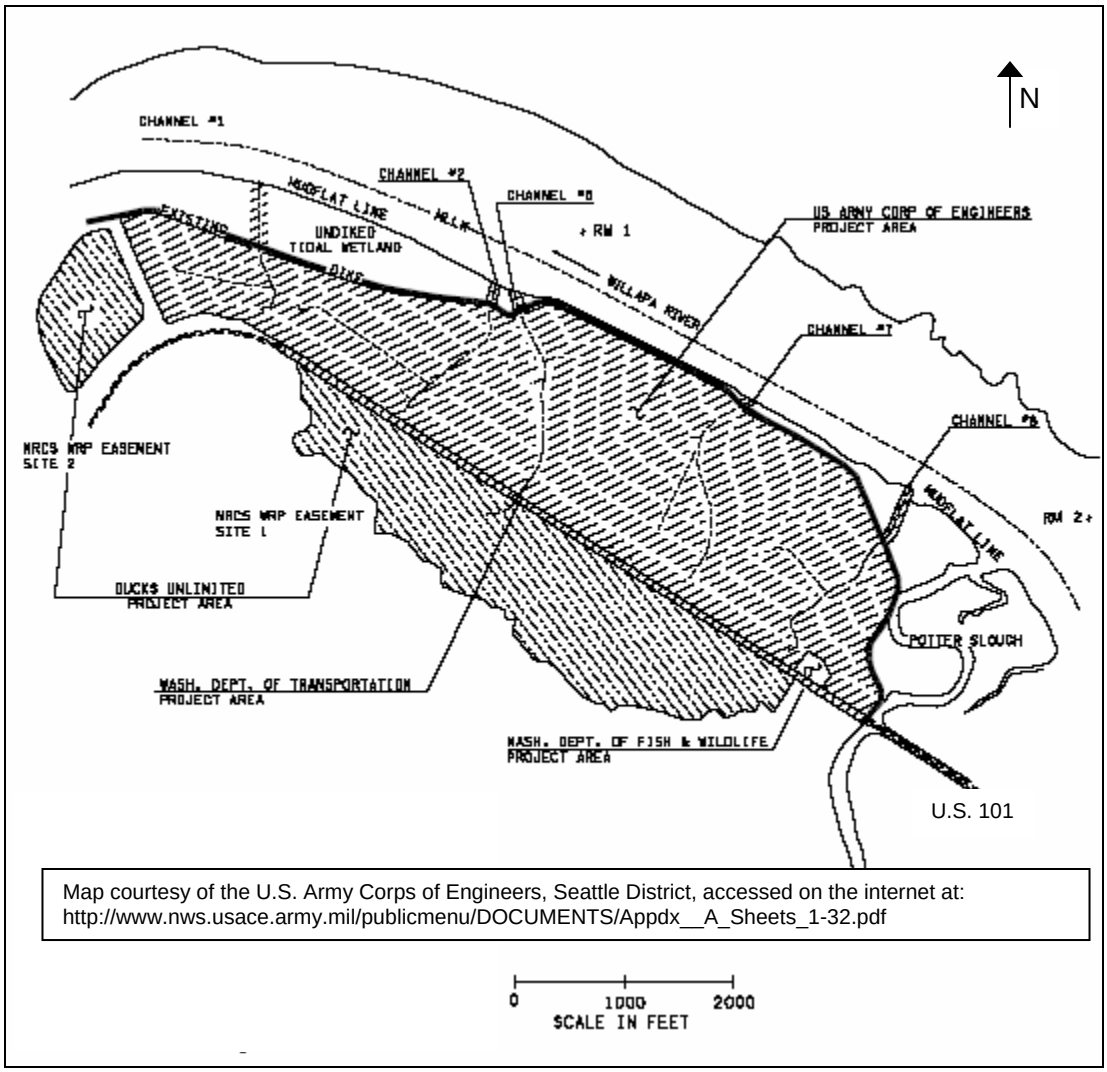


Figure 1. Vicinity map showing the City of South Bend, Washington, and the proposed Willapa River estuarine restoration project site.



Map courtesy of the U.S. Army Corps of Engineers, Seattle District, accessed on the internet at: http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/Appdx__A_Sheets_1-32.pdf

Figure 2. Detailed view of the proposed Willapa River estuarine restoration project site.

When the potential for significant environmental impact resulting from the restoration project is unknown, the National Environmental Policy Act (NEPA) requires federal entities, such as the Corps, to prepare an Environmental Assessment. The Corps prepared and issued a Draft Environmental Assessment (USACE 2002) that described the proposed restoration project and alternatives, and also presented an analysis of the associated potential environmental impacts. Several comments on the proposed restoration project were received during the official comment period and through the public hearing process regarding the possibility that the project would result in increased habitat for mosquito vectors of West Nile (WN) virus.

This report addresses the public's concern about mosquitoes and the potential for the restoration project to increase the exposure of City of South Bend residents to the WN virus. WN virus is a potentially serious illness. Experts believe WN virus is established as a seasonal epidemic in North America that flares up in the summer and continues into the fall (CDC 2005). Most often, WN virus is spread by the bite of an infected mosquito. Mosquitoes are WN virus carriers (vectors) that become infected when they feed on infected birds. Infected mosquitoes can then spread WN virus to humans and other animals when they bite. Mosquitoes can be infected with the virus, and can then transmit the virus, at different rates or

competence levels. The competence of individual mosquito species can be determined in a laboratory setting, and has been ranked for some species as low, moderate, or high. Because the restoration project will alter the hydrology of the site, the mosquito community is expected to shift relative to the life histories and habitat requirements of particular mosquito species found in the region. Information about the abundance and distribution of mosquito species at the project site and in the City of South Bend was extremely limited prior to this study. Therefore, the goals of this research were to 1) characterize the existing conditions for mosquito breeding in the South Bend area, 2) identify the mosquito species that currently use the proposed mitigation site and associate them with particular habitat types through field sampling, and 3) evaluate how these mosquito populations could change following restoration and alter the potential exposure of South Bend residents to the WN virus.

2.0 MATERIALS AND METHODS

2.1 Characterization of Existing Mosquito Conditions in and around the City of South Bend

A number of methods were employed to characterize existing mosquito conditions in and around the City of South Bend. These included 1) adult mosquito trapping and larval dipping, 2) a one-time examination of the contents of Mosquito Magnets® owned by the City, 3) interviews with mosquito control personnel employed by the City of South Bend and conversations with City of South Bend residents, 4) visual observations made and recorded by the researchers while driving and walking around the City, and 5) acquisition of local and regional climatological data.

2.2 Identification of Mosquito Species and Associated Habitat

Following conversations with the sponsor to refine the goals of the research, a preliminary survey of the proposed restoration site and the surrounding area was conducted in July 2004. The preliminary site visit enabled the researchers to locate various habitat types at the proposed restoration site and make decisions regarding mosquito sampling stations, both at the restoration site and at other locations that would further the purpose of making predictions of mosquito species composition and abundance following restoration.

2.2.1 Site Selection and Characteristics

Two sites were sampled to provide documentation of mosquito species present in and around the City of South Bend (Figure 3). These were the Wastewater Treatment Plant and South Bend Alder House sites. Four mosquito trap sites were selected to represent the variety of habitats existing on the proposed Willapa River restoration site (Figure 3 and Figure 4). These include the Outside Dike, Mudflat, Inside Dike, and Freshwater sites. Three sites were sampled to represent possible future conditions of the site as it progresses through various stages of restoration and recovery (Figure 5). These included the natural salt marsh reference site at Potter Slough, as well as two previously restored salt marsh sites at Elk River and North River. When present, standing water near the trap sites was sampled for mosquito larvae. Brief descriptions of the trap and larval dip sites are presented below. Global positioning system (GPS) coordinates are provided in UTMs.

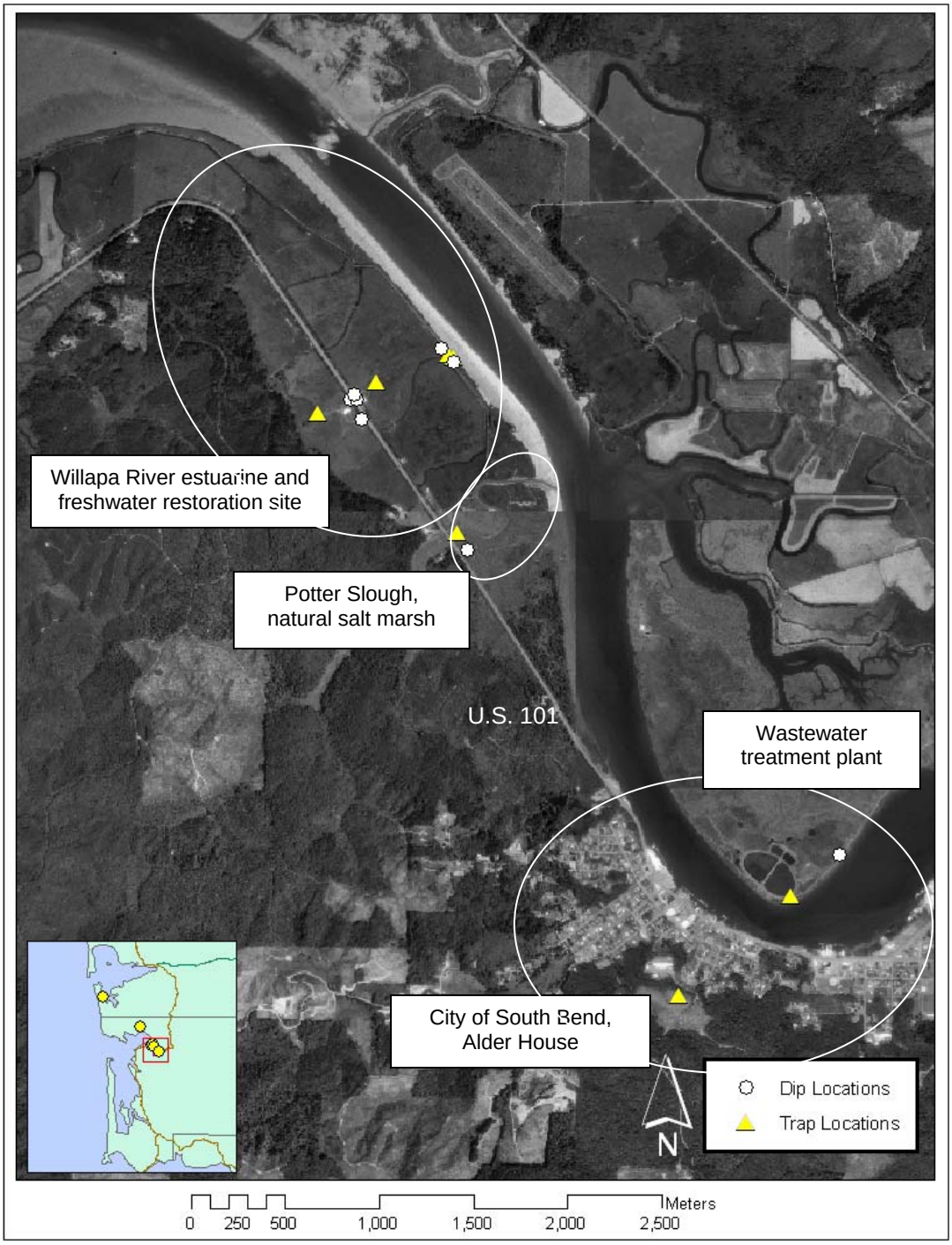


Figure 3. Mosquito trapping and larval dipping locations at the proposed Willapa River estuarine restoration site and near the City of South Bend, Washington, 2004.

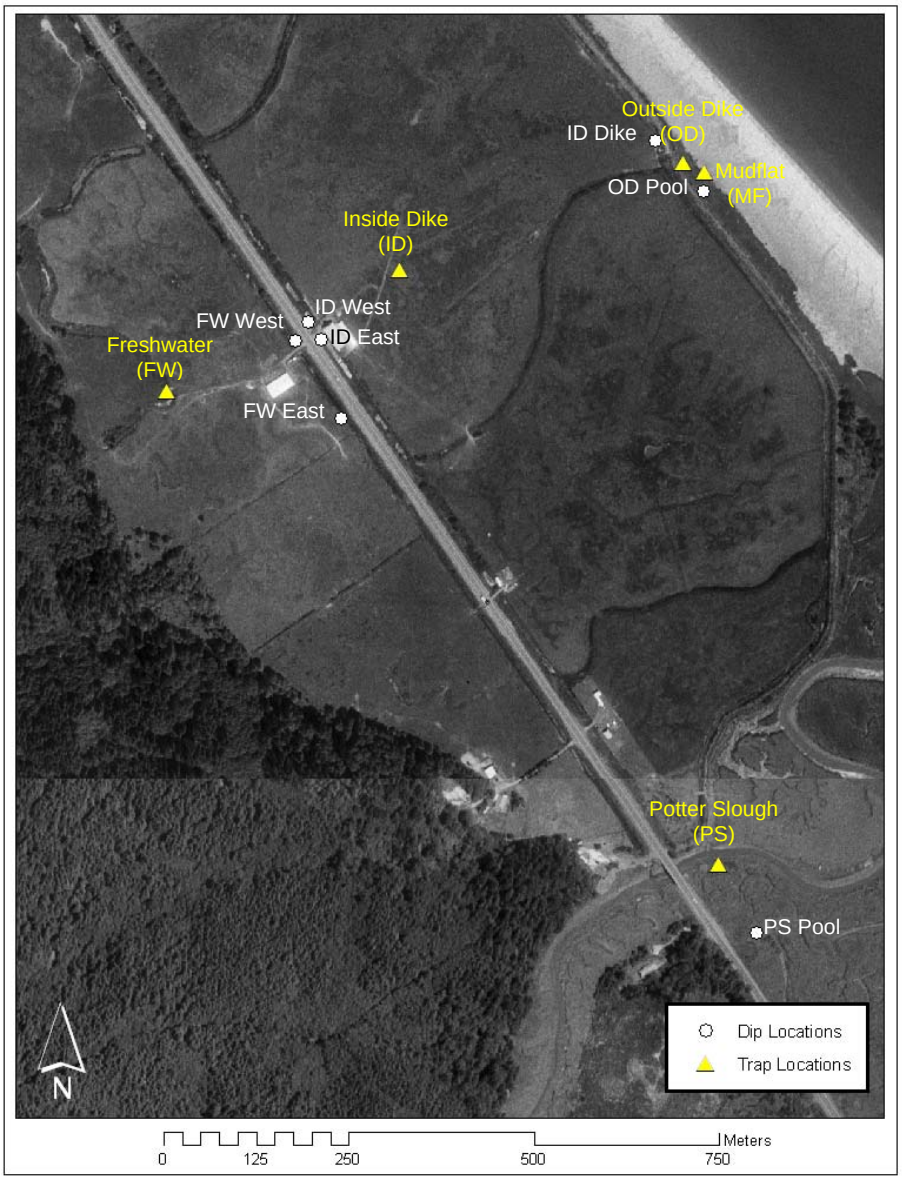


Figure 4. Detailed map indicating locations of mosquito trap and larval dip sampling stations at the Willapa River restoration site and at Potter Slough, 2004.

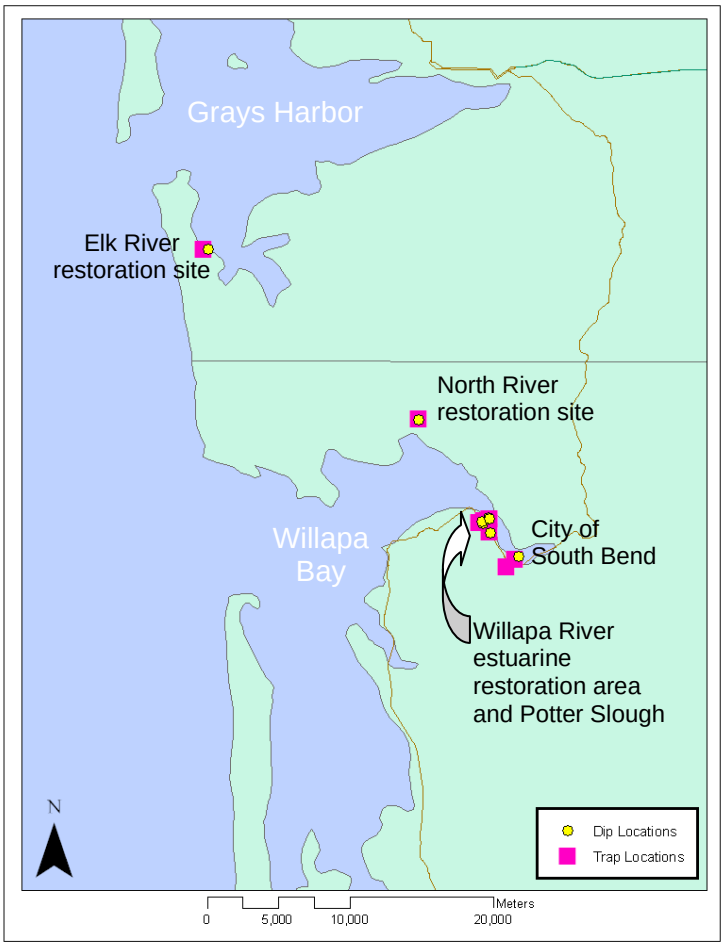


Figure 5. Overview of all trapping and larval dipping locations in Pacific and Grays Harbor Counties, Washington, 2004.

2.2.1.1 Sampling Sites Associated with the City of South Bend, Washington

2.2.1.1.1 Wastewater Treatment Plant (WW)

Trap and Larval Dip under trap: 10T 438543 5168389
Larval Dip Roadside (example): 10T 438796 5168608

The wastewater treatment plant for the City of South Bend is located directly across the Willapa River from the city center (Figure 3). The wastewater site is reputed to be a prime source of mosquito production (Terry Whitworth, personal communication). Hence, this site was selected to assist us in characterizing existing mosquito breeding habitat in and around the City of South Bend.

At extreme high tides, the plant is essentially on an island and is inaccessible except by boat (Figure 6). During most of the year, the site is accessible by a private road that skirts the river's edge. Tides periodically inundate the road, leaving small pools of standing saline water along the roadside (Figure 7).

These areas were sampled for larval mosquitoes. Vegetation associated with these roadside pools included soft rush *(J. effusus),* seaside arrowgrass *(T. maritima)* and saltgrass *(D. spicata).*

The trap was placed adjacent to the treatment pond where it was directly across the river from the City of South Bend. This site was on high ground and was never inundated by the tide (Figure 8). However, a low spot under the trap collected rainwater during the latter part of our study and was also sampled for the presence of larval mosquitoes. Cows were allowed to roam most of the area outside the fence surrounding the treatment pond and were observed on every site visit. On early site visits, the cows were observed inside the treatment pond fence, and before the fence was repaired, even circled our trap, as evidenced by a number of hoof prints. Vegetation near the trap included soft rush *(J. effusus)* and blackberry *(Rubus* spp.*).*

Figure 6. **Wastewater Treatment Plant site at high water (~ +10 ft MLLW).**

Figure 7. **Wastewater larval dip areas.**

Figure 8. **Wastewater Treatment Plant trap.**

2.2.1.1.2 South Bend – Alder House (SBA)

Trap: 10T 437943 5167860

The Alder House is an assisted living facility located above the City of South Bend, next to the Willapa Harbor Hospital (Figure 3). Because the elderly are most susceptible to WN virus, we wanted to sample the existing mosquito population in a place where seniors are likely to be concentrated. Like the Wastewater Treatment Plant site, the Alder House Site was selected to provide data in support of our overall characterization of existing conditions in and around the City of South Bend. The trap was located above the Alder House where it would be out of sight of the residents (Figure 9). Vegetation surrounding the trap included blackberry *(Rubus* spp.*),* morning glory *(Convolvulus* spp.*),* bracken fern *(Pteridium aquilinum),* and an unidentified grass. There was no standing water near the trap site during the sampling period. A surface water runoff retention pond next to the driveway entrance to Alder House was filled with water during our last site visit (Figure 10). It was sampled for larval mosquitoes, but no coordinates were recorded to document its exact location.

Figure 9. **South Bend Alder House trap.**

Figure 10. **Pond sampled for mosquitoes near the Alder House facility.**

2.2.1.2 Willapa River Restoration Site Sampling Stations

The sampling stations at the proposed restoration site included Outside Dike (OD), Mudflat (MF), Inside Dike (ID), and Freshwater (FW), as shown in Figure 5.

2.2.1.2.1 Outside Dike (OD)

Trap: 10T 436719 5171264
Larval Dip: 10T 436747 5171226

This site consisted of tidal marsh associated with the proposed restoration site and adjacent to the Willapa River. It is outside (northeast of) the existing dike (Figure 4 and Figure 11). The area is completely inundated during high tides (those generally above 8.5 ft mean lower low water [MLLW]) and contains salt marsh vegetation. Plants observed in the vicinity of the trap included saltgrass *(Distichlis spicata),* pickleweed *(Salicornia virginica),* seaside arrowgrass *(Triglochin maritmum)*, tufted hairgrass *(Deschampsia cespitosa)* and isolated patches of reed canarygrass *(Phalaris arundinacea).* Vegetation on the dike itself consisted primarily of blackberry (*Rubus* spp). No sedge (*Carex* spp.) were present. A pool of standing water approximately 26 ft x 33 ft (8 m x 10 m) was present during every site visit (Figure 12). Vegetation bordering the pool consisted of pickleweed, saltgrass, and seaside arrowgrass. The pool was sampled for larval mosquitoes during each sampling visit. Like the reference site at Potter Slough (see description below), this site could be used to indicate probable mosquito use of the restored salt marsh area at its maturation.

Figure 11. Outside Dike trap.

Figure 12. Outside Dike larval dip location relative to the trap.

2.2.1.2.2 Mudflat (MF)

Trap: 10T 436748 5171252

We placed a trap in the mudflat along the south bank of the Willapa River, approximately 25 ft (8 m) offshore from the vegetated bank and 50 ft (15 m) from the Outside Dike trap site (Figure 4 and Figure 13). There was very little vegetation near the trap; only a non-native seagrass *(Zostera japonica)* and a small clump of the exotic salt marsh species, *Spartina alterniflora.* There was very little standing water on the mudflat, so larval dipping was not performed in association with this trap site. Because the plant species that currently exist inside the diked area northeast of U.S. 101 will not be able to tolerate the daily inundation and increased salinity that will result following dike removal, there will be a transition period during which the existing plants die off and are replaced by salt-tolerant species. During this transition, bare areas of mudflat are expected to dominate the site for several years (Thom et al., 2002). Thus, sampling mosquitoes over the mudflat provides some estimation of what to expect in mosquito species and relative abundance during the first few years following dike removal.

Figure 13. Mudflat trap.

2.2.1.2.3 Inside Dike (ID)

Trap: 10T 436337 5171121
Larval Dip near dike borrow ditch: 10T 436683 5171296
Larval Dip northeast of U.S. 101, east side borrow ditch: 10T 436231 5171027
Larval Dip northeast of U.S. 101, west side borrow ditch: 10T 436213 5171051

This site was chosen to represent that part of the existing restoration site located inside the dike (Figure 4 and Figure 14). This area is covered with native pasture grasses (e.g., *Agrostis* spp.*)* intermixed with rushes and sedges. Plant species observed in the vicinity of the trap site included soft rush *(Juncus effusus),* spike rush *(Elocharis* spp.*)*, salt marsh bulrush *(Scirpus maritimus)*, thistle *(Cirsium* spp.*)*, bird's foot trefoil *(Lotus corniculatus)*, and fireweed *(Epilobium angustifolium)*. Mosquito species and their relative abundance at this site will be compared with sites that represent future stages of salt marsh succession to determine the likely changes in species dynamics as this area reverts to salt marsh. Although it is, in its current condition, more representative of a freshwater habitat than a salt marsh habitat, the Inside Dike trap site did not have standing water nearby and, therefore, is not used to represent future conditions of the freshwater habitat that will be enhanced on the south side of U.S. 101.

Three sections of borrow ditch on the north side of U.S. 101 were sampled for mosquito larvae on various site visits. These samples were mostly freshwater and are used to represent future conditions of the proposed freshwater habitat that will be enhanced on the south side of U.S. 101. The borrow ditch nearest the dike (ID-Dike, approximately 33 ft (10 m) northwest of the dike overpass used to access the OD trap site) was always full of water covered with algae (Figure 4 and Figure 15). Sections of the borrow ditch nearest U.S. 101 on either side of the vehicle access point were only periodically filled with water (ID-East [Figure 16] and ID-West [Figure 17]). Vegetation associated with this roadside ditch included small duckweed (*Lemna minor*), common cattail (*Typha latifolia*), bittersweet nightshade (*Solanum dulcamaria*), and reed canary grass (*P. arundinacea).*

Figure 14. Inside Dike trap.

Figure 15. Inside Dike-Dike larval dip sampling location.

Figure 16. Inside Dike-East borrow ditch: larval dip sampling location.

Figure 17. Inside Dike-West borrow ditch: larval dip sampling location.

2.2.1.2.4 Freshwater (FW)

Trap and Larval Dip near Trap: 10T 436023 5170957
Larval Dip southwest of U.S. 101, east side borrow ditch: 10T 436259 5170920
Larval Dip southwest of U.S. 101, west side borrow ditch: 10T 436197 5171026

A trap was placed on the southwest (landward) side of U.S. 101, which in general consists of mixed vegetation similar to that associated with the Inside Dike trap station (Figure 4 and Figure 18). The trap, however, was intentionally placed near two adjacent depressional areas that hold standing water during a significant part of the year, as evidenced by the thick stand of common cattail (*T. latifolia*) within each area. Plant species near the trapping station were somewhat different from those found near the Inside Dike trap and, besides cattail, included blackberry (*Rubus* spp.), reed canarygrass *(P. arundinacea),* thistle *(Cirsium* spp.*)*, bird's foot trefoil *(L. corniculatus),* Pacific silverweed *(Potentilla pacifica)*, native pasture grasses *(Agrostis* spp.*),* and soft rush *(J. effusus).* The mosquitoes trapped at this site are expected to be representative of the species of mosquitoes associated with the proposed freshwater wetland cells and amphibian ponds at the Wetland Reserve Program Site 1.

Water was sampled for larval mosquitoes from the borrow ditch running along U.S. 101 on the southwest side of the highway, on either side of our access point. Approximately 500 m eastward of the access point, dipping was conducted among the common cattail (*T. latifolia*) (Figure 4 and Figure 19). Other vegetation at this site included blackberry *(Rubus* spp.*)*, soft rush *(J. effusus),* and native pasture grasses *(Agrostis* spp.). Of note at this larval dip location were the many fish observed and identified as the threespine stickleback *(Gasterosteus aculeatus).* Similar vegetation was present at the dip site approximately 5m west of the access point (Figure 20). Standing water was present and was sampled for larval mosquitoes among the cattails near the trap only during the last two sampling trips.

Figure 18. Freshwater trap.

Figure 19. Freshwater-East borrow ditch: larval dip sampling location.

Figure 20. Freshwater-West borrow ditch: larval dip sampling location.

2.2.1.3 Natural Salt Marsh Reference Site and Previously Restored Salt Marsh Sampling Stations

2.2.1.3.1 Potter Slough (PS)

Trap: 10T 436768 5170318
Larval Dip Pool: 10T 436818 5170226

This site is a natural tidal marsh and slough complex located immediately southeast of the proposed restoration site (Figure 4 and Figure 21). The marsh has not been diked and is used as a mature salt marsh reference area for this study. Native salt marsh vegetation extends from approximately +4 ft to +10 ft (NAVD 88 datum). The trap was placed in a low-lying area (approximately +4 to +8 ft) in a stand of Lyngby sedge *(Carex lyngbyei)*. We placed the trap at this elevation because it was closest to that predicted for the proposed restoration site shortly after restoration takes place. At higher elevations surrounding the trap location, plant species included tufted hairgrass *(D. cespitosa)*, saltweed *(Atriplex patula)*, pickleweed *(S. virginica)*, and seaside arrowgrass *(T. maritimum)*.

Small amounts of standing water were sometimes observed in the sloughs and sampled for larval mosquitoes. The channels were mostly mud with *D. spicata* and *S. virginica* common along the edges. A more permanent pool of approximately 5-ft (1.5-m) diameter was later located adjacent to one of the slough channels and approximately 330 ft (100 m) east of the trap location, where it was inundated at high tides (Figure 4 and Figure 21). This pool was immediately surrounded by *S. virginica, D. cespitosa, D. spicata,* and *A. patula* and was sampled for immature mosquitoes.

2.2.1.3.2 North River (NR)

Trap: 10T 431769 5178225
Larval Dip (example): 10T 431767 5178200

This site was located along the margin of a channel excavated approximately two years ago after a dike was breached along the lower portion of the North River (Figure 5 and Figure 23). The project was designed to restore daily tidal inundation to the area. The site is located on private property, and permission of the landowners was obtained prior to setting up the trap station (Figure 24). The vegetation at the site was in a state of transition between freshwater and salt-tolerant vegetation. For example, there were dense areas of fat-hen saltbush *(A. patula)*, an early colonizer of tidally restored marshes. In mature tidal marshes, it is rarely a dominant species and is found only at the upper edges of the marsh (Spear Cooke 1997). Lyngby sedge (*C. lyngbyei*) and Pacific silverweed (*P. pacifica*) were colonizing the bare substrate immediately adjacent to the channel. Although this site is not adjacent to the proposed restoration site, it is expected to provide some indication of what mosquito species and relative abundance could be expected at a recently restored tidal marsh.

Low-lying areas above the excavated channel filled with water during high tides (Figure 25). It appeared that water was exchanged in some of the pools almost daily, whereas others were replenished only during the highest tides. Those at the higher elevations occasionally dried out. Many pools were present, and most were sampled for larval mosquitoes.

Figure 21. **Potter Slough trap.**

Figure 22. **Potter Slough larval dip sampling site (foreground), and slough channels (background).**

Figure 23. **North River restoration site.**

Figure 24. **North River trap.**

Figure 25. **North River larval dip areas.**

2.2.1.3.3 Elk River (ER)

Trap: 10T 416717 5190127
Larval Dip (example; others were collected near trap): 10T 417072 5190120

A trap was placed inside a 57-acre salt marsh restoration area at Elk River (Figure 5 and Figure 26). Typical vegetation surrounding the trap location consisted of pickleweed *(*S. *virginica),* tufted hairgrass *(D. cespitosa),* Lyngby sedge *(C. lyngbyei),* saltgrass *(D. spicata),* seaside arrowgrass *(T. maritimum),* and fat-hen saltbush *(A. patula).* In 1987, the surrounding dike was breached in a single location, allowing tidal waters to flow onto the site. Some of the existing tidal channels were excavated, but borrow ditches were not filled. Low-lying areas fill with water at extreme high tides (>10 ft MLLW) (Figure 27 and Figure 28) when water covers much of the site. An adjacent natural tidal marsh is approximately two feet higher than the Elk River restoration site, similar to the elevation difference between the Willapa River restoration site and its adjacent Potter Slough reference site. Grazing activities and the loss of natural accretion processes at the restoration sites are the reason for the subsidence. Mosquito species and their relative abundance at the Elk River restoration site may be indicative of species and abundance at a tidal marsh that has been restored for more than 10 years.

Figure 26. **Elk River trap.**

Figure 27. **Low-lying larval dip areas near the Elk River trap.**

Figure 28. **Elk River site covered with water at high tide (+10 ft MLLW).**

2.2.2 Mosquito Sampling and Identification Techniques

2.2.2.1 Adult Sampling with Centers for Disease Control Traps

Adult mosquitoes were sampled using Centers for Disease Control and Prevention (CDC)-type light traps; specifically the New Standard Miniature Incandescent Light Trap Model 1012 with optional air-actuated gate system from the John W. Hock Company. This trap was selected for the following reasons:

- It captures a diverse range of mosquito species for composition, relative abundance, and population analysis.
- It is an accepted standard for the Washington State Department of Health (WDOH) and other agencies.
- It attracts fewer non-mosquito species than other methods.
- It is practical for use in temporary field locations.

Each unit has a control motor and programmable photo switch. However, we set the fan for continuous operation and removed the incandescent bulb to reduce the capture of other flying insects, such as moths and beetles. This has proven to be an effective technique so long as the trap is baited with carbon dioxide (CO_2), which increases the overall mosquito catch and the number of species captured (Gjullin and Eddy 1972; McNelly 1989). We mounted a single granular media CO_2 sachet to each trap at the start of each sampling period. The sachet is activated by removing a separating spline and mixing two powders together. The result is a 24-hour slow-release plume of CO_2 and water vapor. Each trap was connected to a 6-volt rechargeable sealed gelled-electrolyte battery, which was sufficient to operate the trap continuously for a minimum of 48 hours. The downdraft from the fan ensured that all mosquitoes inside the collection net and cup assembly remained there until they were retrieved.

The traps were hung from brackets attached to 6-ft (1.8-m) metal fence posts. The trap height varied according to how deep the posts were set, but averaged 54 inches (137 cm) above the ground. The traps were set during the day, allowed to run overnight, and checked the following day (Figure 29). This schedule was designed to capture both night- and day-feeding species. On collection, the net and cup assemblies were carefully removed and placed inside a killing jar for several minutes to induce mortality. The mosquitoes from the trap were transferred to a small plastic collection jar that was labeled to uniquely identify the sample date, location, and sampling technique. If another sampling period was scheduled to follow, the empty net and container were reattached to the trap, a replacement CO_2 sachet mounted on the trap, and the battery reconnected, thereby starting the next sampling period. If another sampling period was not scheduled to follow, the trap was removed from the post and carried back to the vehicle. The post was left in place to ensure the same site would be sampled during the subsequent site visit.

2.2.2.2 Adult Sampling with Nets and Collection Jars

Mosquitoes were also captured by hand, either by sweeping a small-mesh net through vegetation and transferring the captured mosquitoes into a collection jar or by capturing them directly with the collection jar.

2.2.2.3 Larval Dipping

A standard white larval dipper (13 cm in diameter with a 350-mL capacity) was used to sample areas of standing water for larval mosquitoes. At each site, a series of dips was made (usually three dips). Salinity of the standing water was measured to the nearest part per thousand (ppt) using a Reichert refractometer. Counts of the number of larvae per dip provide an estimate of abundance and indicate

where breeding occurs. Species identification provides information about which mosquitoes are present. Some of the larvae were raised to adults in the laboratory to simplify species identification.

Figure 29. **Trapped adult mosquitoes.**

2.2.3 Timing

All mosquito collection activities were conducted in 2004. A reconnaissance site visit was conducted July 26 and 27 to determine where to place the CDC traps. Sampling began on August 9 and continued on an approximately biweekly basis through October 17. Table 1 presents the sample dates and techniques used for mosquito collection by site. In general, traps were set on two consecutive nights during five of the six sampling site visits; only one night of trapping was conducted on the first site visit. Mosquitoes were usually netted on the first day of each sampling site visit; larval dipping was conducted on the second day. On occasion, this schedule was not followed because the tidal regime prevented our access to low-lying sites, and this is reflected in the table. The South Bend Alder House site was not sampled until the third sampling site visit (August 31 to September 2), at which point we alternated trapping between the Alder House and the Wastewater Treatment Plant. Splitting this sampling effort between the two sites with a fixed number of mosquito traps provided additional information on mosquito species in and around the City of South Bend.

2.2.4 Species Identification

The principal characteristics distinguishing one adult mosquito species from another are the shape, size, coloration, and scaling of various body parts. A binocular dissecting microscope was used to examine each adult mosquito captured in the trap or by hand. Because a single key for Washington mosquito species has not been written, we used a number of keys (listed below) to identify our mosquito specimens. Voucher specimens were sent to a mosquito expert, Dr. William Walton at the University of California-Riverside, for independent verification.

Keys Used to Identify Mosquito Specimens

- Curtis, L.C. 1967. The Mosquitoes of British Columbia. Occasional Papers of the British Columbia Provincial Museum No. 15, Victoria, British Columbia, Canada.
- Darsie, R.F. Jr., and R.A. Ward. 1981. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. American Mosquito Control Association, University of Utah Printing Services, Salt Lake City, Utah.

- Gjullin, C.M. and G.W. Eddy. 1972. The mosquitoes of the northwestern United States. Technical Bulletin No. 1447, Agricultural Resource Service, United States Department of Agriculture, Washington, D.C.
- Meyer, R.P. 2003. Regional guide to the common mosquitoes of California, I. Coastal California. Mosquito and Vector Control Association of California, Sacramento, California.
- Meyer, R.P. and S.L. Durso. 1998. Identification of the mosquitoes of California. Mosquito and Vector Control Association of California, Sacramento, California.
- Stage, H.H., C.M. Gjullin, and W.W. Yates. 1952. Mosquitoes of the northwestern states. United States Department of Agriculture, Agriculture Handbook No. 46, Washington, D.C.

2.2.5 Supplemental Data Collection

Additional data collected during the sample period or obtained after field sampling was completed included wind speed and direction, precipitation, temperature, tidal elevations, and lunar phases. Hourly wind speed and wind direction data were obtained from the Western Regional Climate Center (WRCC) in Reno, Nevada, for Hoquiam, Washington. This meteorological station is situated approximately 20 miles (32 km) north of the project site in a similar orientation to the coast and is also the nearest station to the study site (geographically) that records hourly wind data. Matlab was used to process the velocity and direction data.

Precipitation and temperature data were also obtained from the WRCC for Raymond, Washington to compare values of these elements recorded during the study period and current year with the period of record averages (i.e., 1980 to 2004). Raymond is approximately 5 miles (8 km) east of the restoration site and, thus, is expected to experience similar rainfall and temperatures as the sample stations chosen for this study.

Tide and lunar phase data for Willapa River, South Bend, were downloaded using Version 1.05 of Tides & Currents, Nautical Software Inc. This program calculates tide predictions using the present constituents obtained from the National Oceanic and Atmospheric Administration (NOAA) and the Canadian Hydrographic Service (CHS).

Table 1. Mosquito sampling by site and date (2004)[a]

Site	Sample Method	August					September								October			
		10	17	18	19	31	1	2	8	9	10	24	25	26	15	16	17	18
Inside Dike	Trap	●		●	●		●	●		●	●		●	●		●	●	
	Dip	●		●			●			●			●			●		
	Net		●			●				●		●	●		●			
Outside Dike	Trap	●		●	●		●	●		●	●		●	●		●	●	
	Dip	●		●			●			●			●			●		
	Net		●			●				●		●			●			
Mudflat	Trap	●		●	●		●	●			●		●	●		●	●	
	Dip																	
	Net																	
Freshwater	Trap	●		●	●		●	●		●	●		●	●		●	●	
	Dip	●		●			●			●			●			●		
	Net		●			●	●			●		●			●			
Potter Slough	Trap	●		●	●		●	●		●	●		●	●		●	●	
	Dip	●		●			●			●			●			●		
	Net		●			●				●		●			●			
North River	Trap	●		●	●		●	●		●	●		●	●		●	●	
	Dip	●		●			●			●			●			●	●	
	Net	●	●			●				●		●			●			
Elk River	Trap	●		●	●		●	●		●	●		●	●		●	●	
	Dip	●		●			●			●			●			●		
	Net	●	●			●				●		●			●			
Wastewater Treatment Plant	Trap	●		●	●		●				●		●			●		
	Dip	●		●			●			●			●			●		
	Net	●	●			●				●		●	●		●	●		
South Bend Alder House	Trap							●		●				●			●	●
	Dip															●		
	Net							●		●			●			●		

(a) Trap dates are listed according to the date mosquitoes were collected from the trap (traps operated approximately 24 hours prior to mosquito collection).

2.3 Predicting Mosquito Population Dynamics and the Potential to Alter Exposure of South Bend Residents to the West Nile Virus Following Restoration

To predict what species of mosquitoes may inhabit the proposed restoration areas (salt marsh and freshwater wetland areas), mosquitoes were trapped at existing salt water marshes, both natural and restored, and on the south side of the proposed restoration area in a region of cattail marsh where standing water is present for part of the year.

Sampling mosquitoes at sites outside the proposed restoration was done to improve assumptions about mosquito use of the site in the years following restoration (e.g., 2 years post-restoration at North River, 17

years post-restoration at Elk River) and at marsh maturity (Potter Slough). Similarities and differences between the previously restored site and the proposed Willapa River estuarine restoration site, such as the extent of dike removal, tidal channel excavation, and marsh elevation all factor into the predictions of how the habitat (and mosquito dynamics) at the Willapa River site are likely to change over time. Sampling mosquitoes at the proposed restoration site provided a baseline characterization of existing conditions, to which the predictions of mosquito species and abundance that are based on the existing salt marsh data can be compared.

Regions of the proposed restoration site that currently hold standing fresh water for some part of the year were chosen to represent both existing and future mosquito species and abundance on the freshwater wetland portion of the restoration site. Restoration plans indicate that boards would be placed in control structures to begin retaining rainwater in September. The boards may or may not be removed in the spring or summer to facilitate drawdown for mosquito control. If the boards are removed, most of the water will drain from the ponds. The wetland ponds are designed to maximize the area with water depths of 6 to 12 inches. This is similar to the amount of water that was observed in the cattail area near the Freshwater trap site during the latter part of the sampling period (September/October) and in several of the freshwater borrow ditches associated with the Freshwater and Inside Dike sites between August and October. The drawdown condition is represented by our sampling of the Freshwater trap site's cattail area during the early part our study (August/September), when there was no standing water present.

A baseline characterization of mosquito species and abundance was done in and around the City of South Bend to help us understand how these variables could change following the proposed restoration. The salt marsh and freshwater wetland portions of the restoration project are expected to have distinctly different impacts on mosquito populations at the restoration site, and therefore could potentially have different impacts on mosquitoes in the City of South Bend. Thus, the individual impacts of the two parts of the restoration project are examined, in addition to considering the overall impact of the restoration project.

The reason for considering the salt marsh and freshwater wetland components of the project separately is based on the fact that individual mosquito species inhabit distinctly different habitats. Individual mosquito species contract and transmit WN virus at different levels of competence. Other factors that determine which mosquito species are likely to be important in WN virus transmission include the species' generally preferred hosts, flying ability, and breeding life cycle. Thus, the salt marsh and freshwater wetland components of the restoration project may produce proportionately more or less potential WN virus vectors than are currently produced at the restoration site or in the City of South Bend.

3.0 RESULTS

3.1 Characterization of the Existing Conditions for Mosquito Breeding in and around the City of South Bend

The City of South Bend is situated in the midst of prime mosquito real estate, both natural and manmade. Saltwater and freshwater are present throughout the area, providing abundant breeding habitat for both freshwater and saltwater mosquito species. Under the current conditions, mosquitoes present a significant nuisance problem for the residents of the City of South Bend. The two sites we sampled that are closest to the city (i.e., Alder House assisted living facility and the wastewater treatment plant) had the two highest mosquito trapping rates for the entire study. Likewise, the larval concentrations in the temporary ponds along the road to the wastewater treatment facility were the highest of any we sampled. Most

(>70%) of the mosquitoes from these two sites were *Ochlerotatus dorsalis* (a predominately saltwater species), but *Culex, Culiseta,* and *Coquillettidia* were also observed.

Within city limits, we observed that many conventional mosquito control practices were not being utilized by residents. For example, we observed discarded tires and miscellaneous debris, uncovered boats, and other places that would hold small amounts of standing water that provide suitable breeding habitat for *Culex* and other freshwater species, including some *Culiseta* (Figure 30). These mosquitoes require only one to two weeks to develop from eggs to adults.

To mitigate the existing mosquito nuisance condition, the residents of South Bend support a self-imposed mosquito control tax. Money raised through taxation had purchased four Mosquito Magnets® that were operated in various parts of town according to where most mosquito complaints originated. We examined the contents of one of the Mosquito Magnets® in August and found that it contained an abundance of *O. dorsalis*. In addition to the Mosquito Magnets®, complaint calls are monitored at City Hall, and a licensed pesticide applicator is called in to spray mosquitoes in those areas after a particular threshold of complaint calls is reached. Most residents report relief from mosquitoes for up to a week following the spraying, but resurgence in mosquito numbers occurs soon thereafter.

Figure 30. **Abandoned tires and other debris located behind the local high school stadium, capable of providing breeding habitat for mosquito species that utilize artificial containers.**

Crans (2003) reports that mosquitoes cannot regulate body heat, and few species can function below 50ºF (10ºC). Therefore, local temperature data can be used to predict the time period when mosquitoes are most active in the region. Weather data collected in Raymond, Washington between 1980 to 2004 indicates that the average mean temperature first approaches 50°F in April and remains above this temperature until October. However, the mean average temperature infrequently drops below 40°F for the remainder of the year, creating the potential for some "cold-weather" species, such as *Culiseta,* to be present nearly year-round. Additional mosquito monitoring in the months outside this study's sampling period would be required to substantiate these generalizations.

Mosquitoes require standing water and/or moist soil to reproduce. Therefore, precipitation and tidal records for the region can provide an indication of the potential for mosquito species to be present and to breed during different times of the year. Precipitation records from 1980 to 2004 indicate that Raymond, Washington receives 84 inches of mean annual precipitation. In general, precipitation is minimal (less than 2 inches) during July and August. Precipitation then increases monthly through November, before beginning to decline and taper off in July (Figure 31). Because of the amount of rainfall that can be expected each month and our observations of standing water resulting from rainfall that supported

mosquito larvae as early as September, it is likely that enough standing water to support mosquito production in low-lying areas around South Bend is present between September and June.

High tides mark the upper limit of potential saltwater habitat for breeding saltwater mosquitoes. Marsh areas that experience inundation only a few times per month, associated with full or new moons, provide suitable habitat for floodwater species. Regions of tidal marshes we sampled that were above +9 ft MLLW were inundated only periodically and thus provided suitable rearing habitat for the *Ochlerotatus* species. From April through October, there were at least two tidal peaks per month that were followed by periods of drying (Figure 32). Assuming suitably warm temperatures, this time period appears to provide a large area suitable for *Ochlerotatus* reproduction in areas adjacent to the Willapa River, including areas in and around the City of South Bend.

Various mosquito species have different flying abilities. Most have difficulty flying against winds greater than 5 mph (8 km/h), but may use wind to enhance downwind dispersal (Bailey et al.1965, in Gjullin and Eddy 1972). In 2004 and over the 1996-to-2004 period of record, prevailing winds from May to October are from the west and southwest (Figure 33). Over the period of record, median wind speed is 9 mph; mean wind speed is 9.6 mph, and the mode is 6 mph.

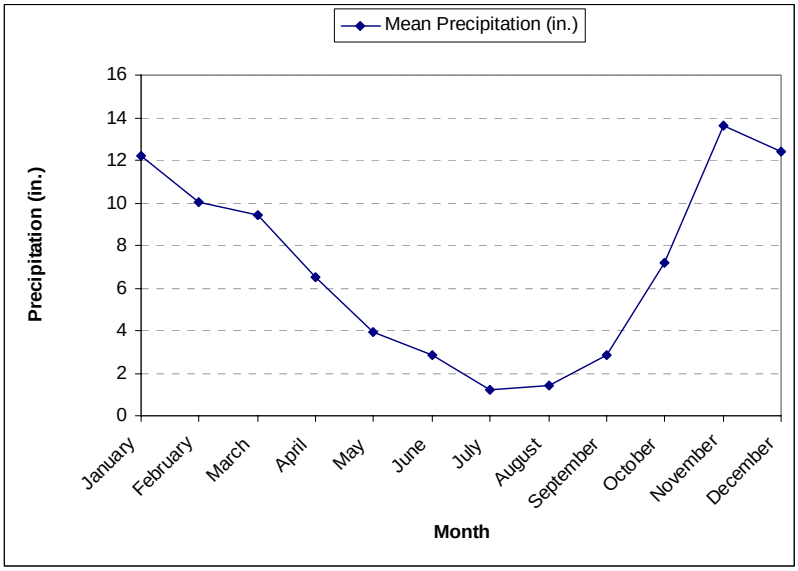


Figure 31. Period of Record Precipitation Summary (1980 to 2004), Raymond, Washington

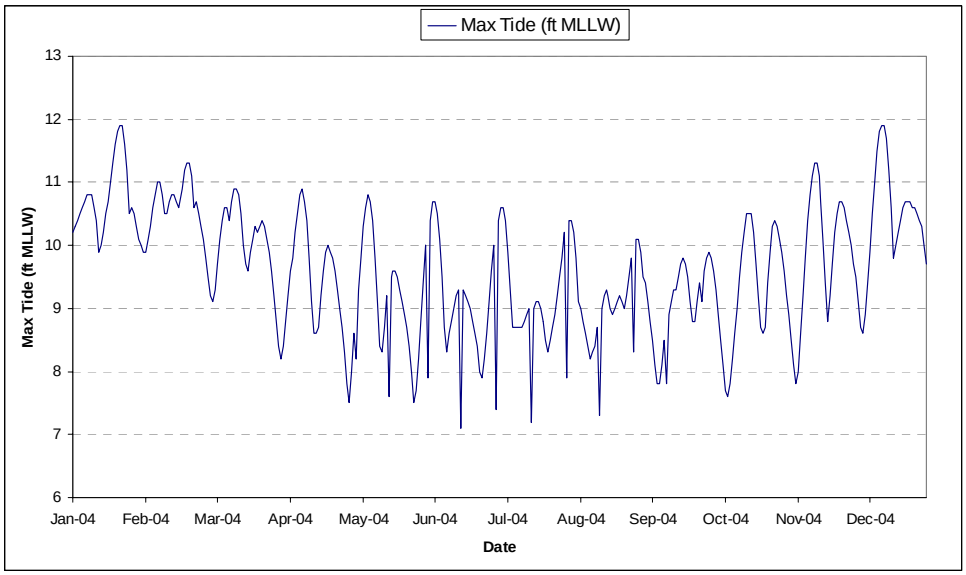


Figure 32. Maximum Tide Elevations (Feet Mean Lower Low Water) on the Willapa River, South Bend.

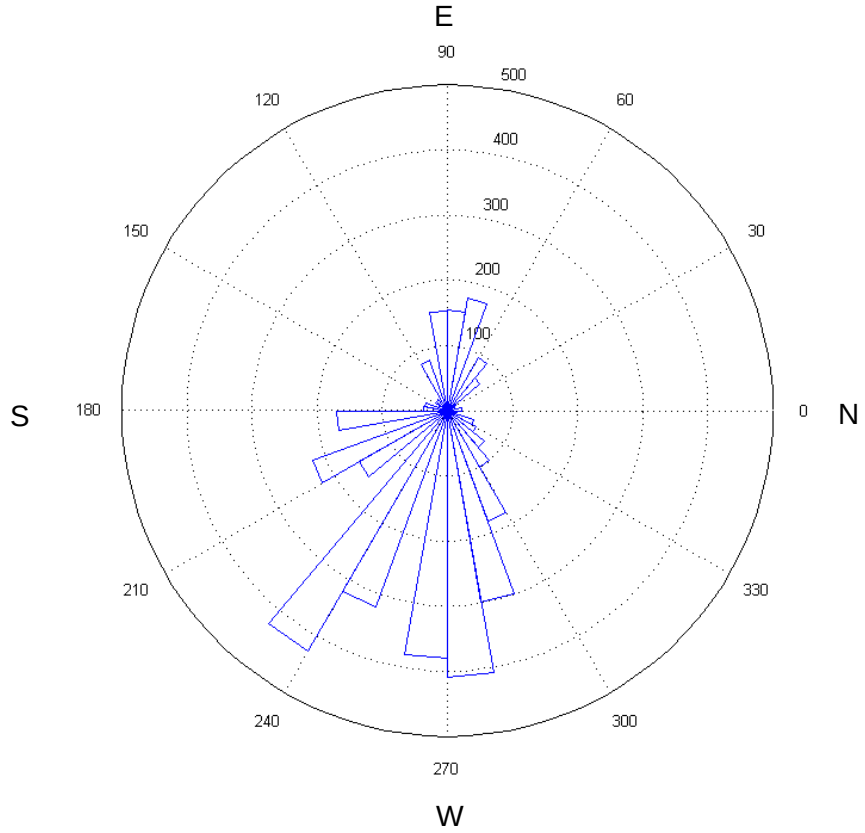


Figure 33. Summary of wind direction for the period of May-October, 2004, measured at Hoquiam, Washington.

(Note: 0 is true north, 90 is east, etc. Units are the number of times the hourly average came from that direction.)

3.2 Identification of Mosquito Species and Associated Habitat

The following sections present the detailed results of adult mosquito trap and net sampling, as well as larval mosquito dip sampling. For reference, a summary of the general habitat types we sampled, the species observed in each habitat type, the relative abundance of each species captured in the habitat, and the potential for the species to carry and transmit West Nile virus, as reported in the literature, is presented in Table 2.

Table 2. Habitat types sampled during this study with their associated mosquito species, and the relative abundance and reported potential of these species to carry and transmit West Nile virus.

Habitat Type	Species Observed	Relative Abundance[1]	Potential to Serve as an Enzootic Vector (i.e., to birds)[2]	Potential to Serve as a Bridge Vector (i.e., to humans)[3]
Mudflat & Recently Restored Salt Marsh[4]	*Cq. perturbans*	●	+	+
	Cx. pipiens	●	+++++	+++
	O. dorsalis	●	-	++
Natural Salt Marsh[5]	*Cx. pipiens*	●	+++++	+++
	O. dorsalis	●●●	-	++
	O. melanimon	●	-	++
Previously Restored Salt Marsh[6]	*Cx. pipiens*	●	+++++	+++
	O. dorsalis	●●●●	-	++
	O. melanimon	●	-	++
Fresh Water Ponds and Ditches[7]	*Cs.incidens*	●	+	+
	Cs. inornata	●	+	++
	Cx. pipiens	●●●	+++++	+++
Pasture Land at Restoration Site[8]	*Cs.incidens*	●	+	+
	Cx. pipiens	●●	+++++	++
	O. dorsalis	●●●●	-	++
	O. melanimon	●	-	++
City of South Bend[9]	*Cq. perturbans*	●	+	+
	Cs. particeps	●	unknown	unknown
	Cx. pipiens	●	+++++	+++
	O. dorsalis	●●●●	-	++
	O. melanimon	●	-	++

[1] Based on the number of mosquitoes captured and their relative percent species composition within the habitat type. ●,=few; ●●●●= many.

[2] (from Turell et al. 2005;Goddard et al. 2002). Enzootic vectors transmit WN virus to birds. Based on field virus isolations, vector competence, feeding behavior, etc. - = little or no risk; +++++= species may play a major role.

[3] (from Turell et al. 2005 and Goddard et al. 2002; *Cx. pipiens* data augmented based on Kilpatrick et al. 2005). A bridge vector transmits WN virus to birds and mammals. Based on virus isolations from the field, vector competence, feeding behavior, etc. - = little or no risk; +++++ = species may play a major role.

[4] Mudflat and North River.

[5] Potter Slough and Outside Dike.

[6] Elk River.

[7] Freshwater borrow ditches and cattail pool, Inside Dike borrow ditches.

[8] Inside Dike trap, Freshwater trap.

[9] Wastewater Treatment Plant and South Bend Alder House.

3.2.1 Trapped Mosquitoes

A total of 1017 adult mosquitoes from four genera were captured during this field study (Figure 34). The samples were dominated by *Ochlerotatus* spp., which comprised 74.1% of all the mosquitoes captured in the CDC traps. Within this genus, most were *O. dorsalis* (91.6%), whereas the remainders were *O. melanimon* (0.8%) or were unable to be confirmed to species because they were missing some body component necessary for positive identification (7.6%). *Culex* was the second most dominant genus (13.9% [27.3% *Cx. pipiens*; 73.7% *Culex* spp.]), followed by *Culiseta* (0.7% [14.3% *Cs. particeps*; 85.7% *Culiseta* spp.]) and *Coquillettidia* (0.2% [100% *Cq. perturbans*]). Unidentifiable mosquitoes comprised 11.1% of the total trapped mosquitoes. These specimens were missing abdomens, heads, wings, or some other component that made it impossible to confirm even the genus. In general, the percentage of species composition for the unidentifiable mosquitoes can be assumed to follow the percentage of species composition of the positively identified specimens. Species descriptions can be found in Section 4.1.2.

Catch per unit effort (CPUE) was calculated by dividing the number of mosquitoes captured by the number of hours the trap was operational. Over the entire sampling period, the average CPUE ranged from a low of only 0.04 mosquitoes trapped per hour (msq/h) at the Mudflat to a high of 1.12 msq/h at the Wastewater Treatment Plant (Figure 35). Species composition varied by trap location (Figure 36 and Figure 37) and by sampling period (Figure 38). Early September produced the greatest overall CPUE (i.e., September 8 to 10), followed by trapping conducted in mid-August (i.e., August 17 to 19) (Figure 38). Sitewise, mosquito CPUE was greatest during the September 8 to 10 sampling period, with the exception of the Mudflat, North River, and the Wastewater Treatment Plant sites, which had their greatest CPUEs during the August 17 to 19 sampling period (Figure 39).

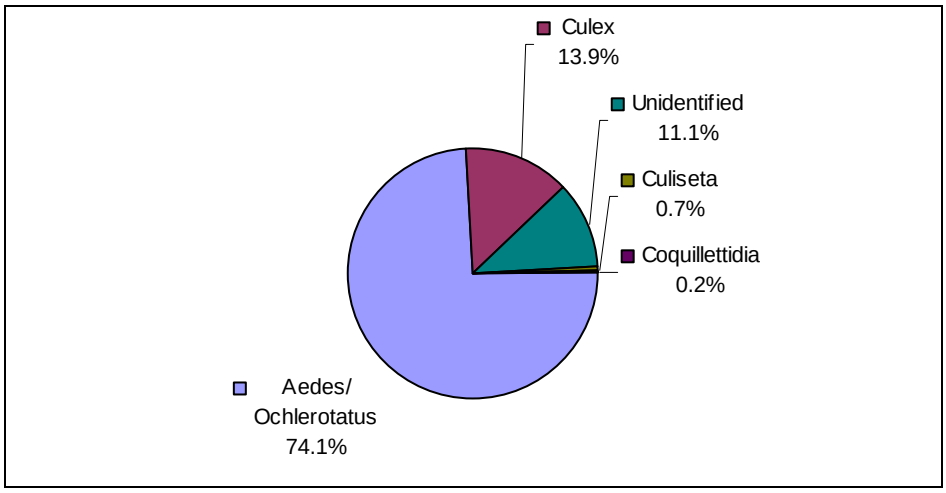


Figure 34. **Overall percentage of composition of mosquito genera captured using CDC traps.**

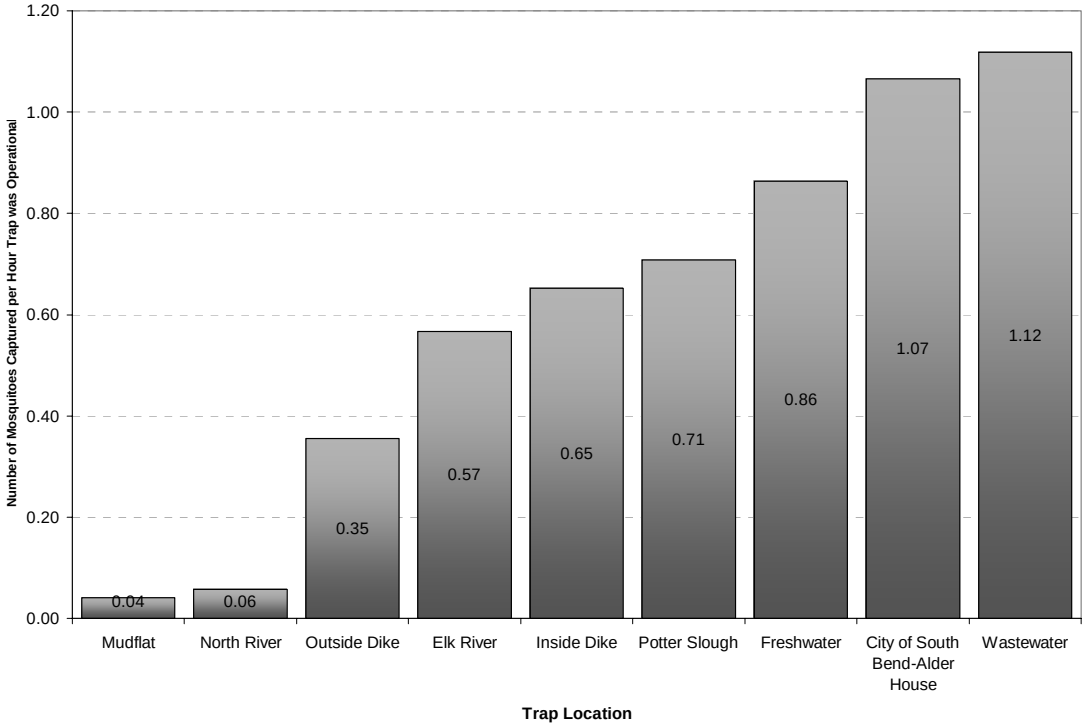


Figure 35. **Number of mosquitoes at a trap site per hour the trap was operational.**

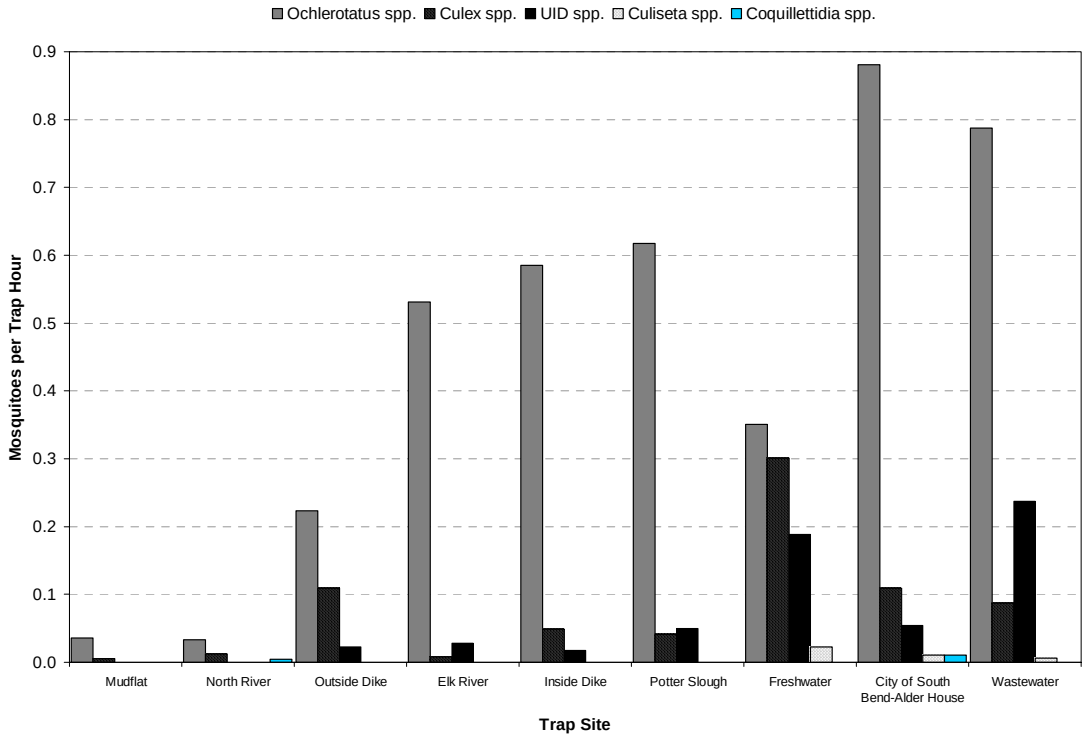


Figure 36. **Average mosquitoes captured per hour, by species, at each trap site per hour the trap was operational**

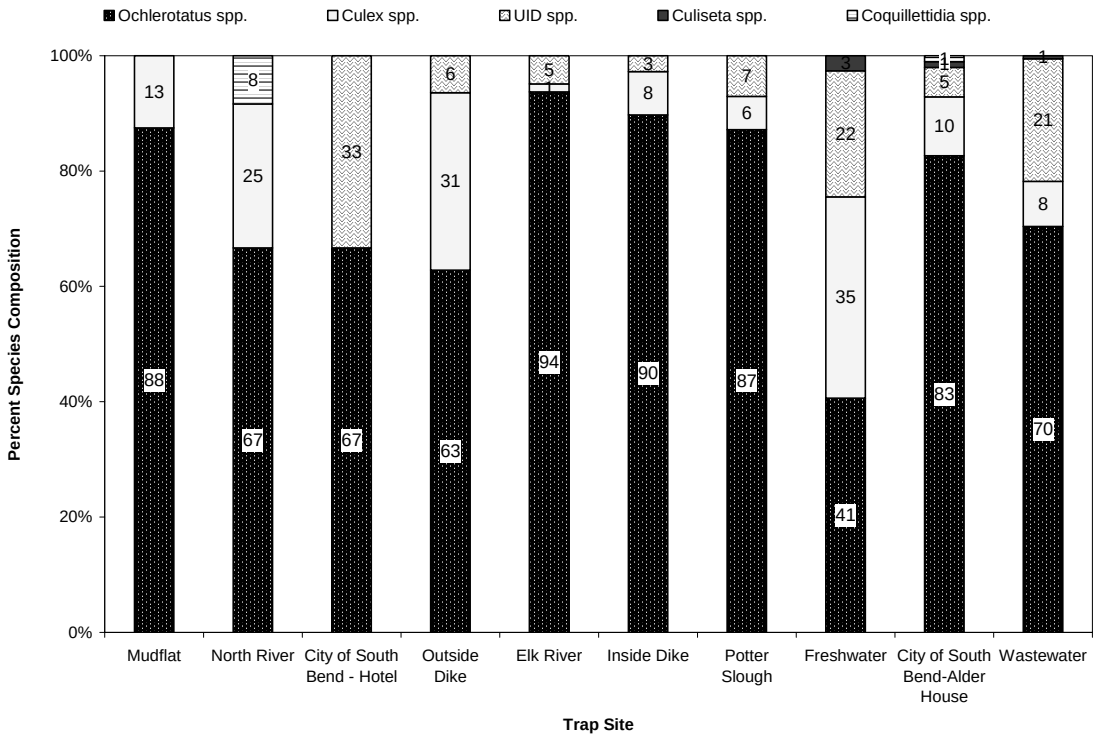


Figure 37. **Site-wise comparison of trapped mosquito genera; percentage of composition.**

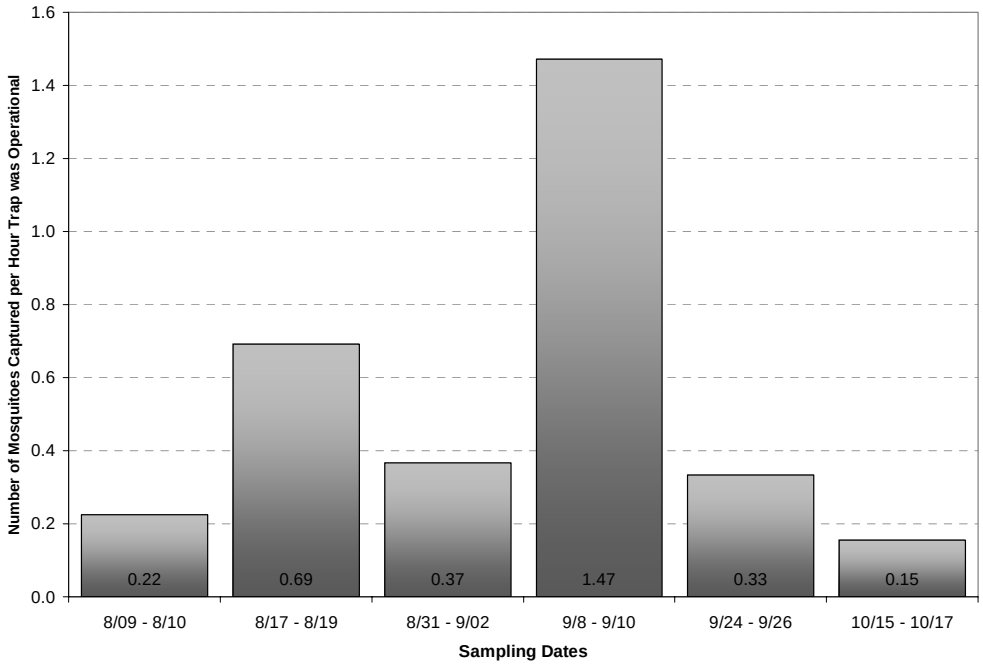


Figure 38. **Number of mosquitoes during a sampling trip per hour the trap was operational, all sites combined.**

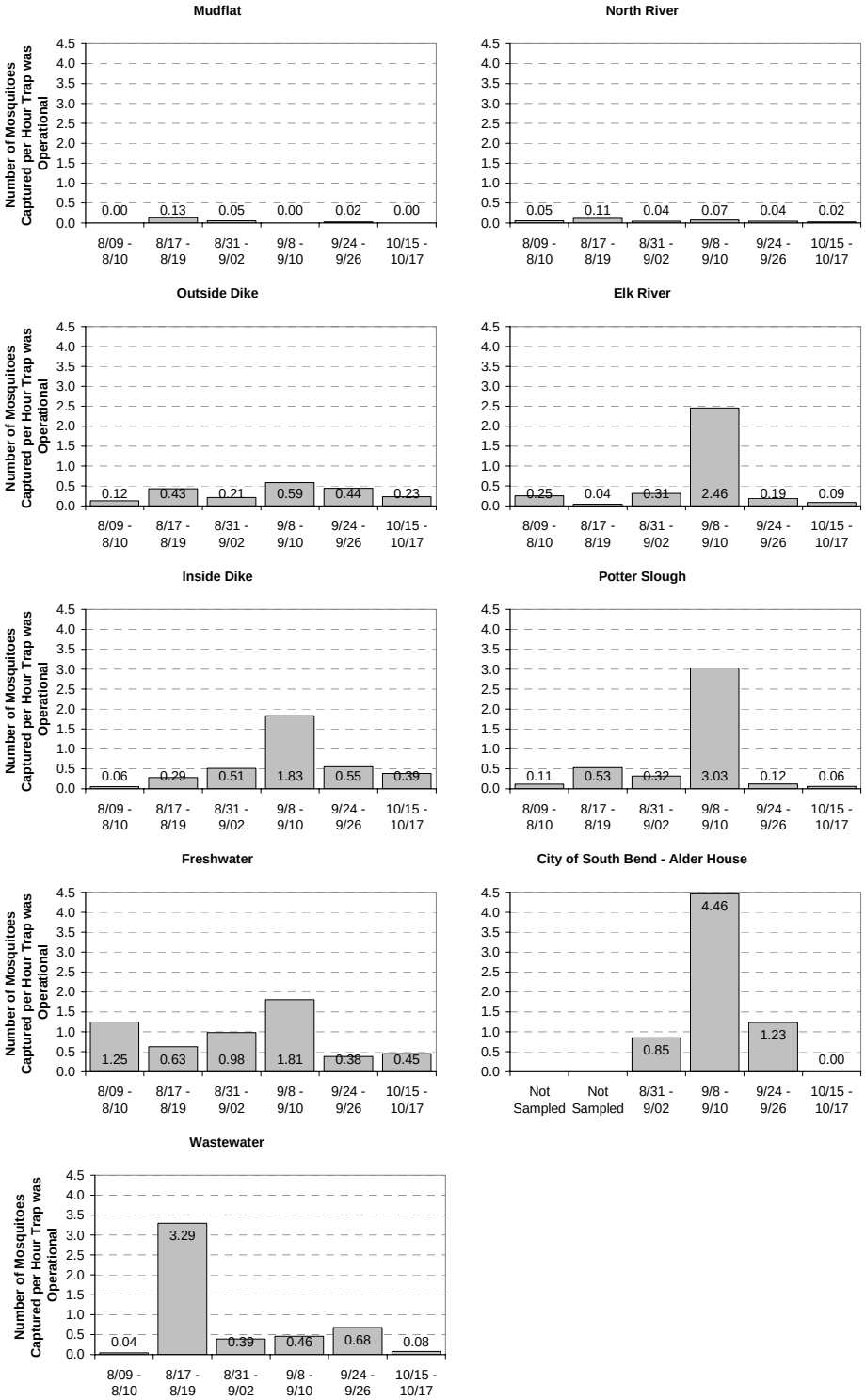


Figure 39. **Mosquitoes captured per operational trap hour during each site visit and at each sampling site (Sampling dates are shown along the x-axis).**

3.2.2 Larval Dip Samples

In Washington, each local health jurisdiction is responsible for establishing its own trigger level, based on larval counts, for initiating larval mosquito control with approved larvicides. The guidance in WDOH (2003) indicates those levels are generally set between larval counts of 0.3 per dip and 5.0 per dip. This density of mosquito larvae is indicative of conditions under which mosquito populations may become a nuisance to people. Although mosquito control in the City of South Bend is not based on larval counts, the larval density guidelines allow us to put the larval sampling data we collected at the various sites into context with what would be considered nuisance levels.

Larval dip samples were obtained whenever standing water was present at or near a trap site. Twelve areas were routinely sampled to document the presence or absence of immature mosquitoes (seven freshwater sites and five saltwater sites; see Table 2). Larvae were found at nine of these sites (four freshwater, five saltwater). Mosquitoes from some samples were successfully reared to emergence, and included the species *O. dorsalis, Cx. pipiens, Cs. incidens, Cs. inornata, and Cs. particeps* (Table 2). Four sites had extremely high densities of immature mosquitoes during one or more sampling periods. These represented both saltwater sites (Wastewater Treatment Plant roadside [maximum 54.3 mosquito larvae per 350-mL dip], Elk River restoration site [maximum 50.7 per dip], and Potter Slough [maximum 26.7 per dip]), and a freshwater site (Inside Dike-East borrow ditch [maximum 35 per dip]) (Figure 40). The other five sites with mosquito larvae present did not exhibit more than 3.7 per dip.

3.2.3 Net Samples

Only two mosquito species were captured with the netting method during formal sampling (Table 2). Every site sampled (this excludes Mudlflat) confirmed the presence of *O. dorsalis.* Two sites (Potter Slough and South Bend-Alder House) also indicated the presence of *O. melanimon.* The *O. melanimon* captured by netting were the only individuals of that species positively identified at these two sites. Capturing only two species by netting may be explained because all net samples were collected during daylight. *Ochlerotatus* are considered day and evening biters, whereas *Culex* and *Culiseta* generally feed at dawn, dusk, or at night. In general, mosquitoes were easy to net during the first half of the study sampling period when adults were abundant and aggressively swarming around the researchers, but were noticeably more difficult to find and to capture toward the end of the season and under certain weather conditions, such as strong winds and driving rain.

3.2.4 Timing

Our study period extended from early August through mid-October. Air temperature was similar to that normal to the region and was conducive to mosquito activity throughout most of this period, although the cooler weather that began at the end of September appeared to generally decrease mosquito activity and aggression. However, it should be noted that we observed some of our first *Culiseta* species, often called "cool weather mosquitoes," in October. Both *Cs. inornata* and *Cs. incidens* were reared to emergence from larval dip samples collected in October. The presence of these species may increase during the fall, but further sampling would be required to determine whether these mosquitoes are ever present in significant numbers. Mosquitoes are expected to become active in the area around April, based on average air temperatures. However, sampling would be required to determine the actual onset of the mosquito season at the project site.

Table 3. Positively identified mosquito species (or genera) according to site and sampling method[a].

Sample Site	Method[b]	*Cq. perturbans*	*Cs. incidens*	*Cs. inornata*	*Cs. particeps*	*Cs.* sp.	*Cx. pipiens*	*O. dorsalis*	*O. melanimon*
Inside Dike	trap						●	●	●
	dip (Fx3)		●	●			●		
	net							●	
Outside Dike	trap						●	●	●
	dip (S)								
	net							●	
Mudflat	trap						●	●	
	dip								
	net								
Freshwater	trap		●			●	●	●	●
	dip (Fx3)								
	net							●	
Potter Slough	trap						●	●	
	dip (S)							●	
	net							●	●
North River	trap	●					●	●	
	dip (S)								
	net							●	
Elk River	trap						●	●	●
	dip (S)							●	
	net							●	
Wastewater Treatment Plant	trap					●	●	●	
	dip (S,F)				●		●	●	
	net							●	
South Bend Alder House	trap	●			●		●	●	
	dip								
	net							●	●

(a) Dark-shaded cells indicate the method was not utilized at that site; light-shaded cells indicate that while mosquito larvae were collected, none were successfully reared to emergence.
(b) Dip samples were taken from freshwater (F) or saltwater (S).

Figure 40. **Mosquito larvae and pupae in a roadside pool at the Wastewater Treatment Plant.**

3.3 Summary of Results by Site

3.3.1 Sampling Sites Associated with the City of South Bend

3.3.1.1 Wastewater Treatment Plant

Averaging 1.12 msq/h, more mosquitoes were trapped per hour at the Wastewater Treatment Plant than at any other sampling station (Figure 35). The trap was situated near vegetation and was surrounded by a variety of habitat types, including the Willapa River, short mown grass, the wastewater ponds, and saltwater marsh that was periodically flooded during above-average tides. Species captured included *O. dorsalis, Cx. pipiens* and *Culiseta* sp. (Table 2). The majority of the mosquitoes were *Ochlerotatus* (at least 70%), whereas 8% were *Culex,* and less than 1% was *Culiseta* (Figure 37).

Two areas were sampled for immature mosquitoes at the Wastewater site. Standing water was present along the road to the treatment ponds during every sampling period. Salinities ranged from 6 ppt to 33 ppt (15 ppt average). Densities of immature mosquitoes in these standing water pools were higher than at any other larval dip site: 48.7 per dip and 54.3 per dip on August 10 and August 17, respectively. Adult mosquitoes present during this period were aggressive biters. Larval densities declined dramatically during following sampling events, reaching a low of 0.3 per dip on September 25. *O. dorsalis* were successfully reared to emergence from samples collected at these ponded areas.

A small depression under the CDC trap was filled with rainwater and held immature mosquitoes when sampled on September 9 (number of dips not recorded), September 25 (3.3 per dip), and October 16 (1.2 per dip). The salinity was consistently 0 ppt. Vegetation surrounding the small pool consisted of *J. effusus* and blackberry *(Rubus* sp.). *Cx. pipiens* were successfully reared to emergence from samples collected September 9 and 25, and October 16. *Cs. particeps* were successfully reared to emergence from samples collected September 25 and October 16. The *Culiseta* pupae were noticeably larger than the *Culex* pupae.

Although this study did not formally sample the treatment ponds, they were observed and dipped for immature mosquitoes on several occasions. No mosquito larvae were ever observed or captured.

Only *O. dorsalis* were netted at the Wastewater site. Near the CDC trap, mosquito numbers were generally moderate and netting was sometimes difficult. The mosquitoes near the trap generally were not overly aggressive and often had to be shaken out of the vegetation. On at least one occasion when the mosquitoes were observed to be staying close to the ground, it was noticed that the majority of them appeared to be male mosquitoes.

3.3.1.2 City of South Bend-Alder House

Mosquitoes were always abundant at the South Bend-Alder House site and averaged 1.07 msq/h (Figure 35). This site did not appear to have any nearby source of standing water, with the exception of a surface water runoff pond that filled up after intense periods of rainfall. There was a great amount of vegetation, however, and it was not possible to determine whether low-lying areas might hold standing water inside the large blackberry thickets surrounding the trap. Although *Ochlerotatus* comprised the majority of mosquitoes trapped, this site had the highest species diversity (Table 2). Species trapped and positively identified included *O. dorsalis, Cx. pipiens, Cs. particeps,* and *Cq. perturbans*. At least 83% of the mosquitoes were *Ochlerotatus,* 10% were *Culex,* and 1% (each) were *Culiseta* and *Coquillettidia* (Figure 37).

Two species of *Ochlerotatus (dorsalis* and *melanimon)* were netted at Alder House. These were the only *O. melanimon* captured at this site. Generally, the mosquitoes at this site were very abundant, aggressive, and simple to net. Only in October did their numbers appear to be on the decline.

3.3.2 Willapa River Restoration Site Sampling Stations

3.3.2.1 Outside Dike

An average of 0.35 msq/h was captured at the Outside Dike site, which is adjacent to the Mudflat trapping station (Figure 35). Natural marsh plants and berry bushes were present at this location, but they were also inundated at the higher high tides, or almost once per day. Mosquito species trapped at this site included *O. dorsalis, O. melanimon,* and *Cx. pipiens* (Table 2). At least 63% of these mosquitoes belonged to the genus *Ochlerotatus;* 31% were from the genus *Culex,* and the remainder was unidentifiable (Figure 37). Ninety-eight percent of the *Ochlerotatus* mosquitoes were *O. dorsalis.*

Immature mosquitoes were observed and sampled from the standing pool of water near the Outside Dike trap during only two sampling periods (August 10 and September 1), even though the pool always contained standing water. Densities averaged 1 per dip and 0.7 per dip, respectively. The salinity in the pool ranged from 0 ppt to 34 ppt, but was generally quite saline and averaged 21 ppt. Several mosquitoes from this site emerged prematurely and were unable to be confirmed to genus or species, although they appeared to most resemble *O. dorsalis.*

Only *O. dorsalis* were netted at the Outside Dike site. These mosquitoes were generally not very abundant and only moderately aggressive. They were often difficult to capture at this site, typically staying hidden in the low vegetation or flying away at any sign of movement.

3.3.2.2 Mudflat

The fewest mosquitoes were trapped at the Mudflat along the Willapa River (n=8; 0.04 msq/h), where very little vegetation was present and where the land is covered by water two times per day during high tide (Figure 35). Species captured were *O. dorsalis* and *Cx. pipiens* (Table 2). Of the mosquitoes trapped at the Mudflat, 87.5% were *Ochlerotatus* and 12.5% were *Culex* (Figure 37).

3.3.2.3 Inside Dike

The trap placed at the Inside Dike sampling station captured an average of 0.65 msq/h (Figure 35). This location is not tidally influenced, and retains standing water only after a period of rainfall. However, adult mosquitoes obviously utilized this site throughout the study period. Species that were positively identified through trapping efforts at Inside Dike included *O. dorsalis, O. melanimon,* and *Cx. pipiens* (Table 2). At least 90% of the mosquitoes trapped at Inside Dike were *Ochlerotatus* (Figure 37), and 98% of the *Ochlerotatus* mosquitoes were *O. dorsalis.*

Larval dipping was conducted at three locations inside the dike. No immature mosquitoes were observed or sampled from the borrow ditch nearest the dike, where salinities ranged from 0 ppt to16 ppt (average was 7 ppt).

Immature mosquitoes were obtained from each of the borrow ditch sections near the highway. The Inside Dike-East sample site was dry during our first site visit, but held water during subsequent sampling events due primarily to rainfall events. The ID-East borrow ditch averaged 10.9 larvae per 350-mL dip, but ranged between 0 per dip on October 16, to 35 per dip on September 9. Water salinity in the east-side borrow ditch ranged from 0 ppt to 8 ppt, but averaged only 2 ppt. Two species of mosquitoes were reared to emergence successfully from this site when the water was 0 ppt; *Cx. pipiens* and *Cs. incidens.* Additional *Culex* and *Culiseta* mosquitoes emerged, but were unable to be identified to species level (Table 2).

The west-side borrow ditch was dry during three sampling events (August 18, September 1 and 9). Following some rain events, the ID-West borrow ditch averaged 0.3 larvae per 350-mL dip, and ranged from 0 per dip on August 10, to 0.7 per dip on September 25. Water salinity from this ditch was always 0 ppt. *Culex* spp. and *Cs. inornata* mosquitoes were successfully reared to emergence from samples obtained at the ID-West borrow ditch (Table 2). It is important to note that the only *Cs.incidens* and *Cs. inornata* mosquitoes positively identified during this study were reared from larval dip samples. However, we are not certain how readily these species are attracted to the CDC-style traps used in this study.

Only *O. dorsalis* were netted at the Inside Dike site. In general, they were observed in fewer numbers and were less aggressive than were experienced at the Freshwater site.

3.3.2.4 Freshwater

An average of 0.86 msq/h was trapped at the Freshwater site, located on the southwest side of U.S. 101 (Figure 35). This trap site was surrounded by upland pasture, berry bushes, and low-lying cattails. The site was devoid of standing water except during the final two trapping periods when rain filled many

depressional areas in the cattails and in the pasture. Mosquito species captured at this site included *O. dorsalis, O. melanimon, Cx. pipiens, Cs. incidens,* and several mosquitoes that could only be identified to the genus *Culiseta* (Table 2). Although still the dominant species trapped, the lowest proportion of *Ochlerotatus* mosquitoes was trapped at the Freshwater site, 41% *Ochlerotatus*, compared to other trap sites. At least 35% were *Culex* and 3% were *Culiseta* (Figure 37). However, we were unable to identify the remainder, even to genus, because of their poor condition.

Approximately 3 to 10 inches of standing water was visibly present in the cattails adjacent to the Freshwater trap during two sampling periods (September 25 and October 15). Larval mosquitoes were found in this standing water and averaged 2.1 larvae per 350-mL dip. In September, we sampled an average of 3.7 larvae per dip. None of the mosquitoes sampled at this location were successfully reared to emergence.

No mosquito larvae were observed or sampled at the Freshwater-East or Freshwater-West larval dip sites. The presence of threespine stickleback *(Gasterosteus aculeatus)* was confirmed in the FW-East borrow ditch. Sticklebacks will eat some mosquito larvae, although at least one controlled experimental study has determined that threespine sticklebacks (*Gasterosteus aculeatus)* are not effective for controlling larval mosquito populations (Offill and Walton 1999). Water salinity was 0 ppt at all three larval dip sites during each sampling period.

Only *O. dorsalis* were netted at the Freshwater site. Mosquitoes were generally abundant at this site, often aggressive, and easy to capture.

3.3.3 Natural Salt Marsh Reference Site and Previously Restored Salt Marsh Sampling Stations

3.3.3.1 Potter Slough

An average of 0.71 msq/h was trapped at Potter Slough, the natural reference marsh adjacent to the proposed Willapa River Estuary restoration site (Figure 35). This saltwater marsh is at a higher elevation than the proposed restoration site. Although its slough channels and the trap site are covered by water on a daily basis, the majority of the salt marsh is covered with water only during the highest tides. The only two mosquito species positively identified through trapping at Potter Slough were *O. dorsalis* and *Cx. pipiens* (Table 2). At least 87% were *Ochlerotatus* (Figure 37).

Immature mosquitoes were collected from a small pool of standing water at Potter Slough. The pool was not located until September 1, so was not sampled during the first two sampling periods. Samples obtained from this pond averaged 7.3 larvae per 350-mL dip, but ranged from 0 per dip on October 16, to 26.7 per dip on September 1. Salinities ranged between 18 ppt and 20 ppt. Mosquitoes reared to emergence from this pool were primarily *O. dorsalis*, though several were unidentifiable.

Two species of *Ochlerotatus (dorsalis* and *melanimon)* were netted at the Potter Slough reference site. These were the only *O. melanimon* positively identified at this site. In general, the mosquitoes at this site were both abundant and aggressive. In October, however, the number of adult mosquitoes had dwindled, and they were very difficult to find and to net. Even shaking the vegetation produced very few mosquitoes during the final sampling period.

3.3.3.2 North River

On average, only 0.06 msq/h were trapped at the North River restoration site, where a dike was breached two years previously (Figure 35). This site was also frequently inundated at high tide, and the vegetation was transitioning between freshwater and salt-tolerant species, with some bare muddy areas present.

Positively identified mosquito species trapped at North River included *O. dorsalis, Cx. pipiens,* and *Cq. perturbans* (Table 2). *Ochlerotatus* represented 67% of the mosquitoes trapped at North River, *Culex* represented another 25%, and *Coquillettidia* the remaining 8% (Figure 37).

Some standing water was present at North River during every sampling event, although several pools were completely dry during some periods (Figure 41). These salt pans occasionally held fish. Immature mosquitoes averaged 1.1 per 350-mL dip, and ranged from 3 per dip on August 10, to 0 per dip during the final three sampling periods (September 9, 25, and October 16 and 17). Salinities ranged from 6 ppt to 33 ppt (15 ppt average). No mosquitoes from North River were successfully reared to emergence.

Only *O. dorsalis* were netted at the North River restoration site. Relatively few adult mosquitoes were observed at this site, and they were usually not very aggressive. The vegetation often had to be disturbed (shaken, kicked) before the mosquitoes would fly at this site.

Figure 41. **Dry pool at North River that contained dead mosquito larvae and threespine sticklebacks.**

3.3.3.3 Elk River

The Elk River restoration area averaged 0.57 msq/h (Figure 35). This site has fully transitioned to salt-tolerant plant species but experiences only periodic flooding at the highest tides, which may occur several times per month. Positively identified species included *O. dorsalis, O. melanimon,* and *Cx. pipiens* (Table 2). At least 94% of all mosquitoes trapped at Elk River belonged to the genus *Ochlerotatus* (Figure 37), and 99% of the *Ochlerotatus* mosquitoes were *O. dorsalis.*

Elk River exhibited one of the highest densities of mosquito larvae at a site, peaking at 50.7 immature mosquitoes per 350-mL dip on August 10. The low-lying areas soon dried out, and on August 18, Elk River had its lowest density of immature mosquitoes: only 1.5 larvae per dip. Throughout the sampling period, Elk River low-lying areas averaged 13.6 larvae per dip. Salinities at these dip locations ranged between 12 ppt and 35 ppt (averaging 24 ppt). All of the mosquitoes successfully reared to emergence from dip samples at this site were *O. dorsalis.* Standing water was located across the highway from the

site, which may have produced the trapped adult *Culex* mosquitoes, though this cannot be confirmed because larval dip samples were not obtained from that area.

Only *O. dorsalis* were netted at the Elk River restoration site. Mosquitoes were generally very aggressive biters at this site. However, during site visits at the end of September and in October, the adults were observed in fewer numbers and did not swarm. Instead, the adults stayed close to the ground and vegetation surrounding the ponded water instead of flying up above the researchers' waists as they had earlier in the season.

3.4 Supplemental Data

3.4.1 Precipitation

We compared the monthly precipitation records (measured in Raymond, Washington) for year 2004 with the monthly average precipitation from the period of record (1980 to 2004) (Figure 42). During this study's period of sampling (August through mid-October), average precipitation was higher than normal, particularly during August and September. Thus, more seasonal standing water pools may have been available for mosquito production during our study period than would normally be present. A further look at daily precipitation values shows that there was an unseasonably high rainfall event on August 21 (Figure 43). It may be expected that periods of high rainfall will produce greater numbers of species associated with temporary water sources, such as *Coquillettidia, Culiseta,* and *Culex.* Thus, the high rainfall in August may have contributed to the higher catch rate for these species during sampling September 8 through 10 at the sites where rainwater had accumulated (e.g., Freshwater, South Bend-Alder House, and Inside Dike).

From the onset of the mosquito season in March or April through July (the period before our study commenced) precipitation was slightly below average for the region and, therefore, fewer of the abovementioned species may have been produced before this study began. However, while the abundance of the various species present during our sampling year may or may not be representative of normal rainfall years, the species that were captured during this study are probably representative of what would be present during other years, because of the variety of habitat types that were successfully sampled.

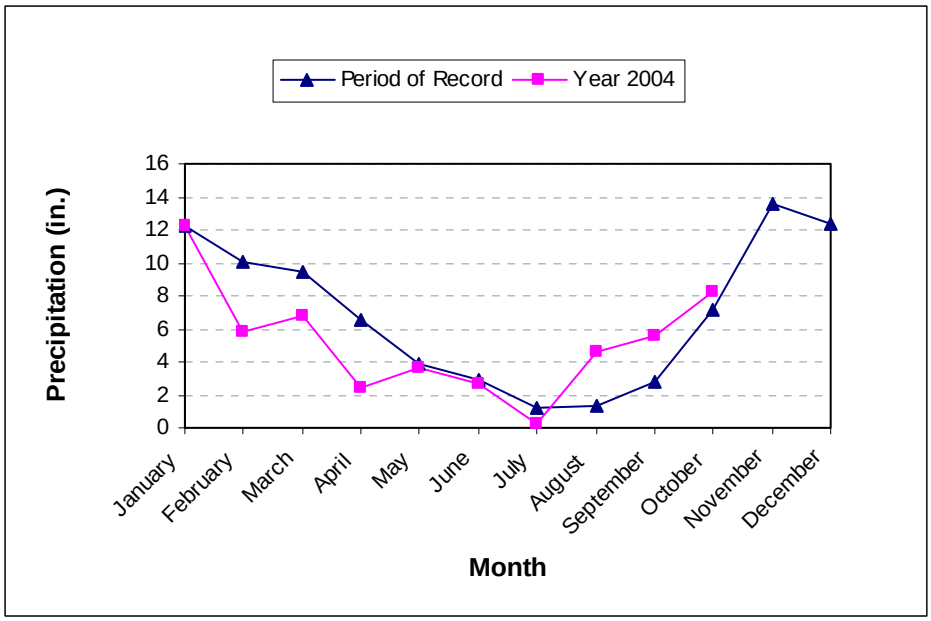


Figure 42. **A comparison of monthly average precipitation for Year 2004 and period of record (1980-2004) for Raymond, Washington.**

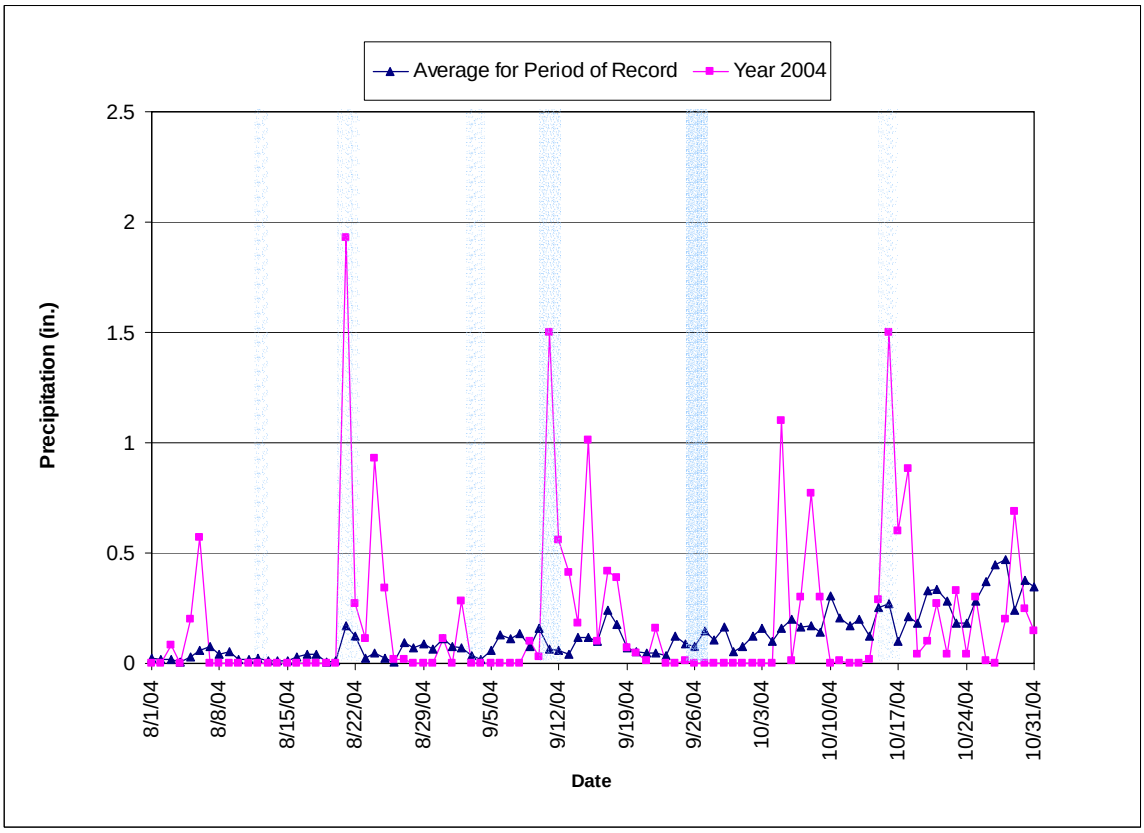


Figure 43. **A comparison of daily precipitation for Year 2004 and daily averages for the period of record (1980 – 2004) for Raymond, Washington.**
(Note: field sampling dates are represented by vertical bars.)

3.4.2 Tidal Cycles

We examined the tidal and lunar cycles for the Willapa River, South Bend, for our sampling period to note the lowest low and highest high tides and to determine the elevations that would provide suitable reproduction habitat for floodwater species, such as *O. dorsalis* (Figure 44).

The tidal marshes we sampled that were above +9 ft MLLW were inundated only periodically and thus provided suitable rearing habitat for the *Ochlerotatus* species. During the period of sampling, high tides exceeded +10 ft MLLW only twice, representing the maximum elevation that experienced inundation suitable for rearing habitat. We also looked at maximum tides for South Bend for the entire Year 2004 (Figure 45). During the active season for *O. dorsalis*, as early as March through October, tides were highest March through July, consistently reaching +10.5 ft to +10.9 ft MLLW. Assuming suitably warm temperatures, this time period may provide a larger area suitable for *Ochlerotatus* reproduction than we observed between August and October. Thus, greater numbers of *O. dorsalis* may have been present earlier in the season.

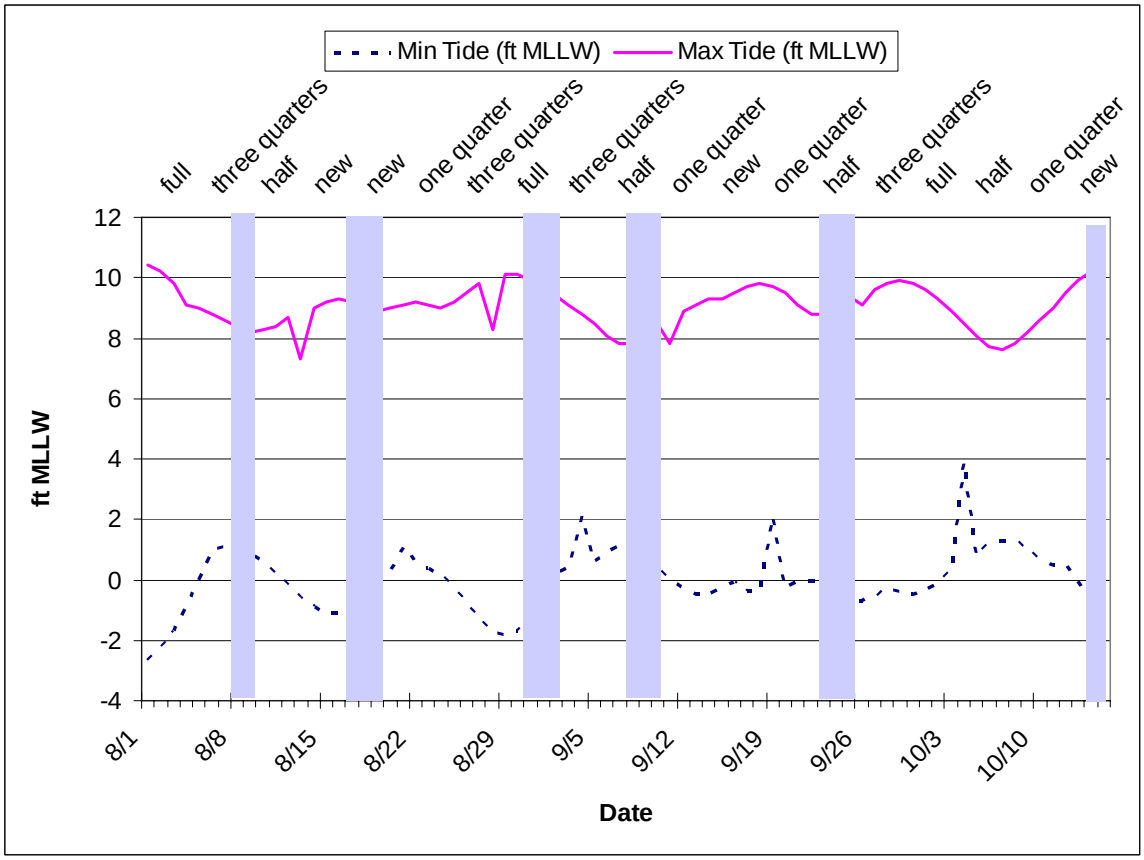


Figure 44. Maximum (high) and minimum (low) tides and lunar phase for the Willapa River at South Bend during mosquito sampling period.
(Note: field sampling dates are represented by vertical bars and lunar stages are provided across the top.)

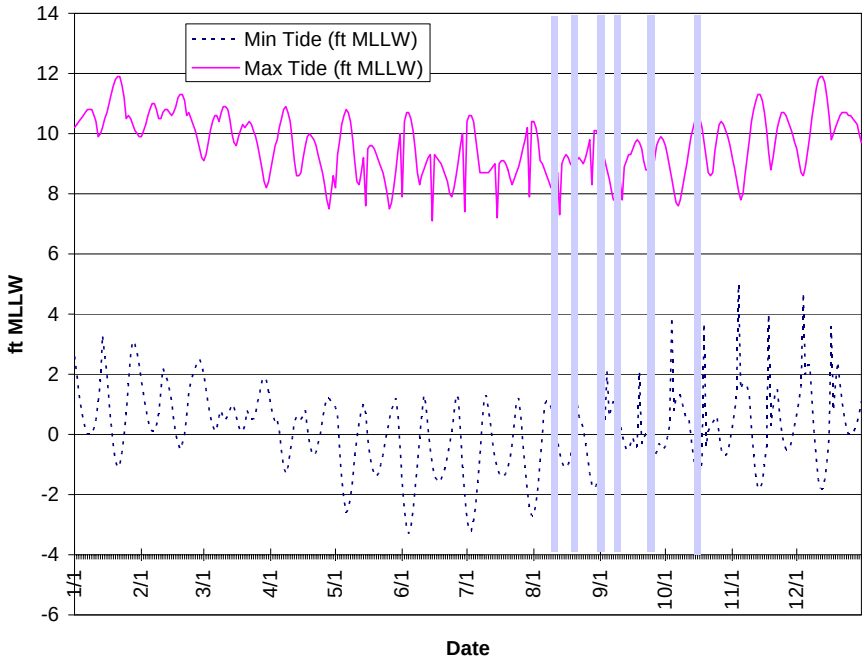


Figure 45. Maximium (high) and minimum (low) tides for the Willapa River at South Bend duringYear 2004.
(Note: field sampling dates are represented by vertical bars.)

3.4.3 Temperature

Both water and air temperature can play a significant role in regulating mosquito populations. Mosquitoes cannot regulate body heat, and few species can function below 50ºF (10ºC) (Crans 2003). Onset of cool weather in the fall will induce hibernation. For those species overwintering as adult females or as larvae in northern climates, a sufficient increase in temperatures in the spring enables the mosquitoes to emerge and either seek a blood meal to develop the eggs that produce the new generation of adults, or to complete their larval development and subsequent life stages. During the course of our sampling period, daytime temperatures were consistently above 50ºF (10ºC), and only during the last part of the study did nighttime temperatures consistently drop below this threshold (Table 3).

We compared monthly average temperatures over our study period with the average highs and lows for the entire period of record to determine whether anomalies (e.g., significantly low temperatures) occurred during our sampling period that may have affected the number of mosquitoes collected. In general, average minimum temperatures were only slightly higher than period-of-record averages (Figure 46). Daily temperatures recorded during our sampling period did not deviate remarkably from period of record daily averages (Figure 47). Thus the number of mosquitoes collected during the 2004 sampling period would not be expected to be significantly different than average years based on temperature.

Table 4. Maximum and minimum air temperatures recorded at Raymond, Washington, during mosquito collection dates in 2004.

Sampling Date	Maximum Temperature F (C)	Minimum Temperature F (C)
8-Aug	72 (22)	48 (9)
9-Aug	93 (34)	52 (11)
17-Aug	75 (24)	61 (16)
18-Aug	73 (23)	56 (13)
19-Aug	76 (24)	56 (13)
31-Aug	75 (24)	52 (11)
1-Sep	71 (22)	54 (12)
2-Sep	67 (19)	52 (11)
8-Sep	72 (22)	48 (9)
9-Sep	68 (20)	53 (12)
10-Sep	69 (21)	45 (7)
24-Sep	67 (19)	42 (6)
25-Sep	72 (22)	44 (7)
26-Sep	63 (17)	46 (8)
15-Oct	58 (14)	46 (8)
16-Oct	57 (14)	48 (9)
17-Oct	57 (14)	43 (6)

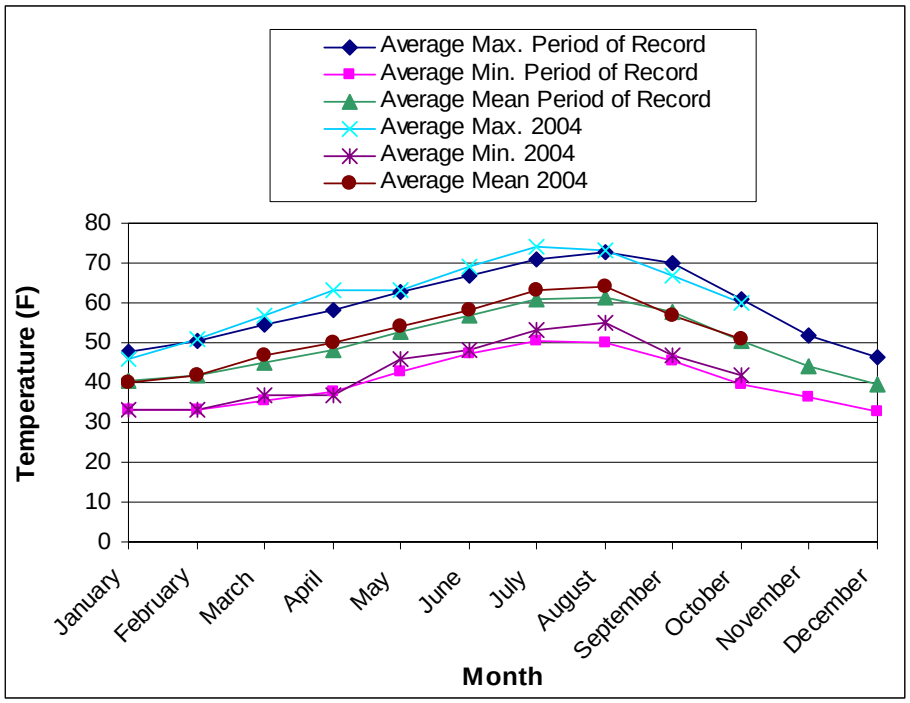


Figure 46. A comparison of monthly average temperatures for Year 2004 and for the period of record (1980 to 2004) for Raymond, Washington.

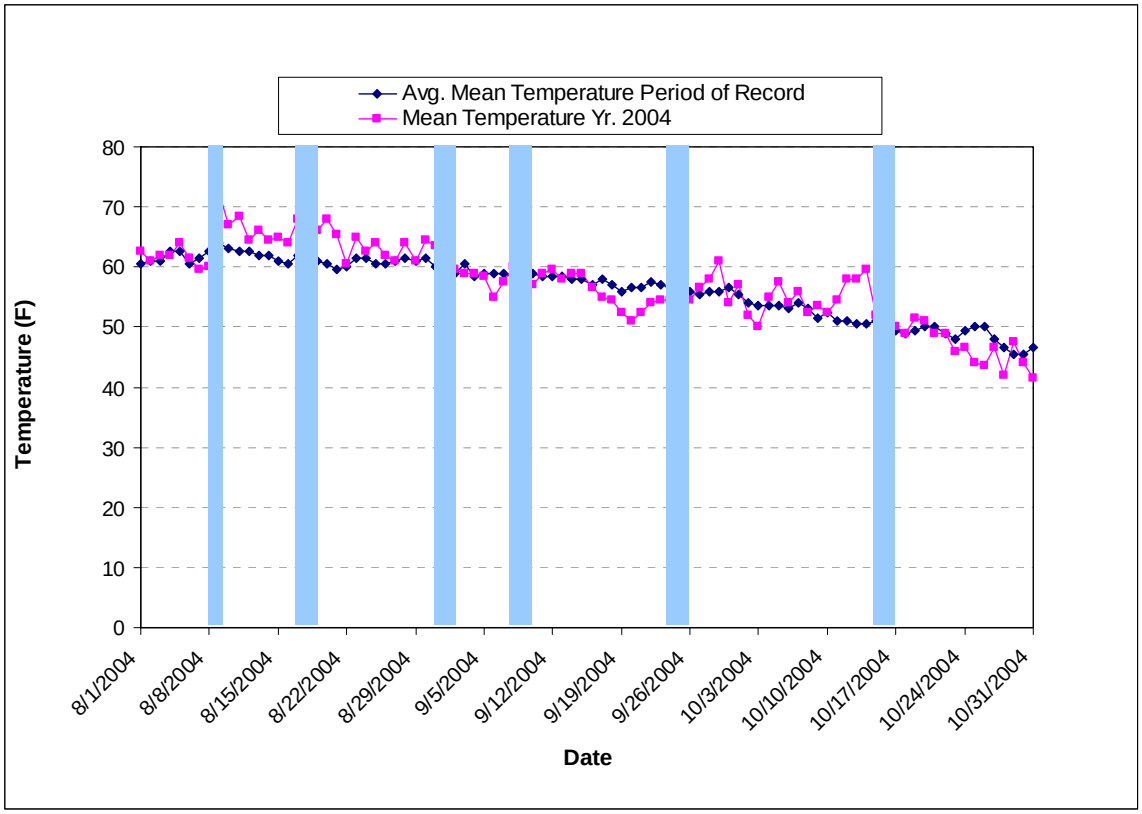


Figure 47. **A comparison of daily average temperatures for the Year 2004 sampling period and for the period of record (1980-2004) for Raymond, Washington.**
(Note: field sampling dates are represented by vertical bars.)

3.4.4 Wind Speed and Direction

We examined maximum and average wind speeds (measured at Hoquiam, Washington) likely to be encountered in the South Bend area (Table 4). The maximum and average speeds recorded would likely restrict upwind movement of mosquitoes (anything greater than 5 mph [8 km/h]) but could enhance downwind dispersal.

We also looked at wind direction for the period of May through October 2004, when mosquitoes are likely to be most active. Prevailing winds were from the west and southwest (Figure 33) and were very similar from May through October over the entire period of record. These data may indicate that wind-induced dispersal from the restoration site is likely to occur more often towards the east and, therefore, toward the City of South Bend. However, regardless of wind speed and direction, it should be noted that the proposed restoration site, about 1.5 miles (2.4 km) from the City of South Bend, is within flying distance of most mosquito species, even unaided by the wind (Table 6).

Table 5. Wind speed summary for Year 2004 measured at Hoquiam, Washington.

2004 Wind Speeds (MPH)			
	Maximum	Average	Standard Deviation
January	32.0	11.2	5.4
February	26.0	10.5	4.3
March	33.0	10.8	5.2
April	26.0	9.2	4.1
May	18.0	9.5	3.9
June	21.0	9.1	3.8
July	20.0	9.1	3.7
August	29.0	9.3	4.4
September	23.0	8.6	4.2
October	28.0	8.7	4.3
November	29.0	8.7	5.1
December	Incomplete	Incomplete	Incomplete

4.0 DISCUSSION and RECOMMENDATIONS

The mosquito monitoring performed for this study provides insight into which mosquito species are most likely to breed at the project site after restoration is complete and how their abundance, at the restoration site and in the City of South Bend, may change following restoration. This section of the report includes a discussion of WN virus and provides life history information for mosquito species considered most likely to transmit WN virus to humans in Western Washington. Predictions are then made regarding mosquito population dynamics and the potential to alter exposure of South Bend residents to the West Nile virus following restoration. Finally, the report concludes with recommendations regarding supplemental mosquito monitoring and project design.

4.1 West Nile Virus and Potential Mosquito Vectors in Pacific County, Washington

4.1.1 West Nile Virus

4.1.1.1 Human Health Risk

West Nile virus, originally from Africa, Asia, Europe, and Australia, has recently spread to North America. It was first detected in New York City in 1999. Since that time, it has extended its range and has been detected in some form in all of the lower 48 States. In the State of Washington, WN virus has not yet been detected in humans; the virus has been detected only in two birds and two horses during 2002, from Island, Pend Oreille, Snohomish, and Whatcom Counties (WDOH 2004b).

Although the risk of human infection with WN virus is generally low in the United States, the perceived threat of infection may cause public alarm and demand for public health action (Knight et al. 2003). Officially, risk is the probability that a harmful consequence will occur as a result of some action that has occurred (e.g., the probability that a person will die from WN virus as a result of wetland restoration). In traditional environmental risk assessment, risk is a function of the hazard and a person's exposure to that

hazard. There must be both a hazard (e.g., WN virus) and exposure (e.g. mosquito-to-human transfer of the virus) for there to be a quantitative risk (i.e., Risk = Hazard x Exposure). A risk assessment is the process that evaluates and predicts the likelihood and the extent of harm that may result from a hazard.

The following example illustrates how a simple calculation of risk is performed. Assuming that automotive deaths will be about the same from one year to the next, one can predict the risk of dying in an automobile accident in a future year. If 43,000 out of a population of 281 million died the previous year, then the rate of death during the following year would be predicted to be approximately 43,000/281 million, or 15 in 100,000 (Evans 2002). Human health risk assessment calculations are generally much more complicated because there are many factors involved, each with areas of uncertainty. An assessment performed by O'Leary et al. (2004) based on data from 39 states with human cases of WN virus in 2002, estimated the relative risk of a person contracting a neuroinvasive WN virus illness as 12 incidences per 1 million people, in those states.

Kilpatrick et al. (2005) recently proposed that the risk that a mosquito species will infect a human with WN virus can be estimated as

$$\text{Risk} = A \times F_m \times P \times C_v$$

where A is abundance, F_m is the fraction of blood meals taken from mammals, P is the WN virus infection prevalence, and C_v is an index of vector competence (the fraction of WN virus-infected mosquitoes that will transmit virus in a subsequent bite). Even if this information were available for mosquitoes in Pacific County, additional variables would have to be considered to make a thorough assessment of risk from WN virus to the residents of South Bend. Some of the other factors to consider are listed below:

- The mosquito (vector) distribution and abundance following the proposed salt marsh and freshwater wetland restoration (unknown)
- The probability of an encounter between an infected mosquito and a person (difficult to assess, but time of day is a factor)
- The age of the person bitten by a mosquito (risk of severe complications is greater for those over age 50; census data available for the City of South Bend for the year 2000 indicate this includes >30% of the total population of approximately 1800 people) (U.S. Census Bureau 2000).
- The percentage of WN virus-infected people that show symptoms
- The percentage of people that die from WN virus (generally less than 1% of people with WN virus get a severe infection).

Because WN virus is, as yet, undocumented in humans in Washington, a quantitative estimate of risk as a result of this wetland restoration project cannot be performed. In other words, the prevalence of WN virus (in humans) in Washington, to date, is zero. The virus has not been detected in humans in neighboring British Columbia, Canada, but has been documented in humans in the neighboring states of Oregon and Idaho. During 2002, two birds and two horses in Washington were also positively infected. In 2002, 77% of counties nation-wide reporting non-human infections never reported human illnesses, while 89% of all counties nation-wide that reported neuroinvasive WN virus had first documented infections in non-human animals (O'Leary et al. 2002). Therefore, the possibility that WN virus will affect some people in Washington in the future cannot be dismissed. To provide some measure of the potential for exposure to WN virus, we assessed the potential for the project to either increase or decrease the number of possible WN virus vectors. Combined with information on the potential of the mosquito species present at the restoration site to act as vectors of WN virus to humans, we can qualitatively evaluate the potential impacts of the wetland restoration activity relative to the potential for human exposure to WN virus in the City of South Bend.

4.1.1.2 West Nile Virus Epidemiology and Virology

A review report by Campbell et al. (2002) states that in humans, most WN viral infections are symptomless. The incubation period is approximately 2 to 14 days for those people who do show symptoms. However, the clinical symptoms are non-specific and a laboratory analysis is required to confirm a diagnosis. Individuals experience different levels of impact from the virus. Most experience no symptoms whatsoever, but others will contract WN fever (a flu-like condition), WN meningitis (inflammation of the lining of the brain and spinal cord), or WN encephalitis (inflammation of the brain). WN virus symptoms vary from headache, sore eyes, high temperature, and stiff neck to muscle weakness and paralysis, disorientation and coma. The Centers for Disease Control and Prevention (CDC 2003) report that most WN infections in humans are mild and do not manifest symptoms of illness. In general, approximately 20% of infections develop WN fever. Severe infections occur in about 1 in 150 cases (or 0.7% of people with the virus). Neurological disease, encephalitis, paralysis, rash, and sometimes death occur from severe infections.

Fatalities from the virus are most often associated with WN encephalitis; however most patients who contract this disease do survive. Mortality from WN encephalitis increases significantly with age, with those over 50 being at greatest risk for severe disease (Hubálek and Halouzka 1999). The median age for severe illness is reported to be around age 70. There is no human vaccine for WN virus available, although several laboratories are conducting vaccine research. Because the incidence of WN virus in humans in most areas is very low, it is generally felt that such a vaccine would not be cost-effective for public health use. To put the risk of dying from WN virus in perspective, we compare it with the risk of dying from influenza, or the flu. The CDC states that, although most people recover from influenza, more than 100,000 people are hospitalized and about 36,000 people die from the flu and its complications every year (NIAID 2004). In contrast, there were 2448 reported cases of WN virus in the United States in 2004, resulting in 87 deaths (CDC 2004).

4.1.1.3 West Nile Virus Cycle in Nature

The WN virus is maintained in nature through a mosquito-to-bird-to-mosquito transmission cycle. Mosquitoes of the genus *Culex* are most often implicated as vectors (organisms that carry or transmit pathogenic agents) (Lincoln et al. 1998), but the virus has been isolated from at least 10 genera (>40 mosquito species) in the United States (Turell et al. 2005). Birds can be infected when a mosquito carrying the virus takes a blood meal from the bird. Likewise, an uninfected mosquito can become infected with the virus if it takes a blood meal from an infected bird. WN virus has been found in at least 200 bird species in North America. The virus was shown to persist in the organs of inoculated ducks and pigeons for 20 to 100 days, suggesting migratory birds can be instrumental in the introduction of the virus to temperate climates during spring migrations (Hubálek and Halouzka 1999). Most birds survive their WN viral infection and develop permanent immunity. However, some individuals become ill and die, and some species are more susceptible than others. In the United States, crows and jays are used in dead-bird-based surveillance programs that detect and track the spread of WN virus.

Some mammals (mostly equines) and humans become infected with WN virus when they are bitten by an already-infected mosquito. Fortunately, mammals are generally considered dead-end hosts, meaning they do not pass the virus to uninfected individuals (including mosquitoes). Although it may not be common, a summary report by Hubálek and Halouzka (1999) reported that frogs can harbor the virus and their donor ability to *Cx. pipiens* has been confirmed, thereby adding another potential player to the WN transmission cycle.

When considering the potential for WN virus to be found in a mosquito population, it is essential to know which mosquito species are present locally. It has been well-documented that different mosquito species

are infected with the virus at different rates. Likewise, the various mosquito species transmit the virus to their hosts at different rates. The "competence" of many mosquito species to pass WN virus to humans, as tested in the lab, has been ranked as low, moderate, and high (Goddard et al. 2002) (Table 7).

Besides vector competence, other factors that may indicate which mosquito species are likely to be important in WN virus transmission include the species' generally preferred hosts, flying ability, breeding life cycle, and preferred habitat. Although nectar is the primary food source for all mosquitoes, the females also take blood to develop their eggs (EPA 2004). Mosquito species that prefer to take their blood meal from birds are more likely to become infected with WN virus than mosquito species that prefer to take their blood meal from mammals.

4.1.2 Potential Mosquito Vectors in Pacific County, Washington

The presence and abundance of mosquito species is primarily determined by environmental factors, such as temperature of air and water, alkalinity or acidity of the water, and associated vegetation and shelter (Stage et al. 1952). Whereas some species have very specific habitat requirements, others are generalists (Table 5). *Cx. tarsalis*, for example, has been found in essentially every water type throughout the Pacific Northwest. *O. dorsalis*, though considered a salt marsh species, breeds extensively in fresh water as well.

The permanence of the water source may also determine the number of generations produced each year. In both temporary and permanent waters, the mosquito species present may change with seasonal changes in the vegetation and water quality. In general, species may be grouped in associations based on where they deposit their eggs, overwintering strategies, preferred hosts, and number of generations produced each year (Stage et al. 1952; Schäfer 2004) (Figure 48). The species in Group 1c contain mosquitoes that are efficient vectors of West Nile virus, in part due to their preference for bird hosts. The species in Group 2 are commonly called floodwater mosquitoes. These are the major nuisance species because of their capacity to produce multiple generations that can result in very large numbers of individuals throughout the entire summer season (Schäfer 2004). Floodwater mosquitoes are adapted for life in temporary habitats. Some produce drought-resistant eggs that can survive for several years until the right conditions cause the eggs to hatch. Other floodwater species are able to accelerate larval development in response to diminishing (drying) habitat. Even after a pool has dried up, some larvae are able to survive in humid soil for periods up to two weeks; long enough for the adults to successfully emerge (Schäfer 2004).

Dispersal of mosquito species is determined by flight capabilities, population pressure, food availability, and wind. Flight capability varies with species and with individuals (Table 5). For example, *O. dorsalis* has been found to travel 22 miles in Utah and in excess of 30 miles in California (Rees and Nielsen 1947 in Romanowski 1989). Conversely, *Cx. pipiens* is considered a weak flier and may remain within a few hundred meters of its larval habitat (Stage et al. 1952). Wind may either increase or decrease the flight range of mosquitoes. Bailey et al. (1965, in Gjullin and Eddy 1972) found *Cx. tarsalis* traveled downwind up to 10 miles within 48 hours with wind speeds of 2 to 7 mph and traveled downwind up to 15.75 miles at wind speeds of 0.3 to 3.5 mph. However, they point out that *Cx. tarsalis* traveled in significant numbers only 2 to 3 miles downwind over 24 hours and only 7 miles over 48 hours aided by wind. Upwind flight was limited against winds as low as 5 mph.

Table 6. Biological data on mosquito species found during sampling period or considered likely West Nile virus vectors in Western Washington.

Species	Larval Habitat	Flight Range *	Peak Biting Activity
Aedes vexans[‡]	Almost any transient water; rainpools in unshaded areas, grassy pools bordering wooded areas, roadside ditches, and vernal pools in open fields.	> 10 miles	Day and dusk
Culex pipiens	Polluted water; artificial containers (storm drains, wastewater ponds, sumps, septic tanks)	< 1 mile	Night
Culex tarsalis[‡]	Irrigated pastures, riparian, woodland pools, foul water, irrigated pastures, salt marsh (salinity up to 10 ppt), freshwater marsh, permanent pools	1 -2 miles	Night
Culiseta incidens	Wide range of fresh and brackish water (isolated creek pools, artificial containers, fish ponds, abandoned swimming pools, water gardens, etc.)	< 5 miles	Daytime in shaded areas and night
Culiseta inornata	Wide range of habitats; marshes (salt and fresh), seepages, ditches, canals, ponds; salinity up to 26 ppt.	< 5 miles	Overcast days, dusk, and night
Culiseta particeps	Cut-off pools of streams and shallow margins of *Typha* spp.-filled pools in wooded and semi-wooded habitat	?	?
Coquillettidia peturbans	Marshes, swamps, or log ponds containing cattails, sedges, or other suitable plant hosts	1 – 2 miles	Dawn and dusk
Ochlerotatus dorsalis	Coastal brackish and salt marshes, inland alkaline areas	< 10 miles	Day and night
Ochlerotatus melanimon	Irrigated pastures and fields, duck club ponds	> 10 miles	Day and dusk

[‡]Species not collected during the present study but indicated as likely WN virus vectors in Western Washington (WDOH 2004a).

[*] Estimates of normal flight range. Seasonal dispersal may be 10 times these values in some species.

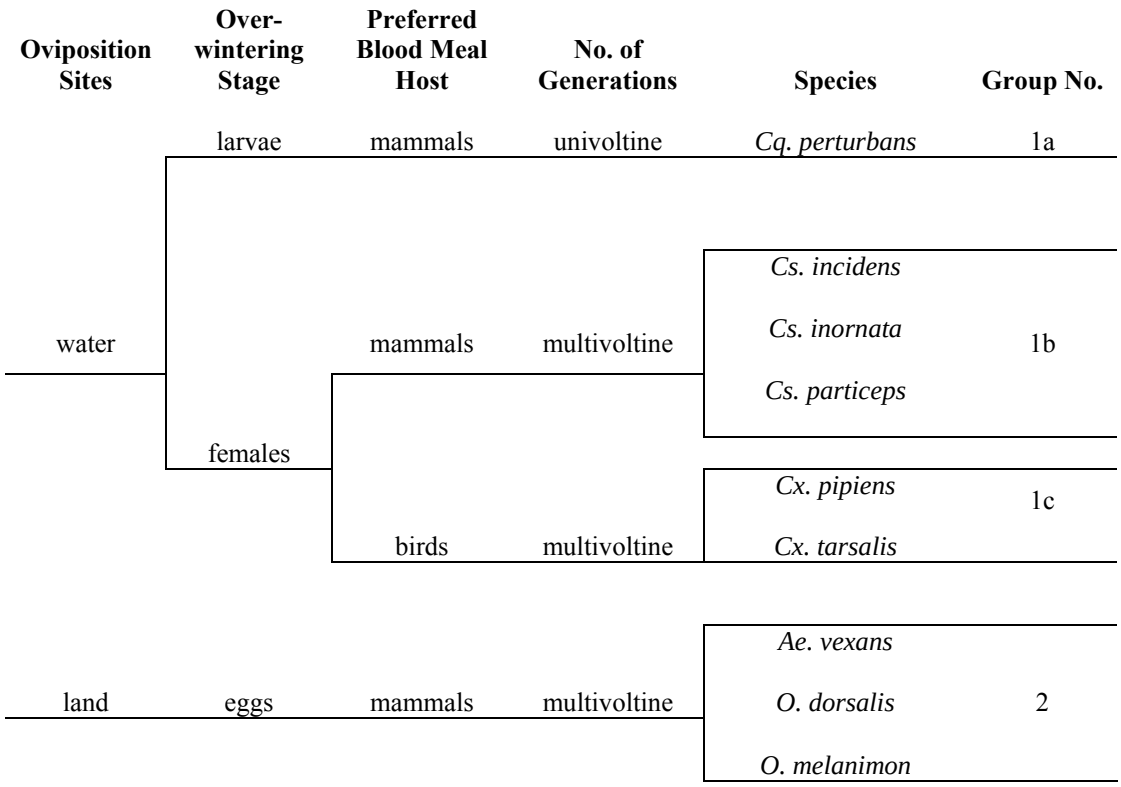


Figure 48. Mosquito species found during the sampling period or considered likely West Nile virus vectors in Western Washington organized into four functional groups based on four biological characteristics.

The WDOH reports that approximately 50 mosquito species of six genera have been identified in the State of Washington. Seventeen mosquito species have been reported from Pacific County (WDOH 2004a, Table 7). Through larval dipping, we confirmed the presence of one additional mosquito species in Pacific County, *Cs. inornata*. In their 2003 report, the WDOH indicated that WN virus has been isolated from several of these species and/or that WN viral ribonucleic acid (RNA) was detected in some of these species in parts of the United States where WN virus is present. However, it is important to note that actual mosquito vector species in the State of Washington can only be determined after the virus is present in humans. Additional studies indicate that other mosquito species are competent for WN virus transmission, and these are also indicated in Table 7.

Table 7. Mosquito Species Documented in Pacific County by the Washington State Department of Health and from this Study

Mosquito Species	Pacific County Species[(a)]	Species Identified During This Study	Potential WN Virus Vector	Efficiency of WN Virus Transmission	Feeding Preference, Biology[(e)]
Aedes cinereus	●		● [(a)]	unknown	mostly mammals[1]
Aedes vexans	●		● [(b),(c),(e)]	low to moderate	mostly mammals[1]
Ochlerotatus aboriginis	●			no information	no information
Ochlerotatus dorsalis	●	●	● [(c)]	moderate	mammals, occasionally birds[1]
Ochlerotatus fitchii	●		● [(e)]	unknown	mostly mammals[1]
Ochlerotatus increpitus	●			low	mammals[?]
Ochlerotatus melanimon	●	●	● [(c)]	moderate	mammals, occasionally birds[1]
Ochlerotatus sierrensis	●		● [(c),(e)]	low	mammals[2]
Ochlerotatus sticticus	●		● [(e)]	low	mammals[1]
Coquillettidia perturbans	●	●	● [(b),(d),(e)]	low	birds, mammals[3]
Culex pipiens	●	●	● [(b),(c),(e)]	moderate to high	mostly birds[4]
Culex tarsalis	●		● [(b),(c),(e)]	high	birds, mammals[4]
Culex territans	●		● [(e)]	unknown	mostly amphibia[4]
Culiseta impatiens	●			unknown	mostly mammals[5]
Culiseta incidens	●	●	● [(e)]	low to moderate	mostly mammals[4]
Culiseta inornata	● [(f)]	●	● [(b),(c),(e)]	moderate	birds, mammals[4]
Culiseta minnesotae	●			no information	no information
Culiseta morsitans	●			moderate	mostly birds[1]
Culiseta particeps	●	●		unknown	mammals[4]

Note: The species not previously documented by the WDOH is in bold font; species documented during this study with at least some potential to be WN virus vectors are shaded.

(a) From Appendix 1 of WDOH (2004a). Mosquito species identified in Washington State, November, 2002.
(b) From Appendix A of WDOH (2003). Potential West Nile virus mosquito vectors in Washington, November, 2002.
(c) From Goddard et al. (2002). Vector competence of California mosquitoes for West Nile virus
(d) From Appendix 2 of WNVWG (2003). Vectoral competence of WNV positive mosquitoes known to occur in Saskatchewan
(e) From Belton (2003)
(f) Not documented as present in Pacific County by WDOH (2004a); identified only by the present study
1 Eggs overwinter, one or two generations per year
2 Eggs and larvae overwinter, several generations per year
3 Larvae overwinter, one generation per year
4 Females overwinter, several generations per year
5 Females overwinter, one generation per year

In summary, six of seven mosquito species captured during this study have at least some potential as WN virus vectors, although most are considered low to moderately efficient vectors (Table 2 and Table 6). Further descriptions of the mosquito species captured during this study are presented below. The species *Aedes vexans* and *Culex tarsalis* are also included, because they have been found by other researchers in Pacific County and are assumed to be two of the four species with the greatest potential to be a WN virus vector in Western Washington (WDOH 2004a). *Cx. pipiens* and *Cq. perturbans*, captured during this study, are the other two species considered most likely to transmit WN virus to humans in Western Washington.

4.1.2.1 Aedes vexans

This "inland floodwater mosquito" is a multivoltine (having several broods or generations per year) species found in a variety of transient water sources (e.g., flooded woodland river bottoms, irrigated pastures, stormwater management ponds, salt marsh impoundments, ditches, dredge spoil sites). However, sunlit rain pools produce the largest broods (Meyer and Durso 1998; Gjullin and Eddy 1972). Overwintering is in the egg stage, and most eggs hatch after the first flooding, some in subsequent floodings. Females feed readily on humans and livestock, but will apparently take advantage of any host available (O'Malley 1990). They feed primarily at night but are known to feed during the day in shaded areas. They have been found infected with Western Equine Encephalitis (WEE) in the wild and have been shown to transmit St. Louis Encephalitis (SLE) in the laboratory (Gjullin and Eddy 1972; O'Malley 1990).

Although not observed during the present study, this species is one of four considered to have the greatest potential to transmit WN virus to humans in Western Washington (WDOH 2004a) and has been documented previously in Pacific County.

4.1.2.2 Culex pipiens

Larvae of this "northern house mosquito" occur in a variety of foul water sources that are high in organic content (i.e., septic tanks, dairy ponds, catch basins). *Culex* species require pooled water to lay their eggs. Eggs hatch within 2 to 4 days and will complete the larval and pupal stages to become adults within about 4 to 14 days, depending on temperature. Therefore, precipitation is critical to creating a variety of temporary water sources that provide suitable reproduction habitat. It may be expected that high rainfall years will produce greater numbers of these mosquitoes. Overwintering is as adult females. Females feed at dusk or later and will aggressively seek out a blood meal. They prefer avian hosts, though they may feed on large mammals, including man (Meyer and Durso 1998). *Culex* species are not considered strong fliers but have been known to fly up to 2 miles (American Mosquito Control Association 2003). Some populations may diapause (a resting phase) or be autogenous (having females that are not required to feed in order to facilitate maturation of eggs). *Cx. pipiens* was known to be a primary vector of WN virus during the 1999 outbreak in New York (Goddard et al. 2002). They are also known to be secondary vectors of the SLE virus.

This species is one of four considered to have the greatest potential to transmit WN virus to humans in Western Washington (WDOH 2004a). It was the second dominant species captured during the present study, comprising 14% of all mosquitoes trapped, and was found at every site. It was trapped at the highest rates at the Freshwater, South Bend-Alder House, Outside Dike, and Wastewater sites, respectively (Figure 31). This species was successfully reared to emergence from dip samples obtained in September and October from a small rainwater pool at the Wastewater site (0 ppt) that was surrounded by *J. effuses* and blackberry bushes. *Cs. particeps* was collected from the same small pool. *Cx. pipiens* was also collected from the east-side borrow ditch inside the dike during September. Salinity was 0 ppt in the borrow ditch, and the water was shallow (<4 in.) and covered with algae, other thick floating and rooted aquatic vegetation (including pondweed, *J. effusus,* and cattail), as well as detritus.

4.1.2.3 Culex tarsalis

Known as the "western encephalitis mosquito," larvae of this species are tolerant of a wide range of water conditions but can be particularly abundant in agricultural sources, alkaline ponds, fresh and saltwater marshes, and secondary treated sewage effluent (Reisen 1993). *Culex* species require pooled water to lay their eggs. Eggs hatch within 2 to 4 days and will complete the larval and pupal stages to become adults within about 4 to 14 days, depending on temperature. Therefore, precipitation is critical to creating a variety of temporary water sources that provide suitable reproduction habitat. It may be expected that high rainfall years will produce greater numbers of these mosquitoes. Females feed mostly at dusk, preferring avian hosts early in the season but often switching to mammals in summer when adult populations are abundant. Like the *Cx. pipiens*, some populations may be autogenous and diapausing.

Cx. tarsalis is considered the most important vector of arboviruses in western North America (Reisen 1993) and was found experimentally to be one of the most efficient WN virus vectors tested in North America (Goddard et al. 2002). Thus, it is included as one of the four species considered to have the greatest potential to transmit WN virus to humans in Western Washington (WDOH 2004a). It was not observed during the present study but has been documented previously in Pacific County.

4.1.2.4 Culiseta incidens

Known as the "cool weather mosquito," this large species is most abundant during cooler months of the year. Breeding sources include such areas as brackish water, snow pools, and artificial containers. *Culiseta* species require pooled water to lay their eggs. Eggs hatch within 2 to 4 days and will complete the larval and pupal stages to become adults within about 4 to 14 days, depending on temperature. Therefore, precipitation is critical to creating a variety of temporary water sources that provide suitable reproduction habitat. It may be expected that high rainfall years will produce greater numbers of these mosquitoes. Overwintering in the mountains or aestivating (passing the summer or dry season in a dormant or torpid state) in lower elevations occurs as adult females. Females actively feed at dawn and dusk on a variety of mammals and are occasionally considered localized pests. *Cs. incidens* is a vector of WEE, SLE and Japanese B encephalitis (JBE) under laboratory conditions.

This species comprised less than 1% of the mosquitoes captured during the present study. This species was captured in the Freshwater trap on September 2 and was also found in an early September dip sample taken from the east-side borrow ditch located inside the dike on the north side of U.S. 101. Salinity was 0 ppt in the borrow ditch, and the water was shallow (<4 in.) and covered with algae, other thick floating and rooted aquatic vegetation (including pondweed, *J. effusus,* and cattail), as well as detritus. Because they feed primarily on mammals, however, this species is primarily considered to be a nuisance or biting pest rather than a threat for conveying WN virus to humans.

4.1.2.5 Culiseta inornata

Widely distributed, this species is prevalent in poorly drained irrigated areas. Larvae occur in a variety of sunlit pools, including duck club ponds, irrigation ditches, rain pools, and salt marshes (Meyer and Durso 1998). *Culiseta* species require pooled water to lay their eggs. Eggs hatch within 2 to 4 days and will complete the larval and pupal stages to become adults within about 4 to 14 days, depending on temperature. Therefore, precipitation is critical to creating a variety of temporary water sources that provide suitable reproduction habitat. It may be expected that high rainfall years will produce greater numbers of these mosquitoes. Overwintering in the mountains or aestivating in lower elevations occurs as adult females. Females actively feed at dawn and dusk primarily on large domestic animals and humans. It is not considered a serious pest of man, but has been found infected with a California encephalitis (CE) group virus in the wild (Meyer and Durso 1998) and is moderately effective at transmitting WN virus in the laboratory (Goddard et al. 2002).

This species comprised less than 1% of the mosquitoes trapped during the present study. This species was found in an October 16 dip sample taken from the west-side borrow ditch located inside the dike on the north side of U.S. 101, where water salinity was 0 ppt. The water was thick with vegetation, including duckweed and cattails. The single large *Cs. inornata* pupa discovered was dipped from a stand of reed canary grass. Other aquatic organisms dipped from that site at the same time included snails, isopods, several mayfly species, and a stonefly. The presence of the mayflies and the stonefly are indicative of overall good water quality.

4.1.2.6 Culiseta particeps

Not a common species in the Northwest, it is found in greater numbers in California, localized in riparian areas and along the coast (Gjullin and Eddy 1972). Larvae occur in wooded and semi-wooded habitat along margins of shaded, clear pools often containing cattails, algae, leaves, and other debris. *Culiseta* species require pooled water to lay their eggs. Eggs hatch within 2 to 4 days and will complete the larval and pupal stages to become adults within about 4 to 14 days, depending on temperature. Therefore, precipitation is critical to creating a variety of temporary water sources that provide suitable reproduction habitat. It may be expected that high rainfall years will produce greater numbers of these mosquitoes. They overwinter or aestivate as adult females. Females prefer large mammals and may feed on humans in shaded places. Adult populations are seldom abundant enough to create a nuisance problem. Little is known about their vector competence (Alameda County Mosquito Abatement District 2004).

This species comprised less than 1% of the mosquitoes trapped during the present study. It was reared to emergence from larval dip samples collected on September 25 and October 16 at the Wastewater site. The pupae were collected from the small pool immediately under the Wastewater trap (0 ppt) that was surrounded by *J. effusus* and blackberry bushes. *Cx. pipiens* was also collected and reared to emergence from the same small pool.

4.1.2.7 Coquillettidia perturbans

Larvae of this species occur in permanent freshwater marshes with emergent cattails, sedges, and tules, as they are dependent on vegetation as host plants during the larval stage. Habitat destruction can make them regionally extinct. *Coquillettidia* require pooled water to lay their eggs. Eggs hatch within 2 to 4 days and will complete the larval and pupal stages to become adults within about 4 to 14 days, depending on temperature. Unlike most mosquito taxa, overwintering is as mature larvae. Females aggressively feed on birds and mammals after sunset, but will also bite during the day. They are localized pests in some areas. They have been shown to transmit eastern equine encephalitis in the wild (Gjullin and Eddy 1972) and are considered potential vectors of WN virus (WDOH 2003). This species is one of four considered to have the greatest potential to transmit WN virus to humans in Western Washington (WDOH 2004a).

This species comprised less than 1% of the mosquitoes trapped during the present study. Two specimens were collected, both on September 1. The first was collected at the North River site. Tidally flooded pools were filled with approximately 4-in. deep water but few larvae were observed. It is unclear whether these larvae were *Cq. perturbans,* as none were successfully reared to emergence, and other species (i.e., *O. dorsalis* and *Culex* sp.) were previously captured at North River. No readily apparent sources of permanent freshwater (preferred *Cq. perturbans* breeding habitat) were present at North River, although lower salinity sources of ponded water were located at the foot of the bank, farthest from the river. Salinities were only 2 ppt in these ponds, indicating they were filled primarily with rainwater and probably only received tidal input during extreme tides. No immature mosquitoes were found in those areas, however, when they were discovered and sampled September 25.

The second *Cq. perturbans* was captured at the South Bend-Alder House site, which was surrounded by blackberry bushes, morning glory, grasses, and bracken fern. The nearest standing water visible at this

site was a surface water runoff pond near the base of the driveway leading to the assisted living facility. This small catchment pond was approximately 16 ft x 33 ft (5 m x 10 m) in size and was surrounded by *J. effusus,* alder, and willow. Salinity was 0 ppt. Unfortunately, it was only discovered and sampled for larvae on October 16. No immature mosquitoes were found.

4.1.2.8 Ochlerotatus dorsalis

O. dorsalis is one of the most widely distributed species in the Northwest (Gjullin and Eddy 1972). It can breed in large numbers throughout the spring, summer, and fall and can produce viable offspring in both salt and fresh water. Female salt marsh mosquitoes lay their eggs on moist substrates in shallow depressions, rather than laying their eggs on the water surface, as do mosquitoes from other genera. These depressions can be very small (inches in diameter) or very large, but generally range from 2 to 10 inches deep. In marshes of the proper elevation, these depressions, or potholes, experience a 10- to 14-day "wet-dry-wet" cycle. The wet portion of the cycle is driven by lunar tides and rainfall; the dry portion by high rates of evaporation during the warm summer months. When the pothole dries, the female mosquitoes lay between 50 and 200 eggs on the moist substrate (Lesser 2004). After several more obligatory dry days with a reduction in the dissolved oxygen within the pool, the pothole is refilled with water and the eggs hatch (Rees and Nielsen 1947 in Romanowski 1989). Thus, marsh areas that experience inundation only a few times per month, associated with full or new moons, provide suitable habitat for this species. Because the pools in which these larvae hatch are typically isolated, the immature mosquitoes are protected from predators. Depending on water temperature, the immature aquatic phase is completed in approximately 7 to 10 days. The adults emerge and mate at the salt marsh. Females may then fly to an upland area in search of a blood meal. Eggs are laid in the salt marsh on moist substrate and the cycle begins again. *O. dorsalis* overwinters in the egg stage. Tidal populations can produce several successive broods in a single season. However, some long-lived females can repeat the egg-laying cycle up to three times before dying. The longer-lived females are the mosquitoes most likely to be capable of transmitting disease to humans, because they have had more opportunities to become infected with a virus during an earlier bloodmeal and then pass the disease on when taking a later bloodmeal. Females are fierce biters, most active in the evening but will also feed during the day. They are strong fliers and will fly great distances to feed on large mammals and man. This species has been found naturally infected with SLE virus and is an efficient laboratory vector of CE virus. Experimentally, it is found to be moderately effective in transmitting WN virus (Goddard et al. 2002).

O. dorsalis was the predominant species observed during the present study, comprising nearly 75% of all mosquitoes trapped. It was found at every site sampled and was successfully reared to emergence from three locations, including Potter Slough, Elk River, and the Wastewater roadside pools. Each of these three areas was representative of typical high marsh for the region and was flooded and dried periodically, associated with the lunar/tidal cycle. Typical vegetation consisted of saltgrass *(D. spicata)*, saltweed *(A. patula)*, pickleweed *(S. virginica)*, seaside arrowgrass *(T. maritimum),* soft rush *(J. effusus),* and tufted hairgrass *(D. cespitosa)*. Waters from which these mosquitoes were successfully reared were always saline and averaged 20 ppt, though pools with larvae observed at these sites ranged between 12 ppt and 33 ppt. At each of these sample locations, *O. dorsalis* was the only species successfully reared to emergence.

4.1.2.9 Ochlerotatus melanimon

This multivoltine spring, summer, and fall species inhabits semi-open, sunlit agricultural areas, especially duck club ponds and pastures (Meyer and Durso 1998). This species is often cited as occurring in association with *O. dorsalis* and has been documented as breeding in upland freshwater areas adjoining salt marshes (Meyer 2003). Females are persistent early evening biters and will travel several miles to feed primarily on large mammals, including man. They will also bite during the day if disturbed. It is considered a local pest, a secondary vector of WEE virus, and the primary enzootic vector of CE group

virus in the Central Valley of California. Experimentally, it is found to be moderately effective in transmitting WN virus (Goddard et al. 2002).

This species comprised less than 1% of the mosquitoes trapped during the present study. It was found at six of the nine sites sampled (Table 6), including sites associated with both salt and fresh water. It did not successfully emerge from any larval dip samples, so breeding areas are not known for certain.

4.2 Predictions of Mosquito Population Dynamics and the Potential to Alter Exposure of South Bend Residents to the West Nile Virus Following Restoration

This section discusses the project plans as presented in the Environmental Assessment (USACE 2004, 2005) and projects how the restoration may be expected to change mosquito populations (species composition and abundance) at the restoration site and, subsequently, in the City of South Bend (see Section 3.1 for a summary of existing conditions in the City of South Bend). Emphasis is placed on potential changes in the abundance of particular mosquito species, because some of the species captured during this study have a greater WN virus vector potential than others. As discussed in Section 4.1.1.1 (Human Health Risk), predicting the potential for South Bend-resident exposure to WN virus is very problematic. In this report, we base our opinions on which mosquito species are likely to be the most competent WN virus vectors in Washington, should the disease come to this state, on data that has been reported from other states.

4.2.1 Impacts of Estuarine Habitat Restoration

This discussion will begin with those parts of the restoration site closest to the Willapa River (i.e., mudflat). The discussion will then proceed to include potential changes to more inland areas, as we associate them with our sampling sites.

4.2.1.1 Mudflat

Existing Conditions
The areal extent of mudflats outside the existing dike varies with the tide, but does not extend landward of the dike. The number of mosquitoes associated with the mudflat during this study was the lowest of any sample site, just 0.4 msq/h (Figure 35).

Conditions following Restoration
The area of mudflat habitat is expected to increase following the restoration process. According to the Environmental Assessment (USACE 2004, 2005), removal of the existing dike will result in the inundation of approximately 300 acres pasture land. Restoration of tidal hydrology (i.e., daily inundation) to this part of the property will result in displacement of the existing vegetation. Over time, the existing plant species will be replaced naturally with salt-tolerant species, but during the transition, much of the site may turn to mudflat. The low numbers of mosquitoes associated with the existing mudflat are expected to be associated with the Inside Dike pasture as it transitions to salt marsh.

Another site that was surveyed with areas of mudflat, the North River restoration site, had the second-lowest catch per unit effort of mosquitoes (Figure 35). The North River restoration project involved breaching the surrounding dike in only one location. An excavated tidal channel at North River facilitated water movement through the restored area. Two years after the North River dike was breached, the North River restoration site was transitioning from a freshwater habitat to a saltwater marsh. Much of the tidally inundated area was mudflat and *C. lyngbyei*. Higher elevations were inundated only several times per month and were covered predominantly with *A. patula.* A number of shallow

depressions appeared to support mosquito production, but for the most part only when the pools had the opportunity to dry out completely between extreme high tides. During most of our study period, it was evident that moderately high tides periodically replaced at least some of the water in these pools. Because of this periodic flushing, the presence of immature mosquitoes in these pools was very low or nonexistent during most of the study period.

Removal of the entire dike along the Willapa River should produce even more dramatic results at that restoration site, as we expect the majority of the Willapa River site to be covered with saline water on a *daily* basis. Within the first several years, the existing vegetation should disappear and be replaced by mud flat and some salt-tolerant species. Based on a comparison of mosquito trapping data from the Mudflat and North River restoration sites (0.04 msq/h and 0.06 msq/h, respectively) to the trapping data from the Inside Dike site (0.65 msq/h), we expect mosquito production at the restored Willapa River estuary site to be lower than under the current conditions, during the early years following dike removal. Therefore, this part of the restoration project is unlikely to increase numbers of mosquitoes, of any species, in the City of South Bend while in the early stages of transition from freshwater to saltwater habitat.

4.2.1.2 Salt Marsh Currently Outside the Existing Dike

Existing Conditions
The vegetated area currently outside the existing dike supported the next lowest number of mosquitoes (0.35 msq/h trapped). This area was frequently inundated by tides, though not completely covered on a daily basis. It provided habitat for breeding *O. dorsalis* in obvious depressions.

Conditions following Restoration
Under a worst-case scenario, mosquito numbers and species would be expected to remain the same in this region. Since these mosquito numbers are already low and were primarily *Ochlerotatus* (not considered likely to be a prominent WN virus vector because of its feeding preference for mammals, rather than birds), increasing tidal hydrology to this portion of the project via the excavated tidal channels is expected to slightly reduce or hold constant the number of mosquitoes supported in this area. Therefore, exposure of South Bend residents to WN virus vector mosquitoes from salt marsh on the river side of the existing dike under existing conditions and post-restoration mosquitoes should remain constant.

4.2.1.3 Pasture Land Inside the Existing Dike

Existing Conditions
Mosquitoes obviously utilized this area as resting habitat, though we did not find any mosquito breeding areas outside the borrow ditches and some of the currently blocked remnant tidal channels. Trap rates were relatively low, but constant, throughout this study's sampling period (Figure 39). Of the mosquitoes captured, 90% were *Ochlerotatus*, a species considered to be a potential, but not predominant vector for WN virus. These mosquitoes were likely bred at nearby high salt marsh areas, possibly west (upwind) of the restoration site. *Cx. pipiens*, one of the more likely WN virus vectors, was also captured inside the dike, but in low numbers. It likely bred in standing water in some of the borrow ditches and blocked remnant tidal channels that cross the site. The potential for mosquitoes currently associated with this site to expose the residents of South Bend to the West Nile virus is considered low. It is likely that any mosquito bred in the pasture land inside the existing dike could fly to the City of South Bend, especially if aided by the wind. However, the proportion of the mosquitoe species that are most likely to be WN virus vectors is small.

Conditions following Restoration
According to the Environmental Assessment (USACE 2004, 2005), removal of the existing dike will result in the inundation of approximately 300 acres of this pasture land. Under the proposed plan, borrow ditches and low-lying areas will be filled, bringing them generally to grade such that water flows off the site toward the river. Excavating and restoring flow to the remnant tidal channels will eliminate the existing standing water habitat and emergent vegetation currently supporting some freshwater mosquito species production (this study, anecdotal data). In addition, the excavated tidal channels will open the area to access by fish and other aquatic species that will have the opportunity to prey on immature mosquitoes, if any exist in these waterways. This increased opportunity for water movement and drainage of the upper elevations will serve to further decrease the opportunity for mosquito production at this site. Over time, the existing plant species will be replaced naturally with salt-tolerant species.

The purpose of monitoring mosquitoes at sites outside the proposed restoration area was to improve assumptions about mosquito use of the site in the years following restoration (e.g., 2 years post-restoration at North River [discussed above, under mudflat], 17 years post-restoration at Elk River, and at marsh maturity [Potter Slough]). Similarities and differences between these sites and the Willapa River restoration site have influenced our evaluation of how restoring the Willapa River site may change mosquito populations, both in the City of South Bend and at the restoration site. Differences in the extent of dike removal, tidal channel excavation, and marsh elevation all factor into the predictions of how the habitat (and mosquito dynamics) at the Willapa River site are likely to change over time.

Potter Slough: a comparison to the Willapa Bay restoration site: The Potter Slough reference site is predominantly a high salt marsh, characterized by its elevation and vegetative community. The tidal slough channels crisscrossing the site filled with the incoming tide, but were observed to drain almost entirely between tidal cycles. The water that was observed standing in small depressions in the slough channels would have been mixed and replaced during the next incoming tide. Thus, the slough channels themselves did not appear likely to support mosquito production by any of the species we encountered during this study. However, because the Potter Slough marsh is not completely inundated on a daily basis, standing water was present in a few of the upper elevation areas where depressions had formed near existing slough channels. At least one such pool supported immature *O. dorsalis*. In time, it is expected that the Willapa River estuarine habitat restoration area will develop tidal channels in addition to those that are excavated, as well as high salt marsh vegetation. Thus, tidal inundation at a mature Willapa River estuarine site will be similar to that at Potter Slough.

The two sites differ in surface elevations. Surveys indicate that ground surface elevations inside the diked area range between +5 ft and +9 ft elevation, or approximately 2 ft lower than the Potter Slough reference marsh area (NAVD 88 datum) (Herrera Environmental Consultants 2002). The surface elevations inside the diked area are also approximately 2 ft lower than the existing marsh on the north side of the dike. To prevent excavation of more fill material than can be utilized throughout the restoration project, the dike may be excavated only to the level of the existing marsh on the north side (USACE 2004). Thus, the only entry for water to the pasture land may be through the five primary channels that will be excavated through the mudflat on the river-side of the existing dike. If these channels are excavated down to the depth of the pastureland currently inside the dike, then water should inundate most of the 300 acres on a daily basis. Under this condition, the Willapa River restoration site would be covered with saline water more frequently, and to a greater depth, than the Potter Slough site (see Figure 49). Under worst-case conditions, assuming the channels are excavated only to the depth of the existing marsh on the river-side of the dike, the pasture land may be inundated only periodically, at approximately the same times as when Potter Slough is inundated. To put this in perspective, we took photos of the Potter Slough reference salt marsh at a very high tide, +10.5 ft MLLW, on October 18. Because Potter Slough is on average about 2 ft higher in ground surface elevation than the area inside the dike, this photo corresponds to what one could expect to see when the tidal elevation is approximately +8.5 ft MLLW on the restored site (Figure 49)

assuming the channels are excavated to the lower elevation. A photo taken at +3.0 ft MLLW (corresponding to approximately +1 ft MLLW tide level in restored area) is provided for comparison.

During 2004, tidal elevations of +8.5 ft MLLW water occurred at least once daily on 320 out of 366 days (46 days). On only 19 days in 2004 did the highest tide fall below +8.0 ft MLLW along this part of the Willapa River.

Kramer et al. (1995) found that enhanced tidal circulation across a restoration site reduced *O. dorsalis* abundance at the study site by impeding the conditioning process of the eggs, by promoting predator circulation, and by decreasing the amount of standing water available for mosquito production. Assuming the restoration site's tidal channels are fully excavated, floodwater mosquitoes are likely to have difficulty finding locations to breed that are dry long enough for the eggs to experience the obligatory drying period before they are re-flooded. Most periods with high-tide elevations below +8.5 ft MLLLW lasted for only 3 to 5 consecutive days in 2004. Potential problem areas will include the fringe of higher elevation vegetation parallel to the highway. The areas nearest the highway will only be covered with water during infrequent extreme high tides and, therefore, may still be used by floodwater species as breeding grounds. The majority of the Willapa River site's salt marsh plain will slowly rise through natural accretion of sediment brought in with each tide. However, it will take years for enough sediment to accumulate and form salt marsh habitat at elevations that are not subject to daily tidal inundation (i.e., habitat more suitable for floodwater mosquito breeding).

Culiseta sp. were the only mosquitoes found at the Inside Dike site that were not found at Potter Slough. They were associated with the fresh water borrow ditches, which will be filled during restoration. Generally, the proportion of mosquito species and their trap rates at the two sites are already very similar (CPUE of 0.71 msq/h at Potter Slough; 0.65 msq/h at the Inside Dike pasture site). The mosquito species composition and abundance at the restoration site are likely to match those of the Potter Slough site eventually, but the restoration site's transition to mature high salt marsh is not likely to happen for several years. The transitional stage is more likely to resemble existing conditions at the Mudflat and North River sites.

<u>Elk River: a comparison to the Willapa Bay restoration site</u>: The Elk River restoration site was a definite source of *O. dorsalis* mosquitoes. Low-lying areas scattered throughout the site were periodically filled with tidal waters and provided optimal larval habitat for salt-tolerant species. Approximately 5 years after the dike was breached, salt-tolerant vegetation dominated the site. Unlike plans for the Willapa River site, only one part of the dike was breached for the Elk River project, and only short tidal channels were excavated to direct the water flow and drain it from the plain. Thus, a limited amount of water enters and floods the site several times per month, then remains ponded in the low-lying areas.

In contrast, plans for the Willapa River estuarine restoration site include the excavation of five remnant tidal slough channels and three side channels. This should initially improve tidal inundation and exchange and will help facilitate the tidal channel formation process across the site. Similar to Potter Slough, the tidal sloughs will play an integral part in draining water from the project site and minimizing the potential for standing water to accumulate where mosquitoes can use it for breeding purposes. Also, to put the mosquito use of the Elk River restoration site in some perspective, the CPUE at Elk River was 0.57 msq/h, less than the Inside Dike trap site (0.65 msq/h). Thus, even after the Willapa River estuarine restoration site begins to develop some mosquito habitat, it is not likely to increase the abundance of mosquitoes on that portion of the site. A slightly greater proportion of mosquito species at the Elk River site were *Ochlerotatus* as opposed to *Culex* or *Culiseta*. *Ochlerotatus* is the least likely of these genera to be a WN virus vector, so it is likely that the potential for South Bend residents to be exposed to WN virus from mosquitoes produced on this portion of the site will remain constant or decrease.

In summary, the estuarine habitat restoration project, as proposed with excavation of the remnant tidal channels to the elevation of the ground surface elevations inside the diked area, should remove most of the 300 inundated acres inside the dike from use by *O. dorsalis* (and any other mosquito species) as a breeding area for several years, while the pasture transitions to high salt marsh habitat. Salt tolerant species were present at the Elk River site within 5 years, but it has taken longer (10+ years) to achieve surface elevations associated with high salt marsh species. It is difficult to predict the amount of time these processes will take at the Willapa River site, because the sediment sources and accretion processes are different. However, even after the restored estuarine marsh reaches maturity, the numbers of saltwater mosquitoes produced on the site is not expected increase significantly over present conditions (based on a comparison between the Inside Dike CPUE and the Potter Slough CPUE). The salt marsh mosquitoes (*Ochlerotatus* spp.) that are expected to predominate following restoration are already the predominant species in the City of South Bend (based on our trapping and examination of the contents of one of the city's Mosquito Magnets®). In fact, the existing CPUE values for the Wastewater Treatment Plant (1.12 msq/h) and for the Alder House site (1.07 msq/h) are greater than the existing CPUE values for the Inside Dike (0.65 msq/h), Mudflat (0.04 msq/h), North River (0.06 msq/h), Elk River (0.57 msq/h), and Potter Slough (0.71 msq/h) sites. Based on these data, the numbers of saltwater mosquitoes in the City of South Bend are not expected to increase as a result of the salt marsh restoration part of the project.

Proportionately more *Culex* species were trapped at the sites associated with the City of South Bend than at the sites associated with the salt marsh portion of the proposed restoration project. Because the habitat currently associated with freshwater mosquito species such as *Culex* and *Culiseta* will be filled in (i.e. borrow ditches) or covered with saline water through tidal inundation, the abundance of these species that breed on this portion of the restoration site are expected to decline. Thus, the numbers of freshwater mosquitoes in the City of South Bend are not expected to increase as a result of the salt marsh restoration part of the project.

Potter Slough when water level is at +10.5 ft MLLW on October 17, 2004. This amount of tidal inundation should be similar to what the proposed restoration area will experience during a +8.5 ft MLLW tide.

Potter Slough when water level is at about +3.0 ft MLLW on October 18, 2004. This is similar to a +1.0 ft tidal elevation at the proposed restoration site.

Figure 49. **Comparative tidal inundation of Potter Slough at +10 ft MLLW and at approximately +3.0 ft MLLW, 2004.**

4.2.2 Impacts of Freshwater Habitat Restoration

Under the proposed project, three wetland cells and six amphibian ponds would be created through grading of the WRP Site 1. The plan takes advantage of the existing natural features of the site, where open ponded areas were historically present. The freshwater wetland cells would be bordered by new levees and would use tide gates and dam boards as water control structures, allowing water to be drained quickly if necessary. They would provide waterfowl habitat and hunting opportunities. The amphibian ponds would be allowed to fill and drain naturally.

The WRP Site 2 is situated on a private 22-acre parcel that is protected by an underlying NRCS permanent wetland easement (USACE 2004). The agreement made between WDFW and the landowner included a provision for WRP restoration actions to enhance waterfowl habitat on the property. To promote this use of habitat, the current plan proposes construction of a 2- to 3-ft high levee across the western and northern boundaries of the site to retain seasonal freshwater. A water control structure installed in the western levee wall would retain water in an old remnant slough and four adjoining swales, providing freshwater habitat supporting wetland dependent species, such as waterfowl, and hunting opportunities. Water levels would be managed to enhance conditions for the plants considered optimal for shorebirds and waterfowl. Open water habitat with islands, or habitat mounds, is a feature of this proposed wetland area.

4.2.2.1 Wetland Ponds

Existing Conditions

The land on the south side of U.S. 101 was dry, with the exception of water in the borrow ditches, from August through mid-September, 2004. In mid-September, rain water began to fill low-lying areas, which is in agreement with statements made in the Environmental Assessment (USACE 2004, 2005) indicating that standing water is present on much of the site during the rainy season. Based on over 20 years of precipitation data, rainfall in the amounts similar to what we observed in September occur in the project area, and are likely to produce small pools of standing water, between mid-September and May. Based on a similar record of temperatures for the area, mosquitoes are likely to be actively breeding between April and October. The overlap of optimal thermal conditions and standing water on the project site would therefore be likely to occur April-May and September-October, or approximately four months time. Currently, the amount of standing water available during that time period is confined mostly to the existing borrow ditches and to several perpendicular channels of cattail marsh that connect to the borrow ditches.

We trapped relatively high densities of mosquitoes at the Freshwater site throughout the August to mid-October study period. The CPUE was the highest for any site within the entire restoration project boundary. Only the sites sampled to represent existing conditions in the City of South Bend (the South Bend-Alder House and Wastewater Treatment Plant sites) had higher trapping rates. One third of the mosquitoes trapped at the Freshwater site were *Cx. pipiens,* one of the four likely WN virus vector species. Although generally linked to degraded water and urban environments, their presence at the Freshwater site and their reputation as poor fliers indicates that the proper conditions for their production exist on the WDFW property.

We captured *Culex* and *Culiseta* mosquito larvae in two intermittently wet borrow ditches and in the cattail marsh located near the Freshwater trap site from mid-August through mid-October, when our field season ended. Each area where the larvae were found was shallow (less than 10 inches) and had been filled primarily with rainwater. The maximum density of larvae from these areas was very high (35 larvae/dip) in mid-to-late September.

Conditions following Restoration
The three seasonal ponds will cover 22 acres, 21 acres, and 17 acres in area. Existing swales will be enhanced and new swales constructed to establish deeper waters within the ponds that may persist during the summer months (USACE 2004). However, the ponds will be graded to move most standing water off the land, through the tide gates and new culverts placed under the highway, and into the excavated remnant tide channels restored during the estuarine habitat restoration phase of the project. The current plan calls for boards to be placed in the water control structures in September. The number of boards placed would determine the resulting water level in each of the three wetland cells. The wetland cells would fill when the fall rains begin, providing habitat for waterfowl and other wetland-dependent species. The proposed action would maximize the area with water depths ranging between 6 and 12 inches. Water in the wetland cells would be allowed to evaporate during the spring and summer, with the option for removing the dam boards reserved for achieving a quick drawdown if needed, to facilitate vegetation management, or to prevent mosquito breeding in freshwater cells early in the season.

Different wetland types present different potentials to produce mosquitoes. Wetlands that are repeatedly wet and dry (such as seasonal wetlands) and have dense vegetation are more likely to produce mosquito populations (Malamud-Roam 2004). However, mitigation strategies can be employed to minimize mosquito production, even in seasonal wetlands. The role of water management is significant. The ability of the wetland to drain efficiently (controllable through design and maintenance), the timing and rate of drawdown (controllable with water control structures), coupled with the timing and amount of rainfall (uncontrollable) determines the amount of mosquito breeding habitat available. Because precipitation levels can only be estimated, the wetland manager must balance the needs of the wetland plants and wildlife the project is designed to benefit with the desire to minimize mosquito habitat when developing the water management plan. The following discussions of potential wetland pond (and amphibian pond) mosquito populations and pond management options are written from a mosquito-centric perspective, because the purpose of this report is to evaluate how mosquito populations could change following restoration and alter the potential exposure of South Bend residents to the WN virus.

In this project, the shallow-water coverage (i.e., 6 to 12 inches deep) proposed provides optimal habitat for the freshwater mosquito species. Mosquitoes produced in the wetland ponds would most likely be freshwater species from Groups 1a-1c (Figure 48), most of which are already present at the freshwater wetland enhancement site. Many are multivoltine species, able to produce multiple broods during the breeding season, so prolonging water retention in up to 60 acres of wetland ponds during the optimal conditions for mosquito breeding at the site (April - October) would be expected to significantly increase mosquito numbers over the current conditions. No mosquito larvae were found in the borrow ditches on the south side of U.S. 101 during our study. This is attributed to a combination of higher salinity water, the daily water movement observed in these channels, and to the presence of fish. Therefore, filling those borrow ditches is not expected to significantly change mosquito production within the freshwater wetland enhancement area.

The combination of the short flight distance and prevailing winds that blow toward the City of South Bend increases the likelihood that mosquitoes produced in the wetland ponds could increase the numbers of nuisance mosquitoes, and potentially the number of mosquito vectors, in the City of South Bend. Dewatering the site will provide the best means for controlling mosquito production at the freshwater wetland enhancement site.

An experiment conducted by Schäfer (2004) found that water level schedule has a significant effect on mosquito larvae survival to adult emergence. Beginning with day-old *Aedes vexans* and *Ochlerotatus sticticus* larvae (similar life histories as *O. dorsalis* and *O. melanimon*), survival to adult emergence was tested under three water level schedules: 1) constant water level, 2) constant water level until Day 4

followed by decreasing water levels on each successive day, and 3) constant water level until Day 4, followed by two days of decreasing water levels and complete drying on Day 6. Under the constant water level schedule, 91% of the mosquito larvae survived to emerge as adults. In the decreasing water schedule, 66% survived, and under complete drying conditions only 3% of the mosquitoes survived to adults. The first adult in the drying treatment emerged six days following water removal; the last adult emerged 14 days following water removal. Schäfer (2004) reached the conclusion that while drying out of mosquito sites is usually considered to make mosquito control unnecessary, the desiccation of a breeding site may not necessarily result in a low adult mosquito population density. Because the mosquitoes in the test showed high survival ability in humid soil, Schäfer (2004) presumed that rainfall within 14 days following desiccation of wetlands could still enable adult mosquito hatching. Even three percent survival could amount to a significant mosquito population. By way of example, assuming one square meter of pond water surface area supports one mosquito larvae (a very low number, provided only for demonstration purposes), 60 acres of water would support 242,000 mosquitoes during just one breeding cycle.

Regardless, design features can enhance mosquito control. For example, Hamman (2003) found that constructing swales can help shallow standing water drain quickly and eliminate mosquito larvae already in a pond. To this end, the current design includes enhancement of existing swales within the three ponds to enhance drainage (USACE 2004). An early, rapid drawdown of water in the spring (e.g., March) and postponement of rainfall retention until late fall/early winter (e.g., November) will result in the least production of mosquitoes in the wetland ponds. Retention of water only between November and March could be expected to result in similar numbers of mosquitoes produced as under present conditions. Water retention outside of these time periods is expected to result in a relative increase in mosquitoes, based on the amount of water that is available for mosquito production. Under the worst-case scenario (no water drawdown in the wetland ponds and full water retention), there could be up to 60 acres of new mosquito habitat available for approximately 7 months.

4.2.2.2 Amphibian Ponds

Existing Conditions
The existing conditions in areas where the amphibian ponds will be located is identical to those discussed above, under wetland ponds.

Conditions following Restoration
The six proposed amphibian ponds would be located outside the levee system next to the hillside bordering the property. Each pond would be 0.08 to 0.29 acres in size with variable depths between 6 and 18 inches. These ponds are designed to fill naturally with fall/winter rains and dry naturally in summer. Standing water would be present long enough for frog or salamander eggs to hatch and for tadpoles to develop to their terrestrial form.

The habitat type associated with the amphibian ponds is one that is most frequently associated with mosquito production. Hamman (2003) reports that most problem mosquito populations are centered around seasonal marshes with poor drainage and around permanent ponds with widely fluctuating water levels. Poorly drained, heavily vegetated areas provide ideal locations for adults to lay their eggs, and areas where water stagnates and slowly disappears promote mosquito production by providing a place for mosquito larvae to reach adulthood (Hamman 2003). Dense emergent vegetation can also physically obstruct predator access to immature mosquitoes (Walton et al. 1998). Additional problematic situations may arise when multiple, smaller wetland sites are dispersed over an area. Malamud-Roam (2004) stated, “In design planning, the fewer shallow, seasonal wetlands on a site, the lower the site’s potential to produce mosquitoes”.

Whether or not the amphibian ponds will increase mosquito abundance in the City of South Bend is difficult to determine definitively, but there is the potential for production of a large number of mosquitoes. One study indicated that *O. dorsalis* can lay between 1,000 and 10,000 eggs per square foot of moist mud habitat (CDEP 1998). *Cx. pipiens* are reported to produce as many as 1000 mosquitoes per week from just one inch of standing water in a coffee can (Ladd and Frankenberger 2003). Under the assumption that the amphibian ponds could create approximately 1 acre of shallow, standing-water mosquito habitat April through May and in September and October, one can see the potential for the amphibian ponds to be responsible for the production of a significant number of freshwater mosquitoes. Some of the species associated with this type of freshwater habitat are potential WN virus vectors (i.e., *A. vexans, O. melanimon, Cq. perturbans, Cx. pipiens, Cx. tarsalis, Cs. incidens, and Cs. inornata*). Of these, *Cs. incidens*, *Cs. inornata*., *Cx. pipiens* and *O. melanimon* were trapped at the Freshwater site and dipped out of freshwater borrow ditches on the north side of U.S. 101. However, all the other species have been documented previously in Pacific County (WDOH 2004a). Birds (i.e., viral hosts) are frequent visitors to freshwater wetlands, and the flight distance between the proposed project area and the City of South Bend is short for both birds and mosquitoes.

In summary, because these ponds are designed without water control structures, there will be no means to dewater them to control mosquito production. The amphibian ponds, created by grading existing low areas to retain more water than they do currently, will increase the number of freshwater mosquitoes produced at this site over what occurs under the present conditions. Under the worst case scenario, the amphibian ponds would add approximately 1 acre of additional standing water habitat over approximately 4 months of the mosquito breeding season.

4.2.3 Combined Project Impact on Mosquito Species and Abundance in the City of South Bend

Relatively low ground surface elevations of the estuarine portion of the site mean that most of the area should be inundated daily. This factor, in combination with proposed management actions of filling low areas and incorporating drainage through excavation of remnant slough channels, will remove almost 300 acres from mosquito production while it transitions from pasture to salt marsh habitat. These same 300 acres will eventually reach a stage of salt marsh maturation similar to the Elk River and Potter Slough sites. The numbers of mosquitoes produced at those two sites are very similar to the numbers produced currently at the Inside Dike site. Thus, as the salt marsh matures, mosquito species and numbers are expected to be similar to what exist currently within the diked area. While the abundance of mosquitoes is expected to remain the same, there may be a decrease in the abundance of freshwater species produced at the restored estuarine site compared to current conditions, because approximately 17 acres of borrow ditches that currently support some freshwater species production will be filled.

Following enhancement of the freshwater portion of the site, mosquito production will increase over current conditions because the amphibian ponds will create approximately 1 acre of mosquito breeding habitat that does not currently exist. The 60 acres of wetland ponds are also expected to contribute significantly to increased mosquito numbers if they contain standing water between April and October. Because mosquito larvae were not discovered in borrow ditches on that portion of the property, filling them is not likely to provide compensation for the shallow-water habitat added from formation of the three wetland ponds. *Culex pipiens*, the predominant vector of West Nile virus to humans in the northeast United States (Kilpatrick et al. 2005), comprised 35% of mosquitoes trapped at existing freshwater habitats on the restoration property. Creation of additional habitat for *Cx. pipiens* and other freshwater species may thereby increase potential exposure to West Nile virus, if and when it arrives in the State of Washington. The extent of the increase in mosquitoes and the subsequent potential to impact residents of the City of South Bend depends, in large part, on how water is managed in the freshwater wetland ponds.

Overall, there is expected to be a net increase in mosquito production as a result of the Willapa Bay Restoration Project. Saltwater mosquitoes are likely to decline or remain constant, but freshwater mosquitoes are expected to increase.

With respect to the potential impacts to residents of the City of South Bend, research cited in a review by Knight et al. (2003) indicates that additional mosquitoes from created wetlands do not significantly add to the nuisance issue in areas with large expanses of existing natural wetlands. The proximity of the City of South Bend to so many natural mosquito production areas that are enhanced by tidal inundation and rainfall means that residents of the City of South Bend will continue to experience high numbers of mosquitoes regardless of the proposed restoration project. These "local" mosquitoes are just as likely to contract and transmit WN virus as mosquitoes from the more distant restoration site. Overall, the baseline probability that a resident of the City of South Bend would contract WN virus is very small (there was less than a 0.4% chance of becoming infected with the virus in the states with human cases reported during 2002 [O'Leary et al. 2004]). The expected increase in freshwater mosquitoes as a result of the restoration project could increase the potential vector population by a small amount (relative to the large existing mosquito population already found in the City of South Bend), and thereby incrementally increase the chance for human/WN virus-vector encounters if these mosquitoes travel to South Bend. However, the overall impact of the restoration project will be extremely small and would most likely represent an insignificant increase in potential risk.

The first human cases of WN virus in the State of Washington, if and when they appear, would provide more insight into which mosquito species are responsible, and whether the restoration project may ultimately contribute any appreciable risk to City of South Bend from exposure to WN virus. However, thoughtful design of the components of the proposed restoration project to limit mosquito production and water management directed toward mosquito control should maintain mosquito populations at the site such that they do not pose a significant risk to the residents of the City of South Bend.

4.3 Final Recommendations

To conclude, the following recommendations are made regarding the Willapa River restoration project, with respect to mosquito production and the potential (at this time, hypothetical) for mosquito vectors from the site to carry the WN virus to the residents of the City of South Bend, Washington:

- Consider supporting additional baseline monitoring (trapping and larval dipping on the Willapa River freshwater restoration sites) to determine the onset of the freshwater mosquito season in the spring and to document mosquito species presence, abundance, and specific breeding habitats.

  This study documented mosquito activity from August through mid-October. However, the mosquito season around the proposed restoration site probably extends from as early as March or April through October. Data from early sampling would provide information needed to make decisions about the timing of water-management practices at the freshwater habitat sites to balance habitat benefits to wildlife while minimizing mosquito production. Additional baseline monitoring at the Potter Slough reference site would provide information on the onset of the saltwater mosquito season and tidal elevations most likely to support salt water mosquito species on the salt marsh portion of the project.

- Monitor mosquitoes through adult trap and larval dip sampling following completion of the estuarine restoration and freshwater wetland enhancement to document mosquito use of the site over time.

  Mosquito data collected throughout the restoration process will provide valuable planning and design information that can be cited for future Pacific Northwest restoration projects.

- Consider eliminating the amphibian ponds from the freshwater habitat restoration project.

  The wetland ponds provide large areas of wildlife habitat and can be drained quickly if required to manage mosquitoes. The amphibian ponds, though small in size, add to the amount of existing mosquito production habitat, may produce a large number of mosquitoes, and have no provision for water management.

5.0 References

Alameda County Mosquito Abatement District webpage. 2004. Accessed on the internet at: http://www.mosquitoes.org/index.htm

American Mosquito Control Association. 2003. "Mosquito Information." Accessed on the internet at: http://www.mosquito.org/info.php

Belton, P. 2003. "British Columbia mosquitoes as vectors of West Nile virus." Accessed on the internet at: http://www.sfu.ca/~belton/summary.pdf.

Campbell, G.L., A.A. Marfin, R.S. Lanciotti, and D.J. Gubler. 2002. "West Nile virus." *The Lancet Infectious Diseases*, Volume 2, pp. 519-529.

CDC (Centers for Disease Control). 2003. "Arborviral encephalitides. Division of vecto-borne infectious diseases." Accessed on the internet at: http://www.cdc.gov/ncidod/dvbid/arvor/arbded.htm

CDC (Centers for Disease Control and Prevention). 2004. "West Nile virus: Statistics, surveillance, and control." 2004 West Nile Virus Activity in the United States (reported as of December 28, 2004). Accessed on the internet at: http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount04_detailed.htm.

CDC (Centers for Disease Control). 2005. "West Nile virus: What you need to know". Accessed on the internet at: http://www.cdc.gov/ncidod/dvbid/westnile/wnv_factsheet.htm Last updated August 10, 2004.

CDEP (Connecticut Department of Environmental Protection). 1998. "Connecticut's mosquito management program." Connecticut, Department of Environmental Protection, Wildlife Division, Hartford, Connecticut. Accessed on the internet at: http://www.fvhd.org/community/mosquito.htm

Contra Costa Mosquito and Vector Control District. 2004. "Guidelines for Creating and Maintaining Waterfowl Habitats." Accessed on the Internet at: http://www.ccmvcd.dst.ca.us/habitats.htm.

Crans, W.J. 2003. "Frequently Asked Questions about Mosquitoes". Fact Sheet FS900. Rutgers Cooperative Extension, New Jersey Agricultural Experiment Station, Rutgers, The State University of New Jersey, New Brunswick, N.J. Accessed on the internet at: http://www.rce.rutgers.edu/pubs/pdfs/fs900.pdf

Curtis, L.C. 1967. *The Mosquitoes of British Columbia.* Occasional Papers of the British Columbia Provincial Museum No. 15, Victoria, British Columbia, Canada.

Darsie, R.F. Jr., and R.A. Ward. 1981. *Identification and geographical distribution of the mosquitoes of North America, north of Mexico.* American Mosquito Control Association, University of Utah Printing Services, Salt Lake City, Utah.

EPA (U.S. Environmental Protection Agency). 2004. *Wetlands and West Nile virus.* EPA 843-F-04-010, U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Evans, L. 2002. "Traffic crashes." *American Scientist* 90(3): 244.

Gjullin, C.M. and G.W. Eddy. 1972. *The Mosquitoes of the Northwestern United States.* Technical

Bulletin No. 1447, Agricultural Resource Service, United States Department of Agriculture, Washington, D.C.

Goddard, L.B., A.E. Roth, W.K. Reisen, and T.W. Scott. 2002. "Vector competence of California mosquitoes for *West Nile virus."* In *Emerging Infectious Diseases* [serial online] 2002 December (accessed 7/22/2004). Accessed on the internet at: http://www.cdc.gov/ncidod/EID/vol18no12/02-0536.htm

Hamman, M. 2003. "Creating a mosquito "un-friendly" marsh". Grassland Today, The Grassland Wetlands Newsletter. Volume 13, No. 2. Grassland Water District, Los Banos, California.

Herrera Environmental Consultants. 2002. *Tide Marsh Restoration and U.S. Highway 101 Improvement Hydrologic and Hydraulic Analysis.* Prepared in association with Philip Williams & Associates and Battelle Marine Sciences Laboratory for the Washington State Department of Transportation, Olympia, Washington.

Hubálek, Z. and J. Halouzka. 1999. "West Nile fever – a reemerging mosquito-borne viral disease in Europe." *Emerging Infectious Diseases,* 5(5). Accessed on the internet at: http://www.cdc.gov/ncidod/eid/vol5no5/hubalek.htm.

Joint Venture. 2004. "West Nile virus strategy and communications plan." San Francisco Bay Joint Venture, San Francisco, California. Accessed on the internet at: http://www.sfbayjv.org/WNV/SFBJV_WNV_STRATEGY.pdf

Kilpatrick, A.M., L.D. Kramer, S.R. Campbell, E.O. Alleyne, A.P. Dobson, and P. Daszak. 2005. West Nile virus risk assessment and the bridge vector paradigm. *Emerging Infectious Diseases,* 11(3): 425-429.

Knight, R.L., W.E. Walton, G.F. O'Meara, W.K. Reisen, and R.Wass. 2003. "Strategies for effective mosquito control in constructed treatment wetlands." *Ecological Engineering*, 21: 211-232.

Kramer, V.L., J.N. Collins, K. Malamud-Roam, and C. Beesley. 1995. "Reduction of *Aedes dorsalis* by enhancing tidal action in a northern California marsh." *American Mosquito Control Association,* 11(4):389-395.

Ladd, B. and J. Frankenberger. 2003. "Management of ponds, wetlands, and other water reservoirs to minimize mosquitoes." *Water Quality*, WQ-41-W, The Purdue Extension Water Quality Team, Purdue University, West Lafayette, Indiana. Accessed on the internet at: http://www.ecn.purdue.edu/SafeWater/Ponds/WQ-41-W.pdf

Lesser, C.R. "Open marsh water management: A source reduction technique for mosquito control. Delaware Mosquito Control Section." Accessed on the internet in 2004 at: http://www.dnrec.state.de.us/fw/mosquito/Final%20Draft%202%20of%20omwmarticleapril.pdf

Lincoln, R.J., G.A. Boxshall, and P.F. Clark. 1998. *A Dictionary of Ecology, Evolution and Systematics.* Cambridge University Press. Cambridge, U.K.

Liu, A.E. 1995. "Restoration of a San Francisco Bay Salt Marsh: Evaluating *Corixid* and mosquito populations." Presented at the Workshop on "Restoration of the San Francisco Bay-Delta-River Ecosystem: choosing indicators of ecological integrity." October 28, 1995. Berkeley, California.

Malamud-Roam, K. 2004. West Nile virus presentation by Dr. Karl Malamud-Roam of the Contra Costa Mosquito and Vector Control District to the San Francisco Bay Area Wetlands Restoration Program Executive Council. Meeting Summary June 9, 2004. Accessed on the internet at: http://www.sfwetlands.ca.gov/060904.pdf

McNelly, J.R. 1989. "The CDC Trap as a special monitoring tool." *Proceedings, 76th Meeting of the New Jersey Mosquito Control Association,* pp. 26-33.

Metzger, M.E. 2004. *Managing mosquitoes in stormwater treatment devices.* ANR Publication 8125. University of California, Division of Agriculture and Natural Resources, Communication Services, Oakland, California.

Meyer, R.P. 2003. *Regional guide to the common mosquitoes of California, I. Coastal California.* Mosquito and Vector Control Association of California, Sacramento, California.

Meyer, R.P. and S.L. Durso. 1998. *Identification of the mosquitoes of California.* Mosquito and Vector Control Association of California, Sacramento, California.

NIAID (National Institute of Allergy and Infectious Diseases). 2004. "Health Matters: Flu." Prepared by the Office of Communications and Public Liaison, National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, Maryland. Accessed on the internet at: http://www.niaid.nih.gov/factsheets/flu.htm

NRCS (National Resources Conservation Service). 2005. Natural Resources Conservation Service Conservation Practice Standard, Wetland Enhancement, Code 300. Accessed on the internet April 8, 2005 at: http://www.pwrc.usgs.gov/wli/constds/wlicp300.htm

Offill, Y.A. and W.E. Walton. 1999. "Comparative efficiency of the threespine stickleback *(Gasterosteus aculeatus)* and the mosquitofish *(Gambusia affinis)* for mosquito control." *Journal of the American Mosquito Control Association,* 15(3): 380-390.

O'Leary, D.R., A.A. Marfin, S.P. Montgomery, A.M. Kipp, J.A. Lehman, B.J. Biggerstaff, V.L. Elko, P.D. Collins, J.E. Jones, and G.L. Campbell. 2004. Research Paper: The Epidemic of West Nile Virus in the United States, 2002. *Vector-borne and Zoonotic Diseases,* 4(1):61-70.

O'Malley, C.M. 1990. "*Aedes vexans* (Meigen): An old foe." *Proceedings of the New Jersey Mosquito Control Association,* pp.90-95. Accessed on the internet at: http://www.rci.rutgers.edu/~insects/sp13.htm.

Reisen, W. 1993. "The western encephalitis mosquito, *Culex tarsalis.*" *Wing Beats,* Vol. 4(2):16.

Romanowski, M. 1989. "*Aedes dorsalis* in New Jersey: larval habitat and identification." *Proceedings, New Jersey Mosquito Control Association.* pp. 58-62. Accessed on the internet at: http://www-rci.rutgers.edu/~insects/sp14.htm.

Schäfer, M. 2004. Mosquitoes as part of wetland biodiversity. Acta Universitatis Upsaliensis. *Comprehensive Summaries of Upplala Dissertations from the Faculty of Science and Technology* 1042. 63 pp. Uppsala. ISBN 91-554-6094-1.

Spear Cooke, S. (Ed.) 1997. *A Field Guide to the Common Wetland Plants of Western Washington and Northwestern Oregon.* Seattle Audubon Society. Seattle, WA.

Stage, H.H., C.M. Gjullin, and W.W. Yates. 1952. *Mosquitoes of the Northwestern States.* United States Department of Agriculture, Agriculture Handbook No. 46, Washington, D.C.

Thom, R.M. 1992. "Accretion rates of low intertidal saltmarshes in the Pacific Northwest." *Wetlands,* 12: 147-156.

Thom, R.M., R. Zeigler, and A.B. Borde. 2002. "Floristic development patterns in a restored estuarine marsh, Elk River, Grays Harbor, Washington." *Restoration Ecology* 10(3): 487-496.

Turell, M.J., D.J. Dohm, M.R. Sardelis, M.L. O'Guinn, T.G. Andreadis, and J.A. Blow. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *Journal of Medical Entomology*, 42(1): 57-62.

USACE (United States Army Corps of Engineers). 2002. *Willapa River Estuarine Habitat Restoration Project, Pacific County, Washington: Draft Ecosystem Restoration Report and Environmental Assessment.* U.S. Army Corps of Engineers, Seattle District, Seattle, Washington.

USACE (United States Army Corps of Engineers). 2004. *Willapa River Section 1135 Estuarine Habitat Restoration Project, Pacific County, Washington: Revised Draft Environmental Assessment.* U.S. Army Corps of Engineers, Seattle District, Seattle, Washington.

USACE (United States Army Corps of Engineers). 2005. *Willapa River Section 1135 Estuarine Habitat Restoration Project, Pacific County, Washington: Final Environmental Assessment.* U.S. Army Corps of Engineers, Seattle District, Seattle, Washington.

U.S. Census Bureau. 2000. Census 2000. Table DP-1. "Profile of General Demographic Characteristics: 2000. Geographic area: South Bend city, Washington."

Walton, W.E. 2003. *Managing mosquitoes in surface-flow constructed treatment wetlands.* ANR Publication 8117. University of California, Division of Agriculture and Natural Resources, Communication Services, Oakland, California.

Walton, W.E., P.D. Workman, L.A. Randall, J.A. Jiannino, and Y.A. Offill. 1998. "Effectiveness of control measures against mosquitoes at a constructed wetland in Southern California." *Journal of Vector Ecology,* 23: 149-160.

WDOE (Washington State Department of Ecology). 2003. Department of Ecology news release – March 20, 2003. Publication 03-046. Accessed on the internet at: http://www.ecy.wa.gov/news/2003news/2003-046.html.

WDOH (Washington State Department of Health). 2003. *Mosquito-borne disease response plan.* Washington State Department of Health, Olympia, Washington.

WDOH (Washington State Department of Health). 2004a. "West Nile Virus nonhuman surveillance in Washington State." Washington State Department of Health, Environmental Health Division, Office of Environmental Health and Safety, Olympia, Washington. Accessed on the internet at: http://www.doh.wa.gov/ehp/ts/Zoo/WNV/wnv03report.pdf.

WDOH (Washington State Department of Health). 2004b. "West Nile Virus-positive cases in Washington, 2002." Washington State Department of Health, Olympia, Washington. Accessed on the internet at: http://www.doh.wa.gov/ehp/ts/Zoo/WNV/Surveillance02.html. Last updated November 15, 2004.

WNVWG (West Nile Virus Working Group). 2003. "Draft Saskatchewan West Nile Virus Response Framework – 2003." Accessed December 29, 2004 on the internet at http://www.health.gov.sk.ca/rr_WNV_Framework.pdf.