Catch Basins as Sources of Mosquitoes and West Nile Virus: An Evaluation of the Abundance of Mosquito Larvae and the Efficacy of Control Strategies

Final Report

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Catch Basins as Sources of Mosquitoes and West Nile Virus: An Evaluation of the Abundance of Mosquito Larvae and the Efficacy of Control Strategies

Final Report

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Executive Summary

In collaboration with Landau Associates, Inc., we quantified the abundance of mosquito larvae weekly within 250 randomly selected catch basins within the City of Seattle from mid-June through September 2006. We monitored a variety of environmental variables within these catch basins, and within an additional set of randomly selected basins examined the efficacy of several mosquito control strategies. Basins were selected using a stratified random design incorporating sector and zone. Our overall goal was to assist the City (Seattle Public Utilities [SPU]) in developing effective strategies to evaluate and control mosquito breeding in catch basins in response to the threat of West Nile Virus that are protective of human health and the environment.

Within the 250 basins monitored weekly, larval abundance began to increase in mid-June and peaked mid-July through the end of August and then decreased sharply with low numbers detected by the end of September. Differences in larval counts between the "early" (Weeks 25-28), "peak" (Weeks 29-35), and "late" (Weeks 36-39) seasons were statistically significant. The overall pattern was similar to that observed by Landau Associates, Inc. in a smaller subset of basins in 2005, but overall weekly averages of the number of larvae were approximately three times greater in 2006. Nearly 90 percent of the 250 basins monitored had mosquito larvae in 2006. Less than 1 percent consistently had water and no mosquito larvae. Similarly, less than 1 percent was consistently dry. Conversely, 99 percent of the basins monitored had water at one point during the season, and 58 percent consistently had water. These statistics were similar among sectors. However, larval counts within the West and Southwest Sectors across seasons were statistically greater than counts within the other sectors (N, NE, E, and S), but during the "peak" season, only counts within the West Sector differed significantly from some of the other sectors (E and SE). Statistical separations among larval counts within sectors were hampered by the high variability within sectors. Larval counts within the Single Family

Zone were statistically greater than those within the Multi-family and Neighborhood Commercial Zones, irrespective of season.

A stepwise regression analysis relating larval counts within the 250 basins to environmental variables indicated that sector, zone, ambient air temperature, basin water temperature, and time (week) were the best predictors of larval abundance. However, the resulting model accounted for only 25 percent of the variation in larval abundance among basins suggesting that other environmental variables within or outside the basins may be important. Precipitation was not a factor in 2006, with the first significant rainfall during the breeding season occurring in early September. A similar regression analysis relating the larval counts within 50 of these basins randomly selected each week to weekly measures of water temperature, water and sediment depth, pH, dissolved oxygen, and conductivity indicated that sector, zone, time (season), and water temperature and conductivity were the best predictors of larval counts. These variables accounted for 18% of the variation in larval abundance. In separate analyses, we compared the extent and composition of debris on the water surface within the 250 basins monitored weekly. Larval abundance was greater in basins with debris coverage greater than 0, but less than 76 percent. High larval counts were associated with basins with any plant material in the first half of the season, and debris that included 1-25 percent plant material later in the season. High counts were also associated with basins in which 1-25 percent of the basin surface or the debris present was other than plant material, paper, and plastic (i.e., styrofoam peanuts, cigarette butts, and other trash). Coverage and composition of debris did not vary among zones. We also compared characteristics of the sediment within five basins with consistently high (>30 larvae per dip) and five basins with consistently low larval counts (<u><</u>2 larvae per dip) within each of the three zones. Characteristics included texture (percent clay, silt sand), percent organic matter, pH, and concentrations (ppm dry weight) of copper, lead, zinc, diesel oil, and motor oil. No statistically significant differences were detected in these characteristics between the "high" and "low" basins.

To evaluate the efficacy and fate of selected control strategies, a new subset of basins was randomly assigned to each of three larvicide treatments: BTI (*Bacillus thuringiensis israelensis*, Mosquito Dunks®, Summit), BS (*Bacillus sphaericus*, VectoLex® WSP [water soluble pouches] VALENT BioSciences Corporation) or Methoprene (Altosid® Briquettes, Wellmark International). Ten basins per larvicide within each of the three zones were treated. Within each each zone, five basins were randomly assigned to determine efficacy of the larvicides and five to determine fate. Formulations and application rates were selected following review of the existing literature and consultations with other municipalities and the product manufacturers. Treatments were according to maximum label recommendations and performed by a licensed pesticide applicator. In addition, 15 control basins (5 per zone) were selected from the 250 basins being monitored weekly.

All treatments resulted in a rapid and dramatic reduction (<u><</u> 7 days) in either the number pupae present (BTI and BS) or in the hatching success of pupae (Methoprene). Both BTI and BS continued to reduce the number of pupae for 7 weeks post-treatment. BS was the

This report includes results of analyses from contracted analytical laboratories received by 14 March 2007. Data on the fate of Methoprene in the basins we treated are still outstanding. Because of this and because this research was conducted in partial fulfillment of the requirements for an MS degree by one of the authors, the data included will be subject to additional analysis and interpretation.

most effective control agent with essentially no pupae detected in basins treated with the bacteria. BTI was almost equally effective with the exception of Week 4 in which the average count approached two pupae. The reduction in efficacy of BTI in Week 4 appears to have been associated with an increase in basin water temperatures that may have shortened time to maturation and therefore also shortened larval exposure to the bacteria. Increases in the number of pupae were also observed in the BS and Methoprene treatments and controls during the same week. Methoprene was effective resulting in an average hatching success of less than 10 percent thru the first 4 weeks post-treatment. The effectiveness of Methoprene was reduced by precipitation events during Weeks 5 and 6, but the growth regulator was again effective in Week 7. Concentrations of Methoprene likely dropped below threshold concentrations following flooding of the basins associated with the rainfall events.

Concentrations of the larvicides within the water to sediment-water interface within the "fate" basins were monitored before and after treatment. Basins treated with BS or BTI were monitored for 5 weeks post treatment and those treated with Methoprene were monitored for 7 weeks. Concentrations of BS and BTI were expressed as numbers of spores per ml. The formulation of BS we used was actually a combination of BS and BTI with no difference in the strain of the latter between the VectoLex® and Mosquito Dunks®. Average spore counts for BS and BTI in the basins treated with VectoLex® and those treated with BTI only (Mosquito Dunks®) were very low pre-treatment, peaked 7 days post-treatment and then decreased during subsequent weeks. Concentrations of BS and BTI in the VectoLex® basins 5 weeks post-treatment were approximately 10 and 17% of peak concentrations, respectively. At this same time, concentrations of BTI within the basins treated with Mosquito Dunks® were 33 percent of peak concentrations. Both products were still effective in reducing the number of pupae present 7 weeks after treatment. Methoprene samples have been submitted to Warren Analytical Laboratory, Greeley, CO, but results are not yet available.

As a potential alternative control strategy, a subset of catch basins was cleaned early in the season within two areas in southern part of the City (West Seattle, Southeast Seattle – Columbia City). Within each area, half of the basins were within streets that were also swept repeatedly. Basins were cleaned and swept as part of the City's "Clean Basin/Street Sweeping Initiative". We randomly selected five basins within each area and treatment (cleaned, cleaned and swept), and five control basins located at least one block outside of each of the study areas, and monitored larval abundance weekly beginning the week of 26 June. Cleaning and cleaning plus sweeping were not associated with a reduction in mosquito larvae. Larval counts within basins that were both cleaned and swept were statistically greater than those within nearby control basins; counts within basins that were only cleaned were similar to controls.

Recommendations for future research include (1) additional efforts to identify factors governing larval abundance; (2) determination of the length of time each of the larvicides is efficacious, including the extended release formulation of Methoprene; and (3) an evaluation of the potential for non-target effects within surface waters associated with an operational control program.

Catch basins studied in 2006 were round-top. White float in bottom picture was attached to a continuous temperature data logger resting on the sediment within the basin. Photos: C. Grue

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Acknowledgments

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Measuring water and sediment depth and water quality before dipping for pupae as part of assessments of the efficacy of mosquito larvicides in 2006. Photo: C. Grue

Introduction

West Nile Virus (WNV) first appeared in the United States (US) in New York City in 1999. It is thought that an illegally imported host, likely a bird, carried a strain of WNV that originated in the Middle East (Bost 2004). In the years following its introduction, the virus has spread quickly throughout the US from east to west. States generally first detect animal infections (birds, horses or mosquitoes); human WNV cases typically appear in the year following the initial detection (www.cdc.gov).

In 2005, Maine and Washington were the only states in the contiguous US without human cases of WNV. In Washington State, the virus had been detected in animals in six counties in 2002. Across the US, 43 species of mosquitoes have tested positive as carriers of WNV. In Washington State, only five mosquito species have been shown to actually transmit WNV from host to host (Bost 2004). Species of particular concern are within the genus *Culex* because they thrive in urban areas and feed on both birds and humans (Bost 2004). Four more mosquito species have been identified as potential vectors because they have tested positive for the virus; but this does not necessarily mean that these particular species are able to transmit the virus (Bost, 2004).

In anticipation of the spread of WNV and with the knowledge that the virus had been detected within Washington State, the City of Seattle (Seattle Public Utilities [SPU] initiated a city-wide research effort in summer 2006. The overall goal of this research effort was to develop effective strategies to evaluate and control mosquito breeding in SPU catch basins that are protective of human health and the environment. Specific objectives were to quantify the abundance of mosquito lavae within storm-water drainage systems (catch basins), identify the environmental factors governing larval abundance, and determine the efficacy of three common larvicides. The City's research effort proved to be timely because Washington State reported its first human cases of WNV in 2006 (www.doh.wa.gov).

Other municipalities along the West Coast have also undertaken research aimed at determining which larvicide product is the most effective. For example, in the summer of 2006, Vancouver, BC treated half of its basins with Altosid® briquettes and the other half with Vectolex® WSP pouches. The results from this study will be available early in 2007 and will likely dictate how the city controls mosquitoes in summer 2007. Sacramento, CA has conducted research on the efficacy of Altosid® briquettes since 2003. To date, they have found that this particular product is effective for up to 95 days. In addition, the city has found that rain does not diminish the effects of either BS or BTI products. Future research will be based on testing a new BTI product, similar to the BTI donut, but which is expected to be effective for longer than 100 days. The city is also planning to conduct studies to determine whether or not temperature has an effect on the degradation of Altosid® products. Additional details on the activities of these and other municipalities can be found in Appendix 1.

Abundance of Mosquito Larvae in Catch Basins

Synopsis — Larval abundance began to increase in mid-June and peaked mid-July through the end of August and then decreased sharply with low numbers detected by the end of September. Differences in larval counts between the "early", "peak", and "late" seasons were statistically significant. This pattern was similar to that observed in 2005, but overall weekly averages of the number of larvae were approximately three times greater in 2006. Nearly 90% of the basins monitored in 2006 had mosquito larvae. Less than 1% consistently had water and no mosquito larvae. Similarly, less than 1% was consistently dry. These statistics were similar among sectors. However, larval counts within the West and Southwest Sectors across seasons were statistically greater than counts within the other sectors, but during the "peak" season, only counts within the West Sector differed significantly. Statistical separations among larval counts within sectors were hampered by the high variability within sectors. Larval counts within the Single Family Zone were statistically greater than those within the Multi-family and Neighborhood Commercial zones irrespective of season.

Objective

Our objective was to quantify the abundance of mosquito larvae in catch basins. Specific tasks were:

- Review the 2005 SPU study report and make suggestions to improve the quality and utility of studies in 2006.
- Recommend and implement a sampling design to monitor abundance of larvae in 2006.

Methods

We reviewed the 2005 study design, data collection protocols, and data analyses, as well as the pertinent literature and efforts by other municipalities (Appendix 1). We identified limitations and suggested improvements. We met with the contractor to review the 2005 effort and discuss our suggestions. We obtained the data and performed additional analyses to help with the planning and study design for 2006. Based on this review, we recommended a sampling design to quantify the abundance of larvae in catch basins by dipping that complemented the other research efforts described in this report. The sampling design shown in Figure 1 (page 17) incorporated three zoning designations (Single Family, Multi-family, and Neighborhood Commercial) within the City of Seattle, adequate spatial coverage within zoning categories, and a sufficient number of catch basins within each zoning category for complementary research efforts. No distinction was made between the two primary basin types (240 and 242) due to the difficulty in identifying the locations of the different basin types. Basins included in the sampling effort were randomly selected using a stratified random sampling design considering sector and zone (Appendix 2). Criteria for basin selection are given in Appendix 3.

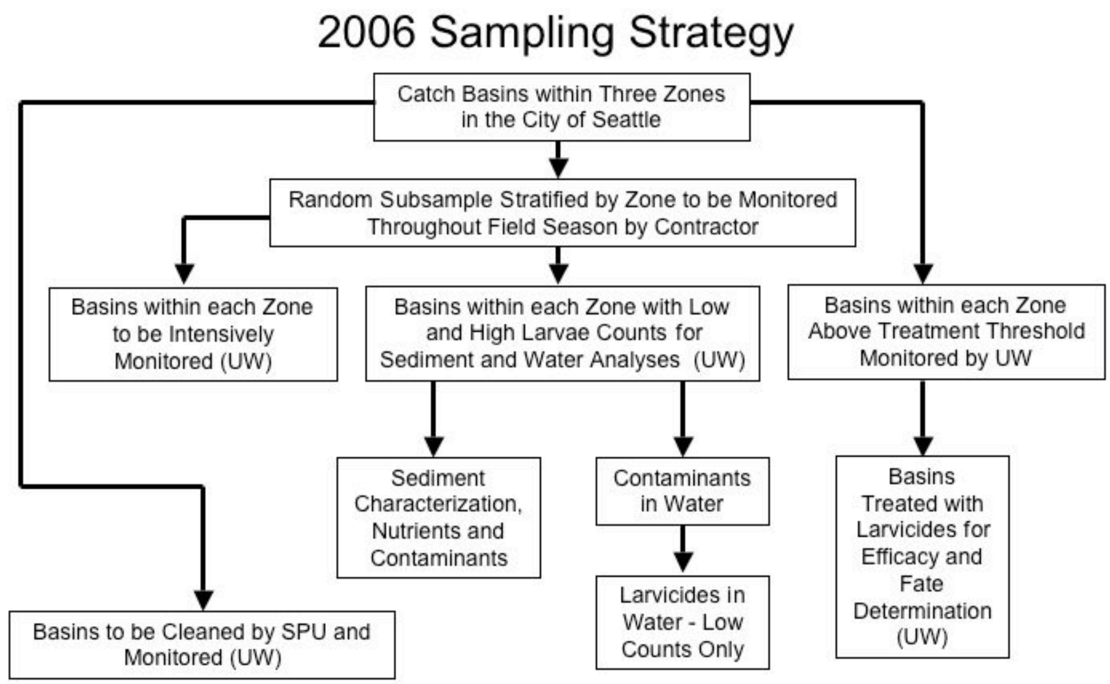


Figure 1. Sampling strategy adopted in 2006.

Protocols for training and data collection to ensure QA/QC and safety of personnel were developed and implemented.

Landau Associates, Inc. quantified the abundance of mosquito larvae within the 250 catch basins selected for study beginning in mid-June and continuing through the end of September. Each basin was visited weekly on a fixed schedule. Three 350-ml dips were collected along the edge of the basin approximately 120˚ apart using a standard dipper (Clarke Mosquito Control Service and Supplies, Roselle, IL) attached to a telescoping pole at a slight angle to accommodate the distance between the opening to the catch basin and the waters edge. To collect the sample, the dipper was slowly lowered below the water surface and allowed to fill to below the rim. Care was taken to prevent over filling and potential flushing of the contents of the dipper. The number of larvae were counted within each dip and recorded. Water temperature (C) was determined at the sediment water interface (Fluke Model 52II, Everett, WA) and water depth was measured to the nearest 0.25 inches) using standardized instruments developed by the Unit. Composition and spatial coverage of debris within each catch basin were noted.

All digital data were proofed by the contractor. A subset (10%) was subsequently checked again by Unit staff. If errors were detected, an additional subset was checked, and the process repeated until no transcription errors were detected.

Statistical analyses were performed using SPSS (V.11 for Mac) and Excel (Office version 2004 for Mac) software. Larval abundance data were expressed as averages of the three dips collected at each basin on each visit. Averages were transformed using an inverse transformation (1/x+1). We used a series of one-way analyses of variance (ANOVAs) to determine the effects of season, sector, and zone followed by a Student Newman-Keul's multiple comparison when the variances were homogeneous and by Dunnett's T3 multiple comparison when they were not. Significance was accepted when $P \leq 0.05$.

Results

Preliminary results of the monitoring conducted by the contractor were summarized in weekly reports to SPU and its cooperators. Data collected by the contractor clearly defined the mosquito-breeding season in the City's catch basins in 2006 (Fig. 2, page 19). Based on these data, we divided the season into "early" season (4 weeks), "peak" season (7 weeks) and "late" season (4 weeks) (Fig. 2, page 19). Subsequent analyses use this delineation.

Larval abundance within the 250 basins monitored weekly increased in mid-June and peaked mid- July through the end of August and then decreased sharply with low numbers detected by the end of September (Fig. 2, page 19). Differences in larval counts between the "early" (Weeks 25-28), "peak" (Weeks 29-35), and "late" (Weeks 36-39) seasons were statistically significant (P<0.000). The overall pattern was similar to that observed by Landau Associates, Inc. in a smaller subset of basins in 2005 (Fig. 3, page 19), but overall weekly averages of the number of larvae were approximately three times greater in 2006. However, a direct comparison between years in the average numbers of mosquito larvae per dip is hampered by the fact that the basins sampled in 2005 were not representative of those within the City (i.e., the selection was not random, J. Starstead, personal communication; Appendix 4). In fact, numbers in 2005 were more similar to those collected within specific sectors (e.g., Northeast) in 2006 (Fig. 4, page 20).

Summary statistics for 2006 are provided in Table 1 (page 20). Nearly 90 percent of the 250 basins monitored had mosquito larvae. Less than 1 percent consistently had water and no mosquito larvae. Similarly, less than 1 percent was consistently dry. Conversely, 99 percent of the basins monitored had water at one point during the season, and 58 percent consistently had water. These statistics were similar among sectors (Appendix 5). However, larval counts within the West and Southwest Sectors across seasons were statistically greater (P<0.0000) than counts within the other sectors (N, NE, E, and S), but during the "peak" season, only counts within the West Sector differed significantly from some of the other sectors (E and SE, P<0.002) (Fig. 4, page 20). Statistical separations among larval counts within sectors were hampered by the high variability within sectors (Appendix 6). Larval counts within the Single Family Zone were statistically greater (P<0.000) than those within the Multi-family and Neighborhood Commercial Zones irrespective of season (Fig. 5, page 21).

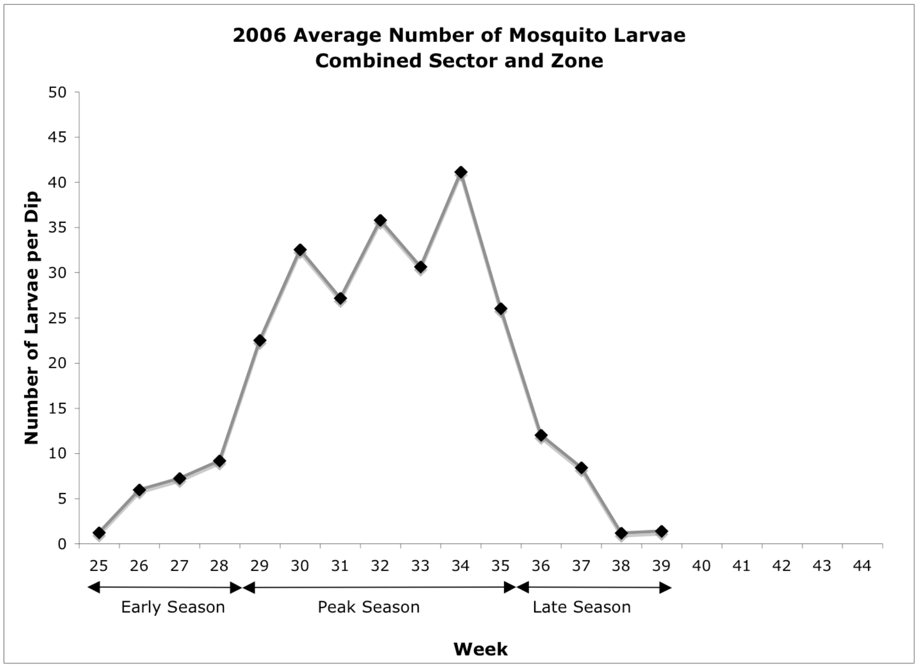


Figure 2. Average number of mosquito larvae per three dips across sector and zone in 2006; weeks of 6/19/06-6/23/06 (Week 25) through 9/25/06-9/29/06 (Week 39).

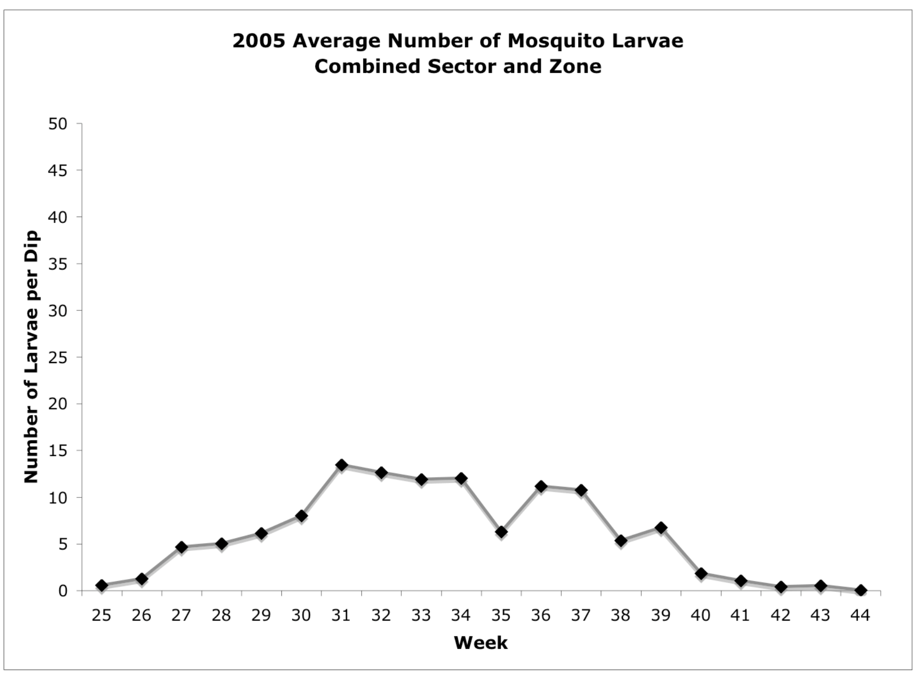


Figure 3. Average number of mosquito larvae per three dips across sector and zone in 2005; weeks of 6/20/05-6/24/05 (Week 25) through 10/31/05-11/04/05 (Week 44).

Table 1. Percent of catch basins with specific mosquito larvae and water conditions during the entire (6/19/06-9/29/06), "early" (6/19/06-7/14/06), "peak" (7/17/06-9/1/06), and "late" (9/4/06-9/29/06) seasons. Statistics were similar among sectors (Appendix 5).

	Entire Season	Early Season	Peak Season	Late Season
Basins with larvae on 1 or more occasion	89.6	71.6	84.4	74.4
Basins consistently had water and no larvae	0.8	14.0	0.8	8.4
Basins consistently dry	0.8	28.4	10.4	0.8
Basins had water on at least one visit	99.2	71.6	89.6	99.2
Basins consistently had water	58.4	77.6	63.6	75.2

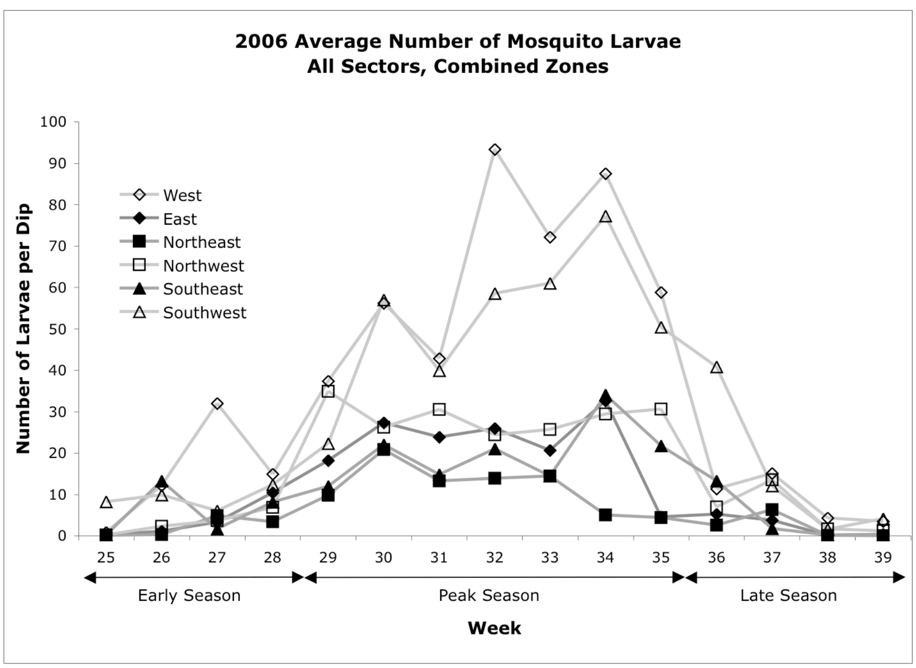


Figure 4. Average number of mosquito larvae per three dips for each sector across zone in 2006; weeks of 6/19/06-6/23/06 (Week 25) through 9/25/06-9/29/06 (Week 39). Larval counts within the W and SW sectors were statistically greater than those in the other sectors across the entire season.

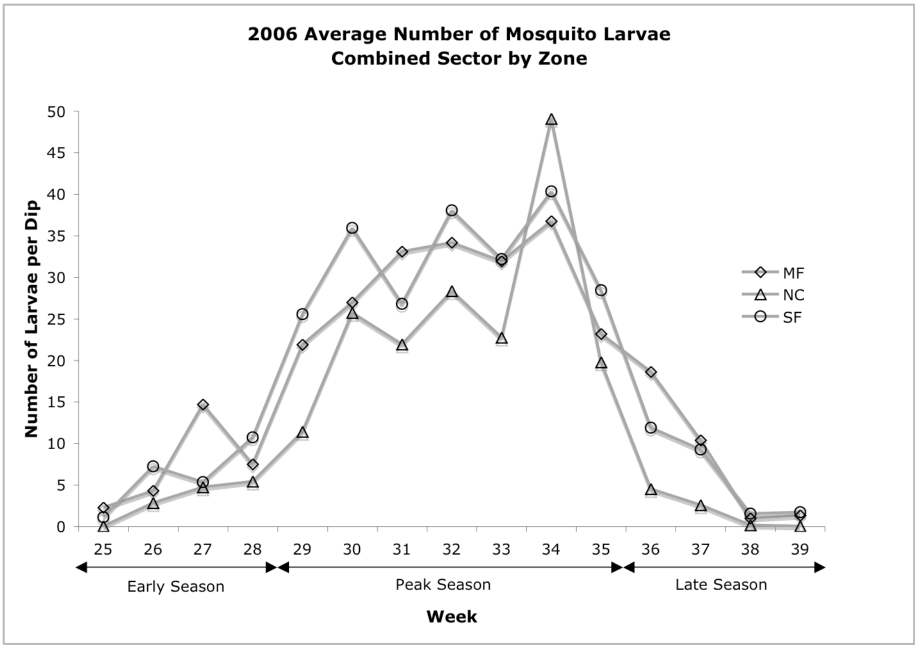


Figure 5. Average number of mosquito larvae per three dips for each zone across sector in 2006; weeks of 6/19/06-6/23/06 (Week 25) through 9/25/06-9/29/06 (Week 39). Larval counts within the Single Family Zone were statistically greater than those in the other zones.

Conclusions

The mosquito-breeding season within the catch basins was defined as mid-June to the end of September with the peak season from mid-July to the end of August. Nearly all (90%) of the basins monitored in 2006 had mosquito larvae. Less than 1 percent consistently had water and no mosquito larvae. Over the entire season, numbers of larvae within the West and Southwest Sectors were statistically greater than counts within the other sectors. Larval counts within the Single Family Zone were statistically greater than those within the Multi-family and Neighborhood Commercial Zones.

Research Needs

Based on the results of the 2006 monitoring and research effort and a review of the research and control programs of other municipalities on the West Coast, we do not see the need for further quantification of the abundance of mosquito larvae within the types of catch basins we sampled in 2006. Furthermore, because of the incidence of human cases of WNV in Western Washington in 2006, it may not be possible for sampling to occur in 2007 within the context of "no control". Efforts would be better focused on identification of factors governing differences in larval abundance among sectors and zones and addressing concerns over the potential non-target effects of control strategies.

Factors Governing Larval Abundance Within Catch Basins

Synopsis — Stepwise regression relating larval counts within the 250 basins to environmental variables indicated that sector, zone, ambient air temperature, basin water temperature, and time (week) were the best predictors of larval counts. However, the resulting model accounted for only 25 percent of the variation in larval abundance among basins suggesting that other environmental variables within or outside the basins may be important. Precipitation was not a factor in 2006, with the first significant rainfall during the breeding season occurring in early September. A similar analysis relating larval counts within 50 of these basins randomly selected each week to weekly measures of water temperature, water and sediment depth, pH, dissolved oxygen, and conductivity indicated that sector, zone, time (season), and water temperature and conductivity were the best predictors of larval counts. These variables accounted for 18% of the variation in larval abundance. Comparisons of the extent and composition of debris on the water surface within the 250 basins monitored weekly indicated that larval abundance was greater in basins with debris coverage greater than 0, but less than 76 percent. High larval counts also occurred in basins with any plant material in the first half of the season, and debris that included 1-25% plant material later in the season. Similarly, basins in which 1-25 percent of the basin surface or the debris present consisted of debris "other" than plant material, paper, and plastic (i.e., styrofoam peanuts, cigarette butts, and other trash) also supported high larval counts. Coverage and composition of debris did not vary among zones. We also compared characteristics of the sediment within five basins with consistently high ($\geq$100 larvae per dip) and five basins with consistently low larval counts ($\leq$10 larvae per dip) within each of the three zones: texture (percent clay, silt sand), percent organic matter, pH, and concentrations (ppm dry weight) of copper, lead, zinc, diesel oil, and motor oil. No statistically significant differences were detected in these characteristics between the "high" and "low" basins.

Objective

Our objective was to identify the environmental factors governing the abundance of mosquito larvae in catch basins. Specific tasks were:

- Characterize the physical and biological environmental conditions within and outside catch basins.
- Correlate environmental conditions within catch basins with surrounding habitat types and weather conditions, including water temperature and precipitation.
- Relate the conditions within and outside the catch basins to temporal patterns in mosquito larvae abundance.

Methods

Working with the contractor, we developed protocols to measure water temperature and water depth, and characterize debris within the 250 catch basins in which the abundance of mosquito larvae was quantified from mid-June thru the end of September. As previously noted, water temperature (C) was determined at the sediment water interface and water depth was measured to the nearest 0.25 inches using standardized instruments developed by the Unit. Composition of debris and spatial coverage of surface water by debris within each catch basin were recorded. Within 50 of the 250 basins, randomly selected weekly (stratified with replacement), we deployed continuous water temperature data loggers for 6 or 7 days. Before and after deployment, we measured sediment depth measured at the center of the basin to the nearest 0.25 inches using the instruments developed by the Unit, as well as dissolved oxygen (mg/L; Hach Model HQ40D, Loveland, CO), pH (Oakton Model 30, Oakton, VA) and conductivity (μS/cm; Oakton EC Tester Low, Oakton, VA). Ambient temperature data were obtained from the Seattle-Tacoma International Airport (SEA-TAC; http:www.beautifulseattle.com/clisea.htm). Precipitation was not a factor in 2006, with the first significant rainfall during the breeding season occurring in early September (Fig. 6, page 25).

At the end of August/early September, we compared characteristics of the sediment within five basins that consistently had water and high larval counts (>30 larvae per dip) and five comparable basins with consistently low larval counts ($\leq$2 larvae per dip) within each of the three zones (Appendix 7). Characteristics included texture (percent clay, silt sand), percent organic matter, pH, and concentrations (ppm dry weight) of copper, lead, zinc, diesel oil, and motor oil. We used a standard dipper to collect sediment at the sediment-water interface. The dipper was rinsed with dechlorinated water and dried between basins. Samples for "standard soil analyses" were placed in plastic bottles and refrigerated (4 C) before shipment to Soiltest Farms Consultants, Moses Lake, WA for analysis. Samples for contaminant analyses were placed in chemically clean glass jars and delivered to Aquatic Research, Inc. in Seattle the day of collection. At this same time during the study, the habitat surrounding the 250 basins monitored by the contractor was visually characterized by taking a digital photo at four cardinal directions at each basin for subsequent analysis.

Statistical analyses were performed using SPSS (V.10 for Mac) and Excel (Office version 2001 for Mac) software. An inverse or log transformation was used when the need for a transformation was indicated; the transformation that performed best for a particular dataset was selected. For all analyses, significance was accepted when $P \leq 0.05$. Specific analyses for each dataset are noted below.

250 Catch Basins Sampled Through Time — A stepwise regression analysis was conducted to examine the potential correlation of nine predictor variables with larval abundance and to obtain a final model. These predictor variables included season, week, sector, zone, basin number, ambient temperature (average weekly high), ambient temperature lagged 1 week, and basin water temperature. Since the same 250 basins were sampled each week, the assumption of independent error terms was likely not appropriate. As a result, the predictor variables selected for the final model through the stepwise regression analysis were then subjected to an autocorrelation analysis. This analysis results in regression coefficients with autocorrelation accounted for in the model.

50 Catch Basins Rotated Through Time — A stepwise regression analysis was conducted to examine the potential correlation of 11 predictor variables with larval abundance and to obtain a final model. These predictor variables included season, week, sector, zone, basin number, water temperature and depth, sediment depth, dissolved oxygen, pH, and conductivity. The 50 basins were randomly selected each week for sampling, therefore the assumption of independent error terms was valid and an autocorrelation analysis was not conducted.

Extent and Composition of Debris and Larval Abundance — Data on the extent and composition of debris on the surface of the water within the 250 basins monitored weekly was quantified by assigning the overall percent cover and that of each type of debris (plant material, plastic, paper, and other [styrofoam, cigarettes, indistinguishable trash] to 0, 1-25, 26-50, 51-75, and 76-100 percent. Because of an omission on the initial data form, overall percent cover was not recorded until Week 33 of the study. At that time, the data sheet was changed such that overall percent cover was included and values for the specific types of debris were recorded as a percentage of the debris present (not the total water surface). Because of this change occurred "mid-season", the cover data were analyzed and their relationship to larval counts determined within the two time periods: Period 1 – Weeks 25-32 and Period 2 – Weeks 33-39.

To analyze the coverage data, each range of percent cover was converted to a categorical variable: "cover index" (1-5), with 1=0 percent and 5=76-100 percent. This conversion was done so that the SPSS Plus Statistical Software® could perform the necessary comparisons between larval abundance and cover type, as ranges are difficult for the software to handle. In order to replace the missing data on total cover within the basins in Period 1, we translated the individual cover data for each type of debris into a "total debris cover index". The debris cover index is the sum of the individual indices for each debris type, with the index ranging from 4 (0% cover) to 11 (100% cover). The total cover index for Period 2 was classified the same way as the cover index for each type of debris (1-5). We again note that values for each type of debris in Period 2 represented the percentage of the total debris cover, not the percentage of the water surface within each basin as was estimated in Period 1.

Statistical analyses were performed separately for Periods 1 and 2. All entries with no larvae were omitted and any blank entries were ignored by the analysis. Ten univariate ANOVAs (α=0.05, Dunnett T3 statistic) were performed relating either the "total cover index" or the cover index for each type of debris to larval abundance in each basin (average of three dips).

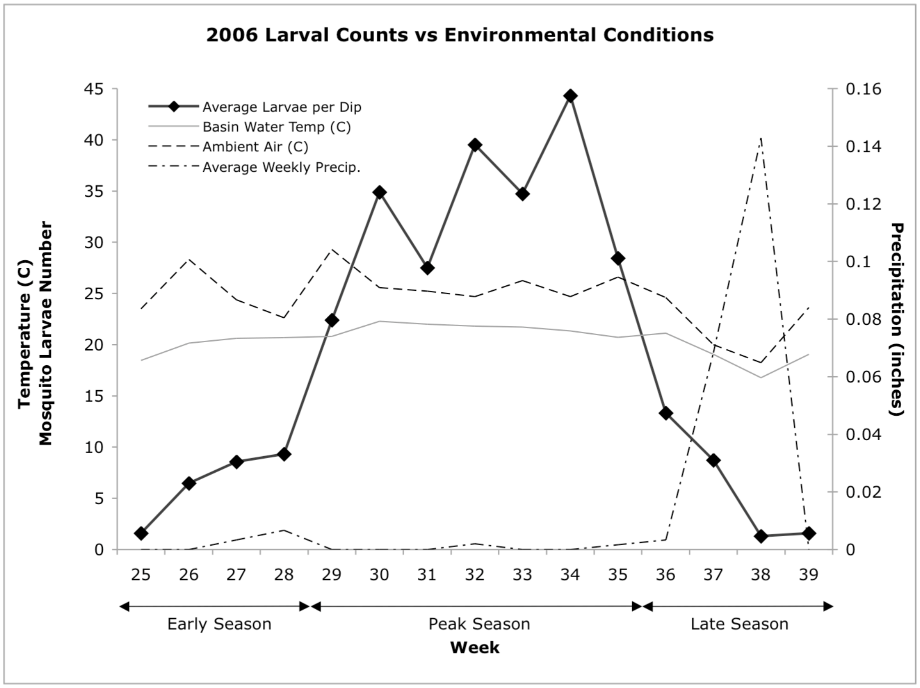


Figure 6. Average number of mosquito larvae per three dips across zones and sectors in 2006; 6/19/06-6/23/06 (Week 25) through 9/25/06-9/29/06 (Week 39) versus ambient air temperature (average weekly high), basin water temperature (sediment water interface), and precipitation (average weekly total).

High/Low Comparisons — Two-way ANOVAs were conducted to determine differences in a number of sediment characteristics and contaminant concentrations between basins with high or low larval abundance within zones. For all analyses, significance was accepted when $p \leq 0.05$ (main effects for ANOVAs) and when $p \leq 0.10$ (interaction effects).

Results

A visual inspection of the relationship between larval abundance and ambient air temperature and basin water temperature (Fig. 6) suggested that the effects of ambient air temperature on basin water temperature lagged by 1 week. Subsequent regression analyses included ambient air temperature offset by 1 week as a predictor of larval abundance.

The stepwise regression analysis relating larval counts within the 250 basins to environmental variables indicated that sector, zone, ambient air temperature (lagged by 1 week), basin water temperature, and time (week) were the best predictors (P<0.003) of larval counts (Table 2, page 26). However, the resulting model accounted for only 25 percent of the variation in larval abundance among basins suggesting that other environmental variables within or outside the basins may be important. As noted earlier,

Table 2. Regression coefficients, values, standard errors and *P*-values for the predictor variables included in the final model for the 250 catch basins sampled weekly. Regression coefficients are negative because of the inverse transformation of larval abundance. Adjusted R^2 for the model = 0.250.

Predictor Variable	Regression Coefficient	Standard Error	*P*-value
Water Temperature	-0.046269	0.003599	0.00000000
Ambient Temperature Lag	-0.033681	0.002151	0.00000000
Week	-0.011118	0.002013	0.00000004
Zone	-0.057457	0.012225	0.00000271
Sector	-0.018430	0.006095	0.00251845
Intercept	2.823213	0.105449	0.00000000
Autocorrelation Coefficient	0.410689	0.016399	0.00000000

precipitation was not a factor in 2006, with no significant rainfall occurring during the study. The regression analysis relating larval counts within 50 of these basins randomly selected each week to weekly measures of water temperature, water and sediment depth, pH, dissolved oxygen, and conductivity indicated that sector, zone, time (season), and water temperature and conductivity were the best predictors of larval counts ($P \leq 0.035$) (Table 3, page 27). These variables accounted for 18 percent of the variation in larval abundance.

We summarized the data on the extent and composition of debris on the surface of the water within the 250 basins monitored weekly within each of the two time periods (Figs. 7 and 8, pages 28 and 29). In both Periods 1 and 2, the majority of basins had debris covering <50 percent of the water surface with <15 percent of the basins containing >75 percent cover. In Period 1, larval abundance was statistically greater ($P<0.05$) in basins with a cover index of 6 and 7 (ca. 2-75% cover) compared to those with an index of 5 (≤25% cover) or 8 (>75% cover). Similarly in Period 2, basins with a cover index of 2-4 supported greater numbers of larvae compared to basins with an index score of 1 (no cover) or 5 (76-100% cover). Larval abundance varied with debris composition. In Period 1, numbers of larvae were statistically greater in basins containing plant debris compared to those with none, and those containing 1-25 percent of “other” debris (compared to those with 0 or 26-50% “other” debris). In both Periods, plant material was the primary debris with >70 percent of the basins in Period 1 having 1-25 percent plant cover. In Period 2, plant material comprised >75 percent of the debris in 40-50 percent of the basins. More than 55 percent of the basins in Period 1 contained “other” debris and most of these basins fell in the 1-25% cover category. In Period 2, two-thirds of the basins contained “other” debris and in about half of these basins “other” debris comprised 1-25 percent of the total debris cover. The presence of plastic or paper was not correlated

Table 3. Regression coefficients, standard errors, and *P*-values for the predictor variables included in the final model for the randomly selected 50 catch basins sampled weekly. Larval counts were log transformed. Adjusted R^2 for the model = 0.178.

Predictor Variable	Regression Coefficient	Standard Error	*P*-value
Water Temperature	0.045080	0.005	0.000
Conductivity	0.000568	0.000	0.000
Season	0.152000	0.034	0.000
Sector	0.006123	0.014	0.000
Zone	0.059750	0.028	0.035
Intercept	-1.095	0.152	0.000

with larval abundance. More than 85 percent of the basins studied did not contain any plastic or paper (Table 4, page 30).

We also compared characteristics of the sediment within five basins with consistently high and five basins with consistently low larval counts within each of the three zones: texture (percent clay, silt sand; Figs. 9 and 10, pages 31 and 32), percent organic matter, pH, and concentrations (ppm dry weight) of copper, lead, zinc, diesel oil, and motor oil (Table 5, page 33). No statistically significant ($P \leq 0.05$) differences were detected in these characteristics between the "high" and "low" basins.

Analysis of the digital photographs characterizing the habitat surrounding each of the basins will be completed in spring 2007.

Conclusions

Of the environmental variables quantified, ambient air temperature, basin water temperature and conductivity, time, sector and zone accounted for the most variation in larval counts. Larval abundance was greater in basins with debris coverage greater than 0, but less than 76 percent. High larval counts were associated with basins with any plant material in the first half of the season, and debris that included 1-25 percent plant material later in the season. High counts were also associated with basins in which 1-25 percent of the basin surface or the debris present was other than plant material, paper, and plastic (i.e., styrofoam peanuts, cigarette butts, and other trash). Coverage and composition of debris did not vary among zones. Characteristics of the sediment including levels of common highway contaminants did not differ between basins with high or low larval abundance. Models predicted no more than 25 percent of the variability in larval counts among basins suggesting that environmental variables external to the basins (e.g., surrounding habitat) may be equally or more important. Analyses of the digital photographs characterizing the habitat surrounding each of the basins have yet to be completed.

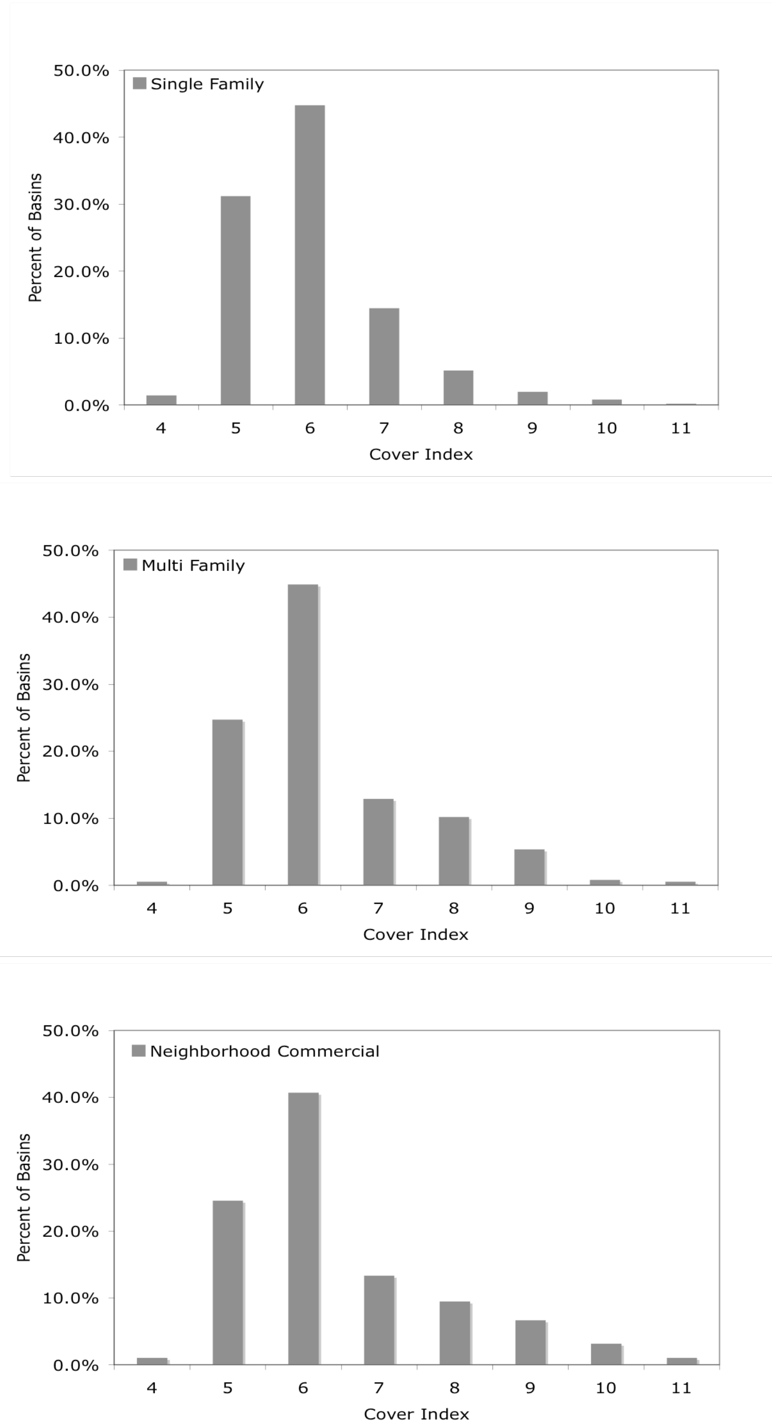


Figure 7. Relative percent cover of debris in catch basins within zones in Period 1 (Weeks 25-32) expressed as a cover index equal to the sum of the individual coverage scores for each debris type: 1=0%, 2=1-25%, 3=26-50%, 4=51-75%, and 5=76-100%. Scores ranged from 4 (0% cover) to 11 (100% cover on the water surface within a basin).

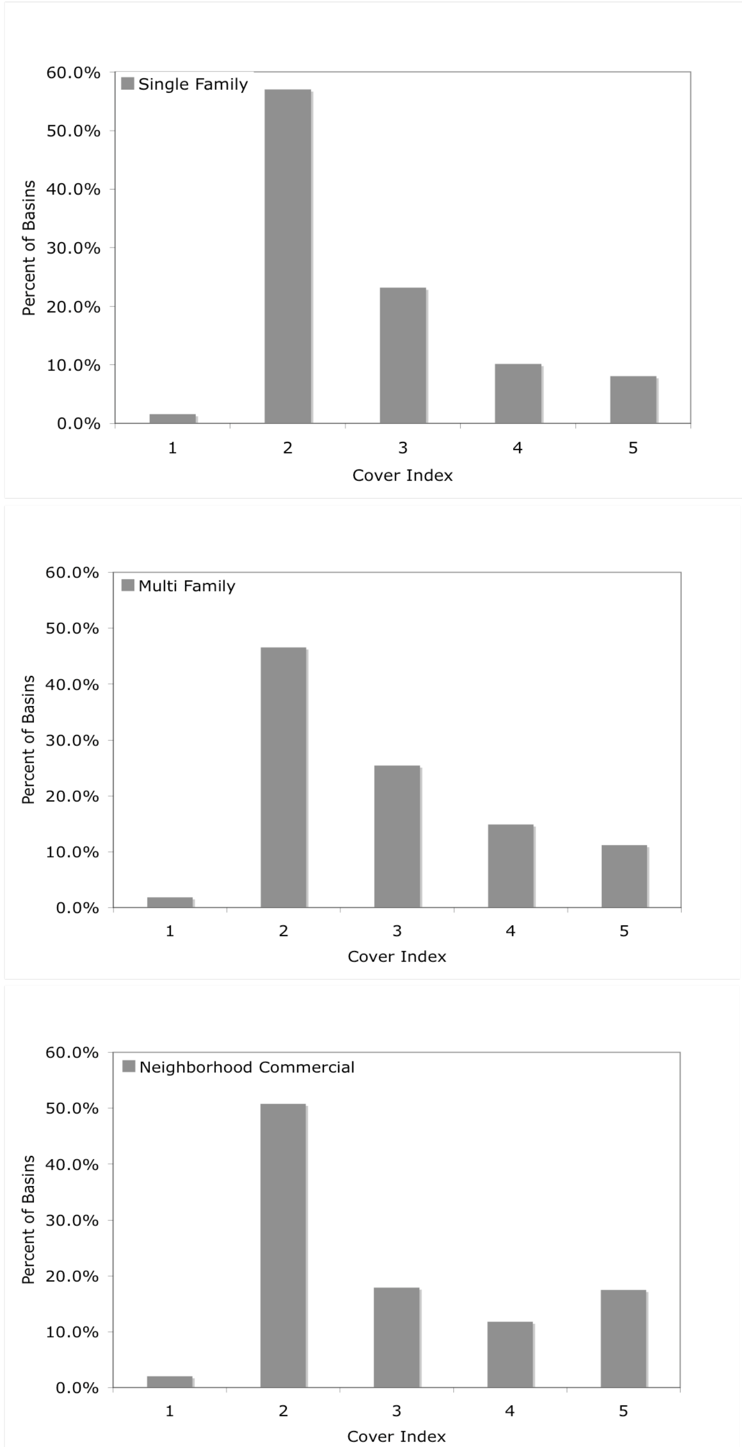


Figure 8. Relative percent cover of debris in catch basins within zones in Period 2 (Weeks 33-39) expressed as the percentage of basins with a cover index of 1=0%, 2=1-25%, 3=26-50%, 4=51-75%, or 5=76-100% of the debris present in a basin.

Table 4. Composition of debris in catch basins within each zone. Time Period 1 = Weeks 25-32; Period 2 = Weeks 33-39. Composition is expressed as a percentage of basins within each cover category. Cover categories in Period 1 are percentages of the water surface in each basin. In Period 2, cover categories are percentages of the debris coverage in each basin. "Other" debris includes styrofoam peanuts, cigarette butts, and indistinguishable trash.

Time Period	Zone	Type of Debris	Percentage of Basins within Relative Percent Cover Categories				
			0	1-25	26-50	51-75	76-100
1	SF	Plant Material	3.7	73.6	15.0	6.1	1.6
		Plastic	92.6	7.3	0.1	0	0
		Paper	95.5	3.8	0.4	.4	0
		Other	44.8	52.5	1.6	.4	0.6
	MF	Plant Material	2.2	73.4	13.7	6.7	4.0
		Plastic	90.3	9.7	0	0	0
		Paper	94.4	5.4	0.3	0	0
		Other	36.8	54.6	5.4	1.3	1.9
	NC	Plant Material	3.5	71.6	12.6	7.7	4.6
		Plastic	86.3	13.7	0	0	0
		Paper	91.2	8.8	0	0	0
		Other	36.1	53.3	4.9	2.8	2.8
2	SF	Plant Material	3.2	16.1	19.6	14.1	49.7
		Plastic	92.0	7.2	0.7	0.1	0
		Paper	94.2	4.7	0.7	0.2	0.2
		Other	36.1	33.1	17.8	5.0	4.3
	MF	Plant Material	3.7	15.6	21.1	16.2	43.4
		Plastic	86.5	12.8	0.6	0.3	0
		Paper	90.8	8.9	.03	0	0
		Other	30.0	34.9	23.9	6.4	4.9
	NC	Plant Material	4.0	17.9	16.3	17.9	43.8
		Plastic	86.5	11.6	1.6	0.4	0
		Paper	85.7	12.7	1.2	0.4	0
		Other	33.5	33.5	20.3	6.0	6.4

Research Needs

Regression analyses reinforced the importance of sector and zone in influencing the abundance of mosquito larvae within catch basins. Additional efforts to identify those environmental factors contributing to differences among sectors and zones may be warranted. In addition to the pending analyses of the digital photographs characterizing the habitat surrounding each of the basins, conventional and/or digital aerial photographs or satellite imagery may also be helpful. Initial efforts could be focused on those basins that consistently supported high or low larval counts. Reasons for the stepwise regression model's inclusion of both ambient air temperature and basin water temperature, factors that one would expect to be highly correlated, further support the hypothesis that factors external to the basins may be important.

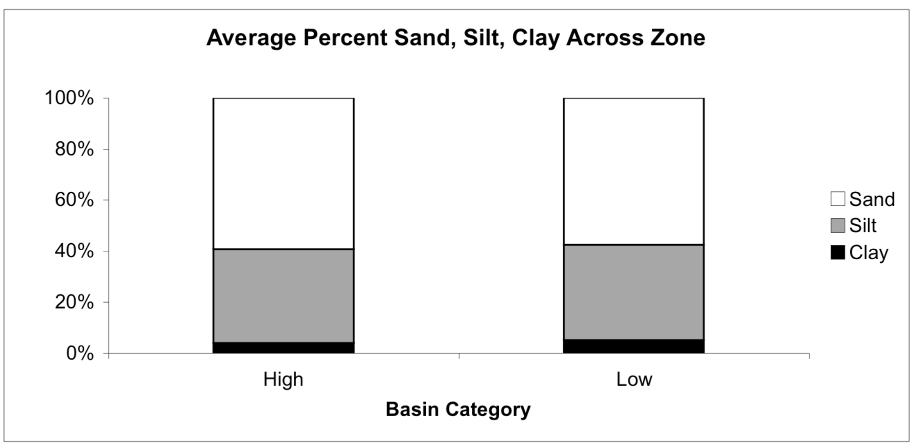
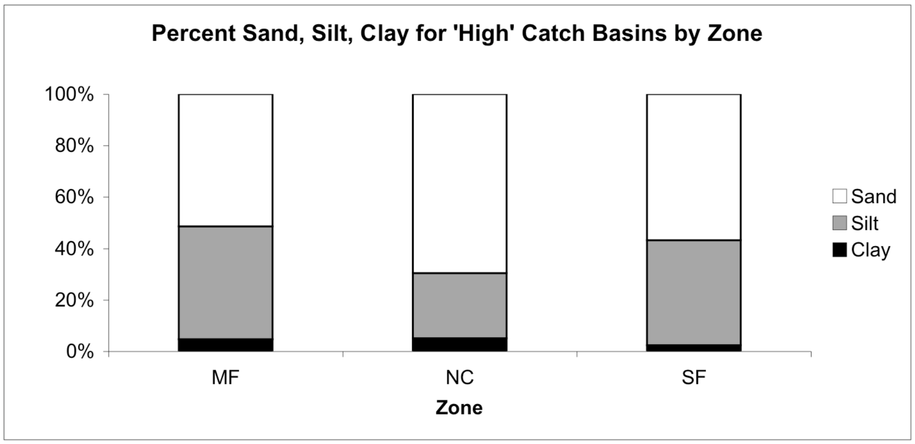


Figure 9. Average measures of texture (% percent sand, slit and clay) of sediments within basins across zones with high or low counts of mosquito larvae. Differences between high and low basins were not statistically significant (P>0.05, n=15 high and low basins).



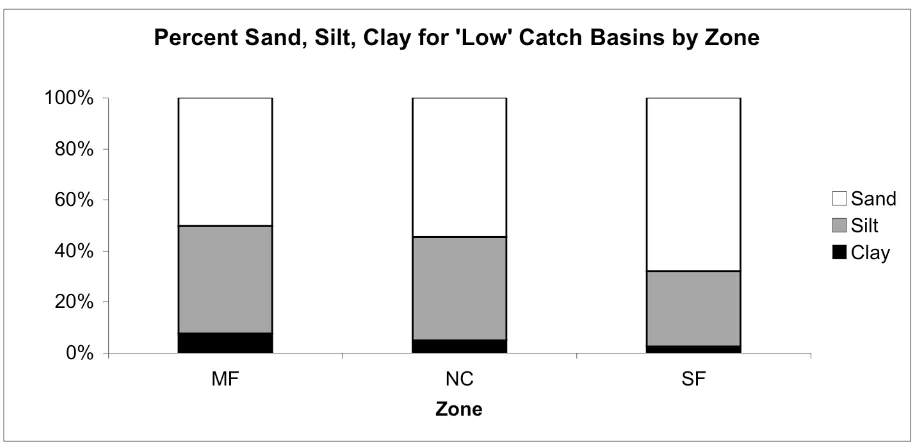


Figure 10. Average measures of texture (% percent sand, slit and clay) of sediments within basins with high (top graph) or low (bottom graph) counts of mosquito larvae. Differences between high and low basins were not statistically significant (P>0.05, n=5 high and low basins within each zone).

Table 5. Average pH, percent organic matter, and contaminant data (ppm dry weight) within sediment from catch basins with either high or low larval counts. Contaminant concentrations are presented as geometric means. N = 5 high and low basins within each zone.

High/Low Larval Category	Soil Characteristics	Single Family	Multi-Family	Neighborhood Commercial
HIGH	pH	7.02	6.98	6.96
	% Organic Matter	23.1	17.9	15.9
	Copper	131	45.0	63.7
	Lead	72.9	65.3	131
	Zinc	310	225	319
	Diesel	38.0	29.1	23.4
	Motor Oil	2,149	1,821	2,108
LOW	pH	7.08	7.10	7.08
	% Organic Matter	13.2	15.4	16.2
	Copper	50.5	87.2	33.9
	Lead	68.7	81.5	54.5
	Zinc	223	292	163
	Diesel	28.3	28.6	34.3
	Motor Oil	1,953	2,066	3,532

Efficacy of Control Strategies

Synopsis — All larvicide treatments resulted in a rapid and dramatic reduction (≤ 7 days) in either the number pupae present (BTI and BS) or in the hatching success of pupae (Methoprene). Both BTI and BS continued to reduce the number of pupae for 7 weeks post-treatment. BS was the most effective control agent with essentially no pupae detected in basins treated with the bacteria. BTI was almost as effective with the exception of Week 4 in which the average count approached two pupae. The reduction in efficacy of BTI in Week 4 was associated with an increase in basin water temperatures that may have shortened time to maturation and therefore also shortened larval exposure to the bacteria. Increases in the number of pupae were also observed in the BS and Methoprene treatments and controls during the same week. Methoprene resulted in an average hatching success of <10 percent thru the first 4 weeks post-treatment. The effectiveness of Methoprene was reduced by precipitation events during Weeks 5 and 6, but the growth regulator was again effective in Week 7. Concentrations of Methoprene likely dropped below threshold concentrations following flooding of the basins associated with the rainfall events.

Cleaning of basins and street sweeping as part of the City's "Clean Basin/Street Sweeping Initiative" were not associated with a reduction in mosquito larvae. Larval counts within basins that were both cleaned and swept were statistically greater than those within controls; those that were only cleaned were similar to controls.

Objective

Our objective was to determine efficacy of selected mosquito control strategies and relate efficacy to environmental conditions within and outside the treated basins. Specific tasks were:

- Evaluate the efficacy of three larvicide treatments: BTI (*Bacillus thuringiensis israelensis*, Mosquito Dunks®, Summit), BS (*Bacillus sphaericus*, VectoLex® WSP [water soluble pouches] VALENT BioSciences Corporation) or Methoprene (Altosid® Briquettes, Wellmark International).

- Evaluate the effectiveness of the cleaning of catch basins and street sweeping as an alternative control strategy.

Methods

Larvicides — At the end of July, prior to the height of the mosquito season (August based on the report by Landau Associates [2006]), we randomly selected a new subset of catch basins within each of the three zones (Single Family, Multi-family, and Neighborhood Commercial) in the Northwest Sector that met previous acceptance criteria

(Appendix 3), exceeded a treatment threshold of >1 mosquito larvae per three dips, and had ≥7.0 inches of water. With only a few exceptions, all three larvicide treatments were replicated at a particular intersection. In a few cases, treatments had to be split between two interections because of a shortage of available basins that met selection criteria. At an interection (or two intersections in the case of the exceptions), selected basins were randomly assigned to one of three larvicide treatments: BTI, BS, or Methoprene. Five basins per larvicide within each of the three zones (45 basins total) were treated during the week of 7 August. Formulations and application rates were selected following review of the existing literature and consultations with other municipalities and the product manufacturers (Appendices 1 and 8). Treatments were according to maximum label recommendations and performed by Unit staff licensed as pesticide applicators. In addition, 15 "control" basins (5 per zone) were randomly selected from the basins being monitored weekly by the contractor that met the above criteria. Individual control basins occurred at different interections.

For BTI and BS, efficacy was determined by counting the number of pupae within three standard larvae dips immediately before treatment and weekly for 7 weeks post-treatment. Because Methoprene (growth regulator) acts by inhibiting the metamorphosis of pupae to adults (i.e., counts of pupae are not a measure of efficacy), we adapted techniques from others for incubating pupae before and after methoprene treatments (Figs. 11 and 12, page 36). Methods were adopted following consultation with the manufacturer. A maximum of 10 pupae were collected from each of the 15 Methoprene-treated basins and the 15 controls before and after treatment. A maximum of three sets of three standard larvae dips were taken from each basin to obtain the larvae. The number collected from the each set was recorded and, if present, 10 pupae were immediately placed in 350 ml of spring water in a 12 oz clear plastic cup to which a styrofoam peanut was added and the cup covered with a domed clear plastic lid with a straw hole at the top. The lid was secured with clear plastic tape and the hole at the top covered with a breathable plastic band-aid. The cup with the pupae was then placed in a covered cooler and secured in a cardboard cup carrier. The cups, lids and carriers were provided by Starbucks Corporation, Seattle, WA. Upon return to the University, any adults that had emerged were counted and the racks with cups placed in an incubator set at 24-26 C (ca. 80° F, Fig. 11, page 36) with a 16 L:8 D light cycle. Adults that successfully emerged in each cup were counted daily for 5 days and the maximum number after 5 days was expressed as a percentage of the original number of pupae. The incubation period was recommended by the manufacturer and confirmed by incubating the pre-treatment samples for 5 days and counting the number of adults that emerged (Fig. 12, page 36). Within each cup, a sub-sample of adults was preserved in 70 percent ethanol for subsequent species identification. Before and after treatment, water quality (all measurements), composition and extent of debris, and water depth were determined. Measurements (including counts of pupae) were conducted weekly for 8 weeks (Week 0 = pre-treatment followed by 7 weeks post-treatment). Separate sets of all sampling equipment we used for each larvicide and the equipment and water quality instruments was thoroughly rinsed with dechlorinated water and dried between each use (basin).

Figure 11. Incubation cups and chamber for assessing the efficacy of Methoprene for controlling the production of mosquitoes in catch basins in Seattle. Larvae were incubated for 5 days at 24-26 C to determine hatching success. Photo: C. Grue

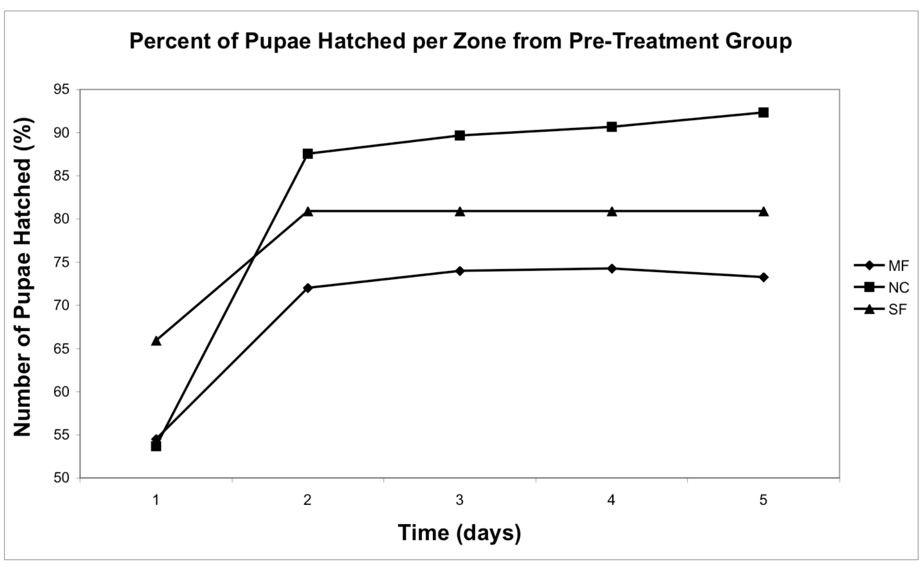


Figure 12. Percent of mosquito pupae hatched per zone before treatment during the week of 8/7/06 (n = 9-10 bioassays [basins] per zone).

Basin Cleaning and Street Sweeping — As a potential alternative control strategy, a subset of catch basins was cleaned early in the season within two areas in southern part of the City (West Seattle, Southeast Seattle – Columbia City). Within each area, half of the basins were within streets that were also swept repeatedly. Basins were cleaned and swept as part of the City's "Clean Basin/Street Sweeping Initiative". We randomly selected five basins within each area and treatment (cleaned, cleaned and swept) and five control basins at least one block outside of each of the study areas and monitored larval abundance weekly. Each week, we measured water temperature at the sediment water interface, water and sediment depth measured at the center of the basin to the nearest 0.25 inches) using standardized instruments developed by the Unit, as well as dissolved oxygen (mg/L), pH and conductivity (μS/cm). Composition of debris and spatial coverage of surface water by debris within each catch basin were also recorded. Monitoring began during the week of 26 June following consultation with the contractor monitoring contaminants within the sediments in the basins and continued through the end of September.

We compared larval abundance between the two treatments and control basins in each sector using one-way analysis of variance (ANOVA) tests, followed by the Newman-Keul's multiple comparison. Analyses were performed using SPSS (V.11 for Mac) and Excel (Office version 2004 for Mac) software. Larval abundance data were log transformed. Significance was accepted when $P \leq 0.05$.

Results

All treatments resulted in a rapid and dramatic reduction ($\leq$ 7 days) in either the number pupae present (BTI and BS) or in the hatching success of pupae (Methoprene). Both BTI and BS continued to reduce the number of pupae for 7 weeks post-treatment (Fig. 13, page 38). BS was the most effective control agent with essentially no pupae detected in basins treated with the bacteria. BTI was almost equally effective with the exception of Week 4 in which the average count approached two pupae. The reduction in efficacy of BTI in Week 4 appears to have been associated with an increase in basin water temperatures (Fig. 14, page 38) that may have shortened time to maturation and therefore also shortened larval exposure to the bacteria. Increases in the number of pupae were also observed in the BS and Methoprene treatments and controls during the same week (Fig. 14, page 38). The abundance of pupae within the control basins was highly correlated with basin water temperature (Fig. 15, page 39). Methoprene was effective resulting in an average hatching success of less than 10% thru the first 4 weeks post-treatment. The effectiveness of Methoprene was reduced by precipitation events during Weeks 5 and 6, but the growth regulator was again effective in Week 7 (Figs. 16-17, pages 39-40). Concentrations of Methoprene likely dropped below threshold concentrations following flooding of the basins associated with the rainfall events.

Cleaning of basins or cleaning and street sweeping was not associated with a reduction in mosquito larvae (Figs. 18 and 19, pages 41-42). Larval counts within basins that were both cleaned and swept were statistically greater than those within controls in both

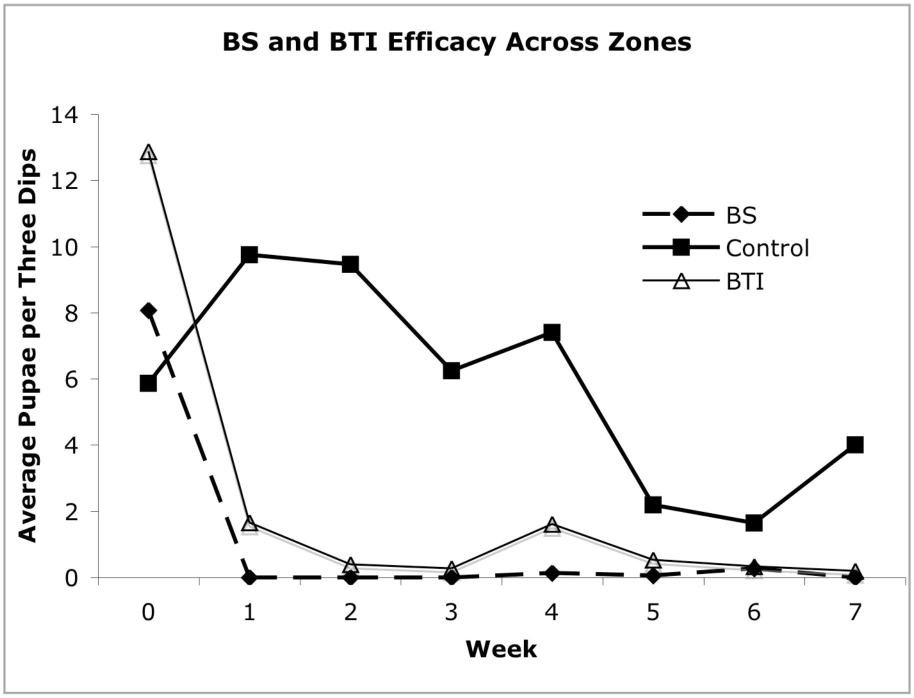


Figure 13. Average number of mosquito pupae within basins treated with BTI or BS or basins that received no treatment (controls). Pre-treatment data were collected at Week 0 before BTI and BS basins were treated. Week 1 = 7 days post-treatment. N = 15 basins per treatment per week.

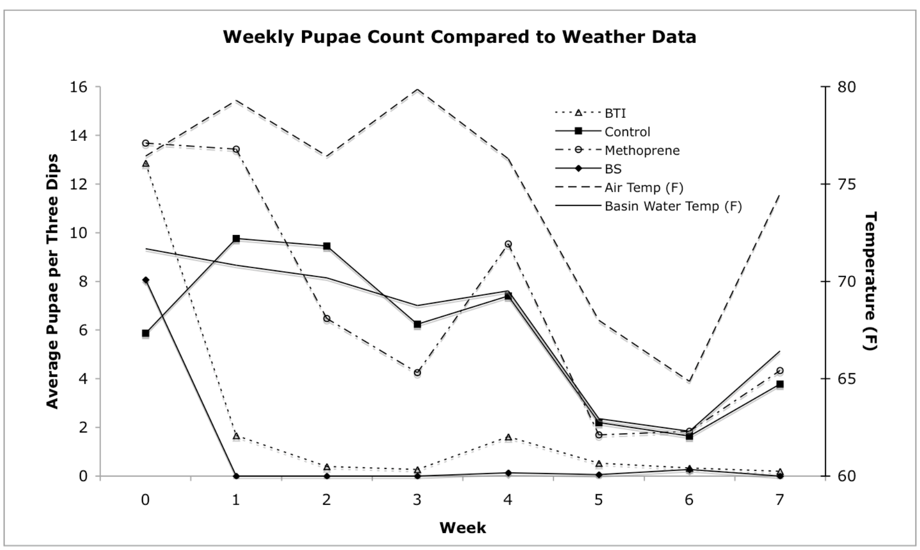


Figure 14. Average number of pupae per three dips from basins treated with one of the three larvicides or negative controls and ambient air temperature and basin water temperature. Week 0 corresponds to pre-treatment.

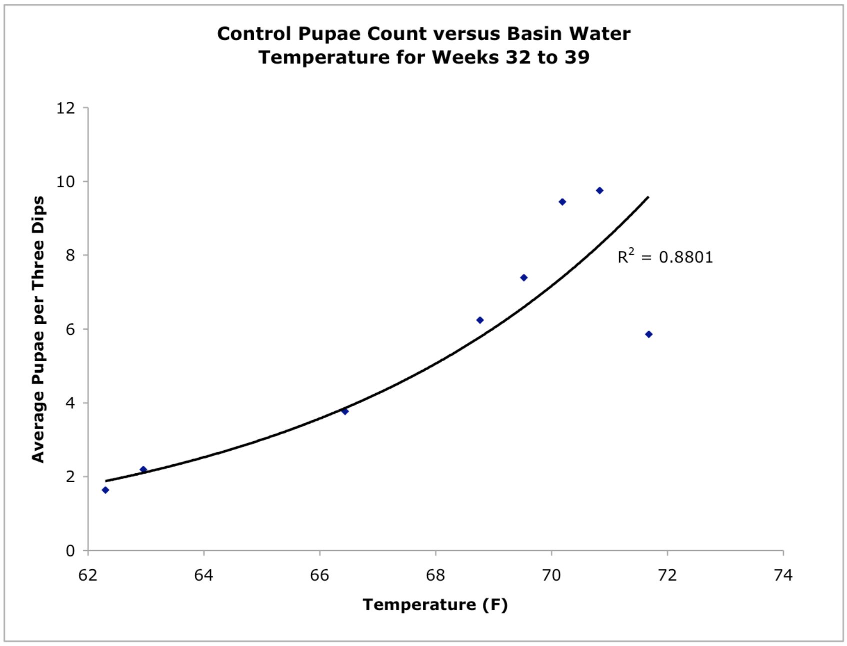


Figure 15. Average number of pupae in the control basins for the efficacy study versus basin water temperature during the entire 7-week study (Weeks 32-39). Fit of the line to the data is a second-degree exponential equation.

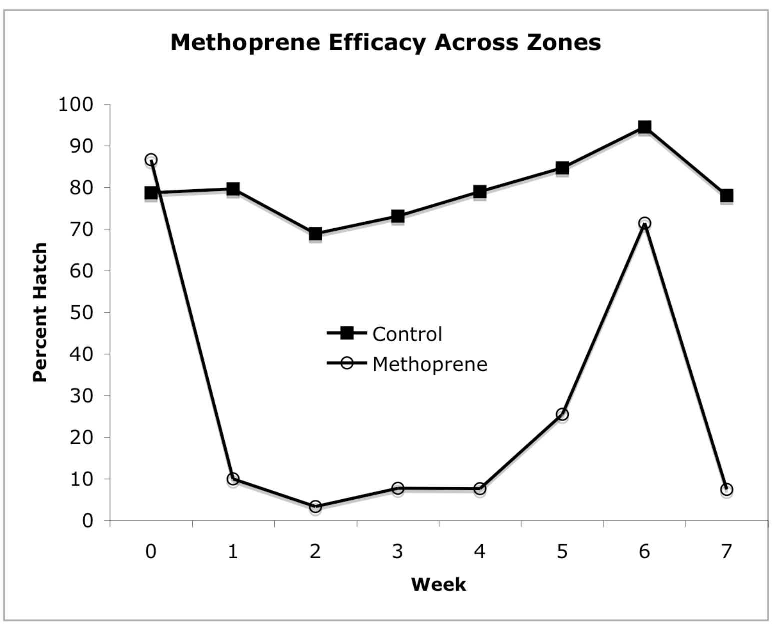


Figure 16. Average hatching success of pupae within basins treated with Methoprene or basins that received no treatment (controls). Pre-treatment data were collected at Week 0 before Methoprene was added. Week 1 = 7 days post-treatment. N = 15 basins per treatment per week.

This report includes results of analyses from contracted analytical laboratories received by 14 March 2007. Data on the fate of Methoprene in the basins we treated are still outstanding. Because of this and because this research was conducted in partial fulfillment of the requirements for an MS degree by one of the authors, the data included will be subject to additional analysis and interpretation.

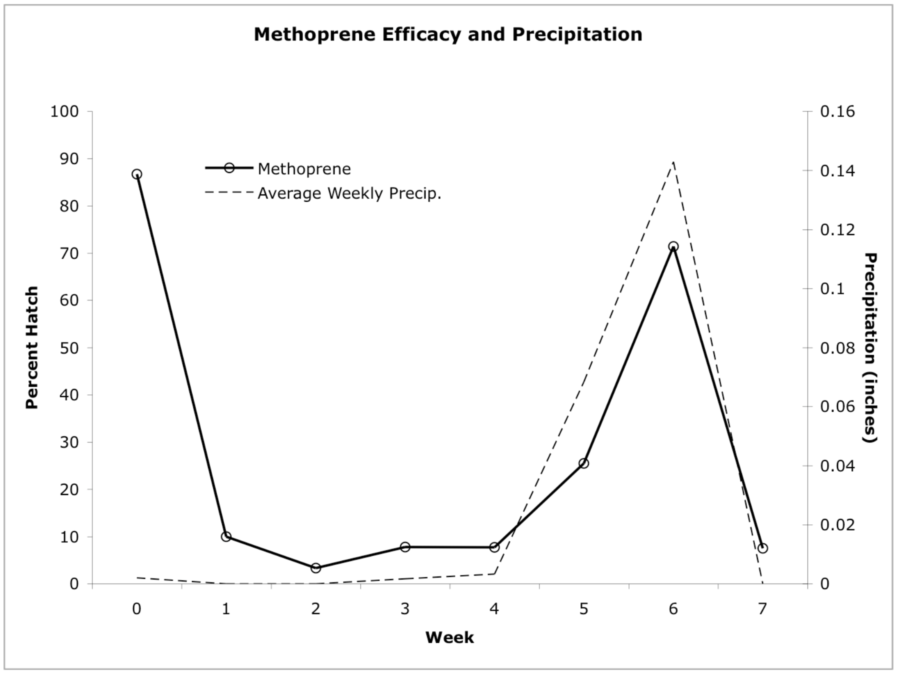


Figure 17. Relationship between the efficacy of Methoprene and precipitation. Week 0 corresponds to pre-treatment.

sectors (1-way ANOVAs, Student-Newman-Keuls, $P<0.01$); those that were only cleaned were similar to controls.

Conclusions

All of the larvicides significantly reduced the number of pupae (BTI and BS) or their hatching success (Methoprene) and performed according to the expectations expressed by the representatives of the manufacturers. The reduction in the effectiveness of Methoprene with precipitation was expected and concentrations of the larvicide were again effective within 7 days. Depending on the frequency, magnitude, and duration of precipitation events during the breeding season, Methoprene may not be as effective as BS or BTI in consistently reducing reproductive success within the City's catch basins. However, the potential for longer-term control (150 days) with the extended release (XR) formulation of Altosid® and the need to incorporate difference control strategies to prevent the development of resistance, may compensate for potential transient reductions in efficacy associated with precipitation events. Other municipalities along the West Coast (Sacramento; Portland; and Vancouver, BC) use an initial treatment of the extended release formulation of Altosid® for these reasons (Appendix 1). As noted in the next section, the fact that VectoLex® WSP contains BTI may complicate efforts to minimize the potential for resistance to develop. Reasons for the continued control by BS and BTI irrespective of the precipitation during Weeks 5 and 6 are not known. Either the dilution of the larvicides within the basins was not sufficient to reduce efficacy and/or infection occurs primarily at the sediment water interface where the concentration of the bacteria may not be as affected by influxes of water as that within the water column.

Cleaning of basins or cleaning and street sweeping was not associated with a reduction in mosquito larvae.

Research Needs

All of the larvicide formulations tested were effective during the 49-day study. How long they would actually remain effective and to what extent precipitation events would alter their efficacy is not known. The extended release (XR) formulation of Altosid® and a new product, Aquaprene®, also with Methoprene as the AI, are reported to be effective for more than 150 and 170 days, respectively. Both products should be tested to confirm the length of time they are effective and the extent to which precipitation reduces their efficacy.

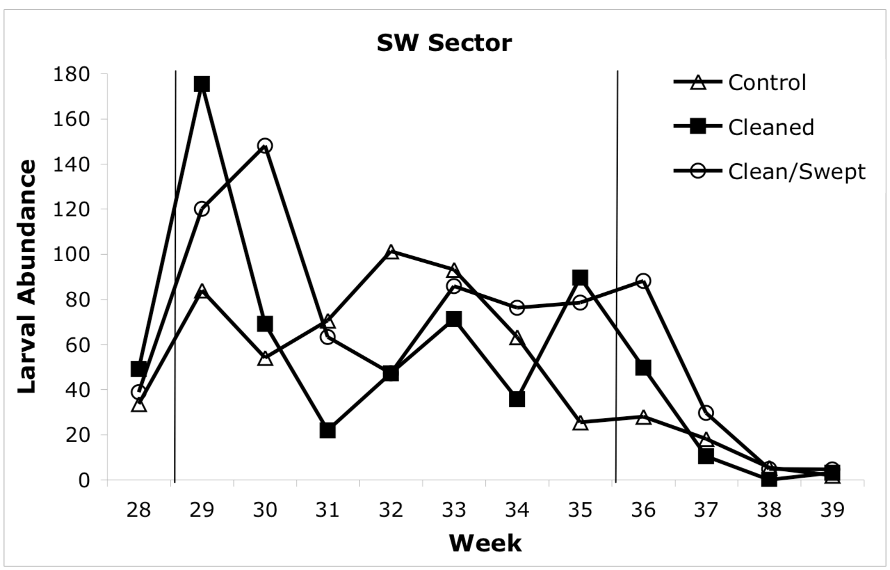


Figure 18. Average number of mosquito larvae per three dips (without log transformation) within basins that were cleaned and streets swept, cleaned only, or unaltered (control) within West Seattle (Southwest Sector). Peak mosquito season corresponded to Weeks 29-35. N = 5 basins per treatment per week. Larval counts within basins that were both cleaned and swept were statistically greater than those within controls; those only cleaned were similar to controls.

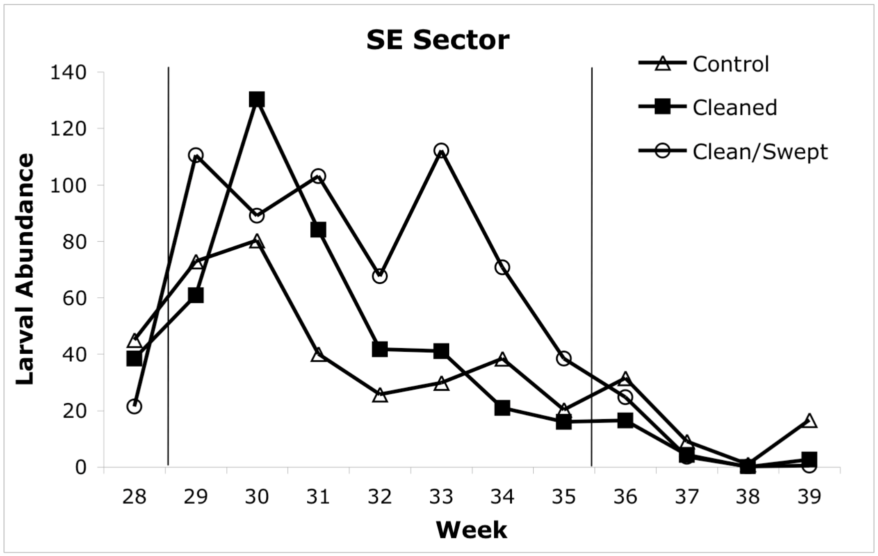


Figure 19. Average number of mosquito larvae per three dips (without log transformation) within basins that were cleaned and streets swept, cleaned only, or unaltered (control) within Columbia City (Southeast Sector). Peak mosquito season corresponded to Weeks 29-35. N= 5 basins per treatment per week. Larval counts within basins that were both cleaned and swept were statistically greater than those within controls; those only cleaned were similar to controls.

Bioassay cup for assessing the efficacy of Methoprene. Photo: C. Grue

Fate of Larvicides

Synopsis — We determined the concentrations of the larvicides BS, BTI, and Methoprene within the water column (water surface to sediment-water interface) in a subset of catch basins. Samples were collected before application, and 7, 21 and 35 days later. Methoprene basins were also sampled 7 weeks post-treatment. BS and BTI were identified using DNA fingerprinting and concentrations expressed as numbers of spores per ml of water. The formulation of BS we used was actually a combination of BS and BTI with no difference in the strain of the latter between the VectoLex® and Mosquito Dunks®. Average spore counts for BS and BTI were very low pre-treatment, peaked 7 days post-treatment and then decreased during subsequent weeks. Concentrations of BS and BTI in basins treated with VectoLex® were approximately 10 and 17 percent of peak concentrations 5 weeks post-treatment. Concentrations of BTI in basins treated with Mosquito Dunks® were 33 percent of peak concentrations at this time. Both bacterial larvicides were still effective in reducing the number of pupae 7 weeks post-treatment. Methoprene samples have been submitted to Warren Analytical Laboratory, Greeley, CO, for analysis, but results were not available for inclusion in this report.

Objective

Our objective was to determine the persistence of BS, BTI, and Methoprene formulations within catch basins. Specific tasks were:

- Quantify the concentrations of BS, BTI, and Methoprene formulations within catch basins.
- Relate persistence to environmental conditions within and outside basins.

Methods

We selected a set of new basins in the Northwest Sector to determine the fate of the larvicides that was comparable to that selected to determine efficacy. Selection criteria were the same. Five basins per larvicide within each of the three zones (45 basins total) that met previous acceptance criteria (Appendix 3), exceeded a treatment threshold of >1 mosquito larvae per three dips, had ≥7.0 inches of water, and to the extent possible, allowed for each treatment to be replicated at an individual intersection. Basins were treated during the week of 7 August. Treatments were according to maximum label recommendations and performed by Unit staff licensed as pesticide applicators. Pretreatment samples served as controls.

We determined the concentrations of the larvicides within samples that included from the water to sediment-water interface. Samples were collected before application, and 7, 21 and 35 days later. Methoprene basins were also sampled 7 weeks post-treatment. Separate sets of all sampling equipment were used for each larvicide and the equipment and water quality instruments were thoroughly rinsed with dechlorinated water and dried between each use (basin). All samples were placed in sterile (BS and BTI) or chemically clean containers (plastic, BS and BTI; glass, Methoprene) and placed on ice in the field. All samples were collected in duplicate. Samples for BS and BT were transferred to the Institute of Environmental Health Laboratories and Consulting Group's Molecular Epidemiology, Inc., in Lake Forest Park, WA on the day of collection. Methoprene samples were frozen following the addition of 75 ml of GC/MS grade methylene chloride as a preservative. Methoprene samples have been transported to Warren Analytical Laboratory, Greeley, CO for analysis (detection limit = 0.2 ppb) and results should be available in late February 2007.

BS and BTI were identified using agar plate culture techniques and DNA fingerprinting (PFGE: Pulse-Field Gel Electrophoresis; and RFLP: Restriction Fragment Length Polymorphism). Concentrations were expressed as numbers of spores per ml of water. Samples of the formulated products were also analyzed as a positive control and to confirm identification of the specific strains of bacteria used in the products.

Results

The formulation of BS we used is actually a combination of BS and BTI with no difference in the strain of the later between the VectoLex® and Mosquito Dunks® (Table 6). The concentration of BTI was about 5% of that of BS within the formulation. Average spore counts for BS and BTI were very low pre-treatment, peaked 7 days post-treatment and then decreased during subsequent weeks (Figs. 20 and 21, page 45). Concentrations of BS and BTI in basins treated with VectoLex® were approximately 10 and 17 percent of peak concentrations, respectively, 5 weeks post-treatment. Concentrations of BTI in basins treated with Mosquito Dunks® were 33 percent of peak concentrations at this time. Both bacterial larvicides were still effective in reducing the number of pupae present 7 weeks post-treatment (Fig. 13, page 38).

Table 6. Bacterial composition of Mosquito Dunks® (BTI) and VectoLex® WSP (BS).

Product	Composition (colony forming spores/g product)
Mosquito Dunks® (BTI)	290,000,000
VectoLex® WSP (BTI)	140,000,000
VectoLex® WSP (BS)	2,900,000,000

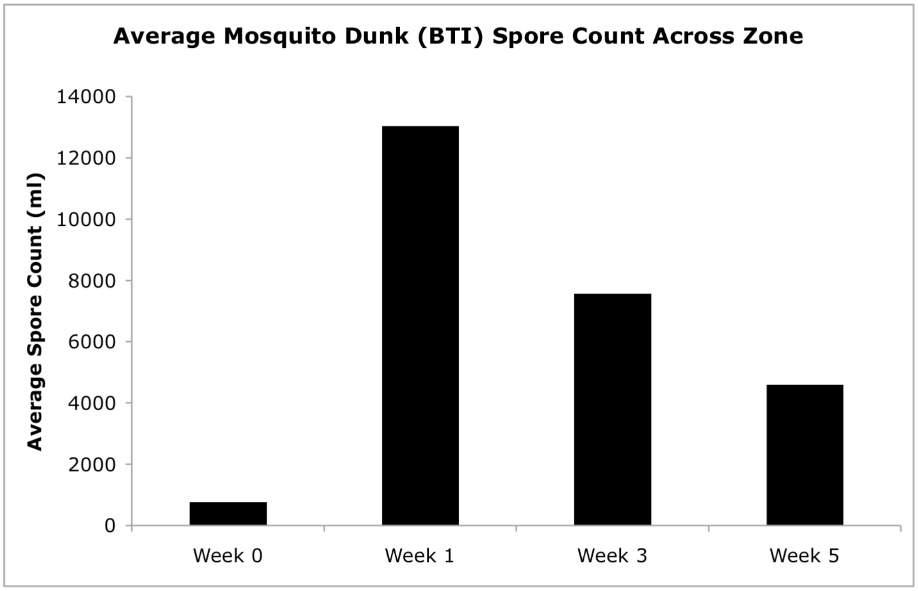


Figure 20. Counts of BTI spores (spores/ml) within basin water treated with Mosquito Dunks® (BTI). Week 0 = before treatment. The larvicide was still effective in reducing numbers of pupae at Week 5 (Fig. 13, page 38).

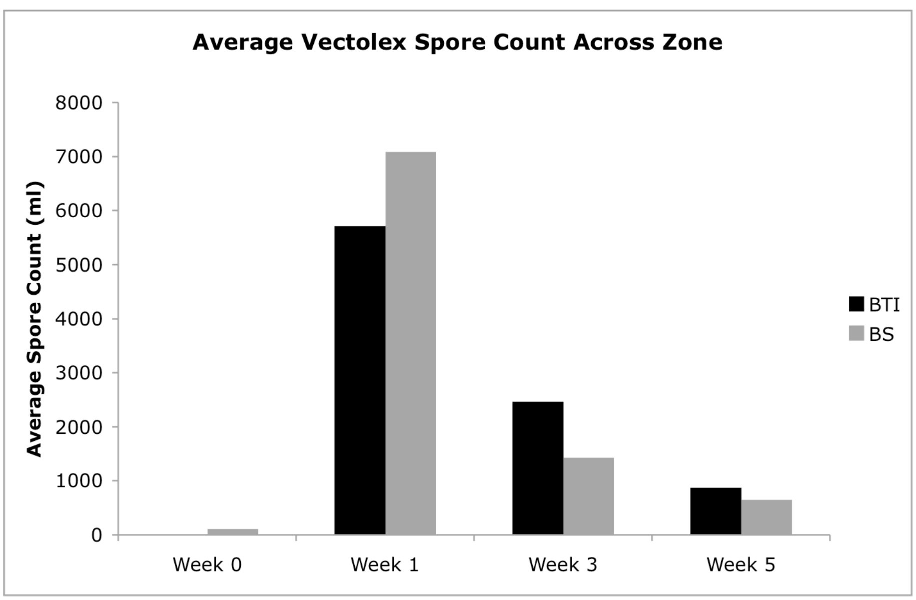


Figure 21. Counts of BS and BTI spores (spores/ml) within basin water treated with VectoLex® WSP pouches. Week 0 = before treatment. The formulated product was still effective in reducing numbers of pupae at Week 5 (Fig. 13, page 38).

Conclusions

The formulation of BS used in this study (VectoLex® WSP) contained both BS and BTI with the strain of BTI the same as that in the BTI product tested (Mosquito Dunks®). Concentrations of BTI and BS (+BTI) were greater than background (pre-treatment) levels 35 days after treatment. Concentrations of the two larvicides did not appear to be significantly affected by the precipitation events during Week 5. Based on efficacy determinations in a companion set of basins treated with the two larvicides, concentrations were still effective in reducing the number of pupae present 7 weeks post-application. Fate data for Methoprene have not been received from the analytical laboratory and will be provided as an addendum to this report.

Research Needs

Both bacterial larvicides were above background concentrations 5 weeks after application. Although fate data for the basins we treated with Methoprene are not yet available, concentrations in the basins 49 days post treatment were still effective in preventing the metamorphosis of pupae to adults, and therefore likely exceeded detection limits. How long levels would have remained elevated is not known and should be determined in the context of efforts to assess the potential for non-target effects in surface waters receiving storm water from treated basins (Appendix 9).

Collection of water for larvicide analyses. Photo: C. Grue

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Appendices

Appendix 1. West Nile virus control strategies of four different municipalities.

Background — West Nile virus (WNV) was introduced to the United States (via New York City) in 1999. The rapid spread of the virus in subsequent years forced affected municipalities along the East Coast to develop control strategies without the luxury of having a large amount of pertinent research from which to draw from. Municipalities along the West Coast have had the advantage of learning from previous mistakes and successes as they designed their WNV control strategies.

Despite the large amount of data on WNV control strategies available from various sources, municipalities across the country have chosen a variety of treatment plans based on proximate environmental conditions driving mosquito abundance and political and financial constraints. For example, rural cities have opted for treatments plans that differ from urban locations and certain urban locations have been more concerned about choosing treatments that will have a relatively low impact on non-target organisms while others are largely unconcerned about non-target effects. Because there is not a standard for data collection or reporting, much of the existing data on efficacy of control strategies are difficult to compare, resulting in locally specific treatment strategies. Therefore, although cities along the West Coast had the benefit of following the lead of those on the East Coast, WNV control strategies vary considerably.

The four municipalities that we have chosen to focus on within this report are Vancouver, B.C., Portland, OR, Sacramento, CA, and Narragansett, RI. Vancouver and Portland were chosen because of their close proximity to Seattle. In addition, Vancouver has investigated a variety of WNV control strategies. Sacramento was chosen primarily because it has a relatively long-standing catch basin treatment program. Narragansett, on the other hand, was chosen because Rhode Island was one of the first states to develop control strategies and has conducted a considerable amount of research on the potential for non-target effects of selected larvicides and the environmental factors governing mosquito abundance.

Vancouver, B.C. and the Greater Vancouver Region — The Greater Vancouver Region, including the City of Vancouver and the City of Richmond among others, has been told by the British Columbia Center for Disease Control (BCCDC) to be prepared to apply larvicide treatments to catch basins and ditches for the first time in 2006; some municipalities began pre-emptive treatments despite the fact that WNV had not been detected within the region. The City of Richmond was put on higher alert than the City of Vancouver and had already started their seasonal catch basin treatment when the first draft of this review was prepared. Numerous reasons contributed to this elevated alert, but the primary reason was the greater number of drainage ditches found within Richmond in comparison to Vancouver, which was mainly concerned with catch basins. Another significant reason why Richmond was at a higher alert level than Vancouver was because of differences in topography between the two regions. Vancouver is largely situated on a hill such that much of the water that would otherwise remain stagnant drains down to the ocean. Richmond, on the other hand, has a much lower elevational gradient. Open ditches with large surface areas that do not have the opportunity to drain create suitable breeding habitat for *Culex pipiens*, the primary mosquito species of concern.

Larvicide treatments will be applied to catch basins in the City of Vancouver if WNV is found at the borders of the region in animals or humans and is coupled by a number of sequential high degree days. Vancouver has been told that they should be prepared to apply larvicides citywide with an advance notice of one week. Despite the fact that the trip point for treatment is not based on the number of larvae using conventional dipping methods, a fair amount of data on larvae numbers have been being collected from catch basins and nearby ditches. Richmond has had a pest management division for many years leading up to the threat of WNV and has been monitoring their catch basins and ditches for the past few years. No formal report on larvae abundance is required from any municipality, although updates are provided to the Vancouver Coastal Health Authority on a weekly basis.

Richmond and Vancouver also have different treatment plans set in place if they are told to apply larvicides. Richmond had already applied Methoprene to 2/3 of its catch basins as of July 12, 2006. The briquette form of Methoprene is being used and was chosen for two main reasons: (1) it can be effective for a relatively long time in comparison to other biological larvicides, and (2) there is insufficient staff to treat catch basins more than once per season.

Vancouver planned to treat half of its basins with Methoprene briquettes and the other half with *Bacillus sphaericus* (Vectolex® WSP pouches). Although informally, the City planned to determine which product is most effective within the city's environment. If *Bacillus thuringensis var. israelensis* becomes a viable treatment option, then Vancouver will use the granular form (Vectobac®, PCP #18158). In the future, both municipalities hope to conduct more formal research on larvicide efficacy and fate. Consequently, Vancouver is also very interested in the results from our study and that in subsequent years wherein we hope to investigate the non-target effects of larvicides on salmonids.

Portland, OR — Portland has had an Integrated Pest Management program since the early 1930s. Despite this history, the prospect of planning a WNV control strategy posed a few problems, largely because of the fact that much of the strategy involved the dependence upon old storm drainage catch basins in great need for repair as the primary treatment environment. Currently, the city has a closed system of catch basins, i.e., the only time anything from the catch basins is deposited into water bodies is when significant precipitation occurs (overflow). The city has devised a plan to restructure all catch basins within the city such that they never directly flow into water bodies. This plan will also help reduce the potential for non-target effects from larvicides entering surface waters. Efforts to restructure outfalls have already begun and are scheduled to be completed by 2016.

To date, Portland has conducted little research on non-target effects, or the efficacy and fate of larvicides. The decision to use the treatment regime that they are now utilizing was based upon the successes of various other municipalities, largely cities in Southern California. No data have been published and no current research is being conducted. In lieu of referring to published literature, Chris Worth, the project manager for Portland's Vector Control Office, provided us with a description of their WNV control strategy.

Portland uses a combination of cleaning basins at the beginning of each summer, treatment of basins (with more than one mosquito) with Methoprene in May and June, and a follow-up treatment approximately 90 days after the initial Methoprene treatment with either *Bacillus sphaericus* or *Bacillus thuringensis var. israelensis*. Methoprene is used first primarily because of its relatively long lifetime in comparison to the biological larvicides, although it is also used because of its low potential for impacting non-target organisms. It is hoped that the initial Methoprene treatment will be sufficient for the season, but a second larvicide treatment around the beginning of September is not rare. Biological larvicides are used for the second round of treatments because they have little or no impact on non-target organisms and the treatment need only last for a brief period (approximately 1 month) as the mosquito season nears its end. The lifespan of most biological treatment options usually range up to 1 month.

Sacramento, CA — Sacramento has had an extensive catch basin treatment program in the downtown area since 2003; prior to 2003 there had been a less organized version of the treatment program that is now in place. According to the catch basin crew field supervisor, Randy Burkhalter, there are approximately 20,000 catch basins in the downtown area alone. The drainage system is primarily a combined system in which larvae are able to survive year-round. Because larvae have been found year-round in certain catch basins within Sacramento and Yolo counties, 500 catch basins have been chosen for monitoring purposes throughout the mosquito season. These catch basins were chosen based on a history of consistently high larvae counts and, therefore, are not randomly chosen. The monitoring season typically begins on February 1st and extends though approximately the middle of November each year.

The catch basin treatment program gets underway when the heavy winter rains have subsided; the treatment program is usually initiated between late March and early April. At this time, every catch basin that can be accessed is dipped; if the dip exceeds one larvae across three dips, then the basin is treated. The catch basins are first treated with Methoprene (Altosid® briquettes). This particular formulation (XR – extended release) is designed to last for up to 150 days. Randy Burkhalter has conducted preliminary, and currently unpublished, efficacy tests and found that treatments have been effective only up through approximately 95 days. Pupae were collected from a number of continuously monitored catch basins, raised in-house using an incubation system, and efficacy was ultimately established using pupae hatch rates. If hatch rates were greater than 20% then the product was deemed ineffective.

A second, and sometimes third, treatment round is always necessary for the City of Sacramento. In order to reduce the possibility of larvicide resistance, the second round involves either the use of a BTI product (Vectobac®) or the use of a BS product (Vectolex® WSP). Because the City of Sacramento has also found that precipitation decreases the efficacy of Methoprene products, after the first rains of the season the bacterial products are added to catch basins. Unpublished reports from the City of Sacramento have claimed that rain does not diminish the effects of either BS or BTI products in comparison to the Methoprene products. Furthermore, highly organic catch basins respond best to BS products.

Randy Burkhalter was also involved in studying different Methoprene and BTI products in the summer of 2006. Specifically, he investigated the efficacy of a new line of larvicide products called Aquaprene®, which targets mosquito larvae in catch basins. The products are expected to be available in 2007. Bob Sjogren, Aquaprene® creator and representative, stated that the products now have EPA approval and are currently in the process of being manufactured. One of the Aquaprene® products has Methoprene as the AI and is effective for up to 170 days (persisting almost twice as long as other brands). Additionally, this slow release product will remain active within a basin even under dry conditions, potentially extending its persistence. Field tests by Randy indicate the product lasts longer than 170 days in the absence of precipitation/other flushing events. In the event of consecutive, large-scale precipitation events, Sjogren stated that the product would last less than 170 days.

A new BTI product will also be studied in Sacramento catch basins during the summer of 2007. The new BTI product will be similar to the BTI donut, but is supposed to be effective for longer than 100 days. Research in 2007 will also include temperature-controlled studies with Altosid products. The City of Sacramento typically has a larger than normal amount of organic debris deposited in their catch basins and, for this reason, will also be looking at how the Altosid products perform with varying levels of organic matter.

Currently, UC Davis is conducting research on the non-target effects of the Altosid® treatments used within the City of Sacramento, a BS, and a BTI product. Dr. Sharon Lawler from the UC Davis Entomology Department is the primary person in charge of the non-target research. According to Dr. Lawler's research, BTI products have the largest non-target window; they are non-toxic to both vertebrates and invertebrates when used according to label recommendations. BTI products are designed to be toxic to insects with a high pH in their gut. In research Dr. Lawler has conducted in salt marshes on BTI and Methoprene, she has found that BTI is non-toxic to tadpole shrimp and that the combination of both BTI and Methoprene has no effects on non-target insects. Moreover, Dr. Lawler's research revealed that Altosid® pellets were active for a longer than the label states and the larvicide had no adverse effects on non-target brine flies.

While Dr. Lawler's research largely indicates that Methoprene and BTI are not toxic to non-target invertebrates in salt marshes, she warns that organisms living in salt marshes are particularly hearty and freshwater organisms may experience adverse effects. Dr. Lawler's subsequent research includes investigating whether or not malformations reported in amphibians may be caused by exposure to a breakdown product of Methoprene, retinoic acid. Furthermore, she stated that she did not know of any research being conducted on the possible effects of Methoprene, BTI, or BS on salmonids.

Narragansett, RI — The WNV Larvicide Program in Rhode Island has generated a great deal of data on the non-target effects of mosquito larvicides, particularly the effects of Methoprene on local lobster populations, as well as well environmental conditions within catch basins that govern mosquito larvae abundance. The majority of the research was conducted in Narragansett between May and November 2002 (Butler et al. 2006a,b).

Butler and her team looked closely at whether or not mosquito abundance varied in storm water catch basins depending on environmental conditions such as temperature, pH, conductivity, dissolved oxygen, and water depth. Total nitrogen (N), total carbon (C), and total suspended solids (TSS) were also measured at each sampling period of the 30-basin study. Mosquito abundance was negatively associated with dissolved oxygen in 3 months, positively associated with pH in 2 months, positively associated with total C during 2 months, and positively associated with total N and TSS in 1 month within the 6-month study. Overall, *Culex* species were most abundant when the water was shallow, had large amounts of C, N, and TSS, and when dissolved oxygen and pH were both low. Whereas the study performed in Narragansett included a large number of variables, the total number of basins (n=30) was relatively small compared to the study being conducted in Seattle and less spatially representative.

Butler (2005) also examined the efficacy of Methoprene (opportunistic sampling) within catch basins in the summers of 2001 and 2002. The study reports that Altosid pellets were effective in killing larvae for approximately 30 days, after which the pellets needed to be re-applied. Furthermore, the report concludes that approximately 3.5 grams of Altosid pellets per catch basin could adequately control mosquitoes for up to 1 month during summers in Narragansett.

Rhode Island has experienced a great deal of pressure associated with the potential for non-target effects of Methoprene on local lobster populations. Under a controlled laboratory setting, nine catch basins were constructed which were comparable in composition (sediment, water, debris) and dimensions to the majority of catch basins within the state. Basins were flushed with a known amount of water after treatment with the specified amount of pellets and the concentration of Methoprene that flowed out of a connecting outflow was determined. The concentration of Methoprene was found to be well below the level known to potentially harm lobsters (Butler 2005).

Based on the results of these studies, the State of Rhode Island currently treats all of their catch basins once a month with Altosid® pellets. Treatment begins in June and continues through September without any in-season dipping to meet a treatment threshold. The State of Rhode Island also treats all catch basins whether or not they are grated.

Appendix 2. Catch basins selected within the stratified random sampling design in 2006.

Table A2-1. Total basins within each zone of interest from which the indicated number of basins (according to proportion) were randomly chosen. Zone totals reflect only those basins which are SPU-owned and that are round-top.

Zone	Zone Total	Proportion	Basins
SF	13,477	0.63	156
MF	4,761	0.22	55
NC	3,312	0.15	38
Total	21,550	1	250

Table A2-2. Zonal basin coverage within sectors in the City of Seattle. All basins were chosen randomly from SPU-owned basins with round tops.

Sector	Zone	Proportion	Basins
NE	SF	0.14	21
	MF	0.07	4
	NC	0.11	4
Total			29
NW	SF	0.23	36
	MF	0.18	10
	NC	0.20	8
Total			54
WEST	SF	0.12	18
	MF	0.14	8
	NC	0.19	7
Total			33
EAST	SF	0.20	32
	MF	0.33	18
	NC	0.24	9
Total			59
SW	SF	0.13	20
	MF	0.17	10
	NC	0.08	3
Total			33
SE	SF	0.19	29
	MF	0.11	6
	NC	0.18	7
Total			42

Appendix 3. Criteria for basin selection.

We utilized various basin selection methods for the 2006 summer field season in order to design a study that would characterize the abundance of mosquito larvae within catch basins within the the City of Seattle. Random basin selection was performed in ArcGIS 9 using specific fields within feature attribute tables as guides for inclusion. As a first cut, we selected basins that were SPU owned and round-top. Additionally, basins were only selected from Single Family (SF), Multi-family (MF), and Neighborhood Commercial (NC) zones. Data files used to compile the list of basins for each task were generated from older city maps in which the basin points had been digitized (cbasin), as well as from updated GPS files (cbgps). The digitized files are less accurate though represent a larger coverage area, while the GPS files are considered to be more accurate though are less spatially comprehensive.

Considerable efforts were made to ensure that each sector of the City (Northeast, Northwest, East, West, Southeast, and Southwest) was sampled. A representative number of basins was selected from the three zones within each of the six sectors in the city, resulting in 18 separate lists (3 zones x 6 sectors) of basins from which to randomly select. For any given task the number of basins randomly selected amounted to two times that which was required in order to provide alternates if basins were excluded (exclusion table) in the field. All basin points were plotted in ArcGIS 9 and were deemed acceptable for the study through visual inspection, as a second cut. Specifically, basins were excluded if they were at the same intersection as a previously chosen basin, if they existed anywhere but within an intersection or at the end of a street, and if they fell less than a block from a fire station.

Appendix 4. Why is it likely inappropriate to compare larval abundances within catch basins monitored in 2005 with those monitored in 2006?

One of the project goals for 2006 was to analyze the mosquito larvae abundance data collected in 2005 by Landau & Associates with the purpose of improving the quality and utility of research in 2006. A related goal was to compare larval abundances in the catch basins monitored in 2005 with those collected in 2006. Based on our knowledge of the 2005 monitoring effort, a quantitative comparison between larval abundances in the two years is not advised because of apparent differences in sampling designs.

The catch basins chosen for monitoring in 2005 were intentionally located near elder care facilities and city tiles were chosen to represent each of the city zones. It is unclear how specific catch basins within the designated tiles were ultimately chosen as neither Landau & Associates nor SPU have claimed responsibility for identifying this set of basins. In addition, the basin type (e.g. round and solid lid vs. rectangular and grated lid) chosen for 2005 monitoring data was never specified; both types of basins were included in the study. Furthermore, we have been unable to determine if the number of basins represented in each zone and sector are proportional to the number present because unique basin identifiers for 2005 are not those which are now included in the SPU GIS shape-file database.

In comparison to the sampling design for 2005, 2006 basin were chosen in a stratified random design in which the number of basins selected in each sector and zone was proportional to the number present. Basins were not selected using a tile layout and only basins that were round and had solid tops were included in the 2006 monitoring effort.

No conclusive statements could be made if the two data sets were compared because of the apparent disparity between catch basin sampling designs. If a determination of the actual representation of basins within sectors and zones in 2005 is desired, then Unit staff must be provided with the 'Grph' numbers for all basins sampled in 2005. Furthermore, it is possible that the physical characteristics of different types of basins may affect mosquito abundance, possibly further hampering direct comparisons.

Appendix 5. Percentage of catch basins with specific mosquito larvae and water conditions within each sector during the entire season (6/19/06-9/29/06), early season (6/19/06-7/14/06), peak season (7/17/06-9/1/06), and late season (9/4/06-9/29/06).

Basin Conditions	Season	Northeast	Northwest	East	West	Southeast	Southwest
Basins with larvae on 1 or more occasion	Entire	93.1	90.7	84.5	90.9	95.1	90.9
	Early	82.1	68.5	71.2	87.1	68.3	93.1
	Peak	92.3	95.9	92.2	96.6	95.1	92.9
	Late	72.4	79.6	69.0	69.7	85.4	72.2
Basins consistently had water and no larvae	Entire	3.4	3.7	0.0	0.0	4.8	0.0
	Early	17.2	24.1	20.3	6.1	31.0	6.1
	Peak	6.9	3.7	0.0	0.0	4.8	0.0
	Late	17.2	11.1	10.2	18.2	9.5	12.1
Basins consistently dry	Entire	0.0	0.0	1.7	0.0	3.0	0.0
	Early	3.4	0.0	10.2	6.1	3.0	12.1
	Peak	10.3	7.4	13.6	9.1	3.0	9.1
	Late	0.0	0.0	1.7	0.0	3.0	0.0
Basins had water on at least one visit	Entire	100.0	100.0	98.3	100.0	97.0	100.0
	Early	96.6	100.0	89.8	93.9	97.0	87.9
	Peak	89.7	92.6	86.4	90.9	97.0	90.9
	Late	100.0	100	98.3	100.0	97.0	100.0
Basins consistently had water	Entire	86.2	83.3	72.9	63.3	90.9	75.8
	Early	89.7	90.7	83.1	75.8	97.0	81.8
	Peak	86.2	83.3	74.6	66.7	93.9	75.8
	Late	89.7	90.7	78.0	81.8	90.9	78.8

Appendix 6. Variability in larval counts within the Southwest Sector in 2006.

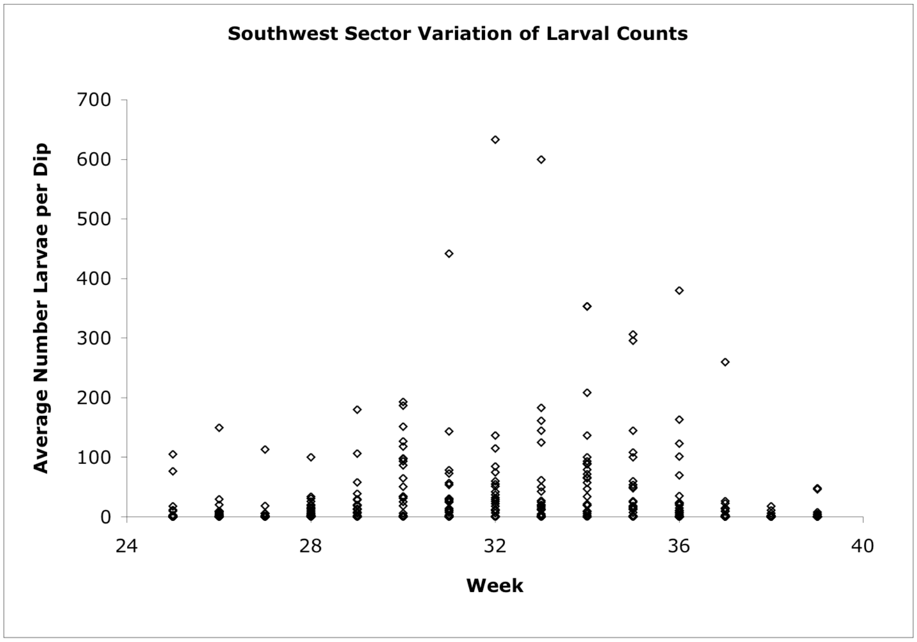

Figure A5-1. Variability in larval counts within the Southwest Sector in 2006

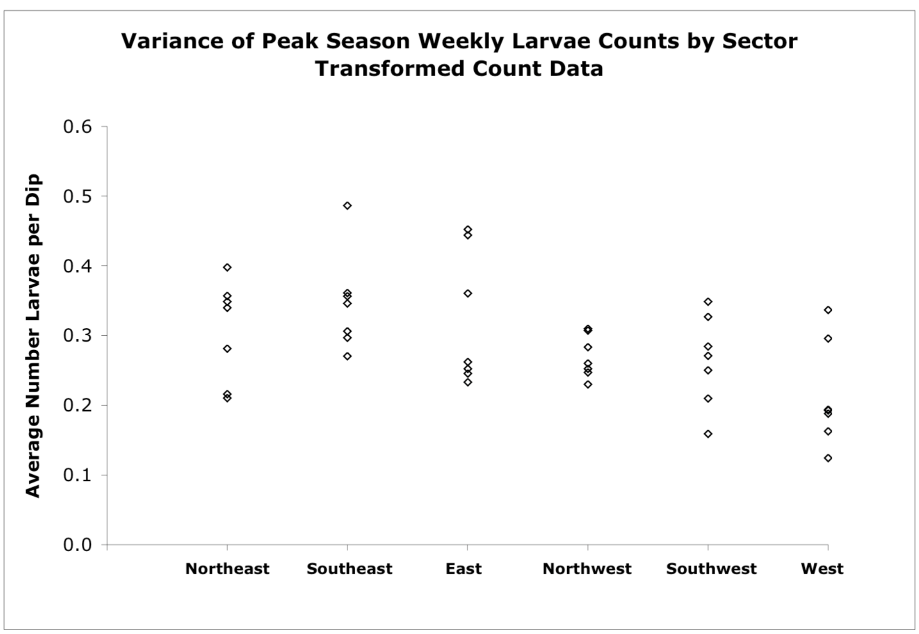

Figure A5-2. Variability in weekly larval counts (inverse transformation) during the peak season (Weeks 29-35) within each sector in 2006. Note the greater overlap among all of the sectors except the West, in which the weekly larval counts were statistically greater than those in the East and Southeast sectors during the peak season.

Appendix 7. Selection of basins with "high" or "low" larval counts for sediment characterization.

All high/low basins were selected on 26 September 2006, utilizing basin survey data available at that time. The data utilized were generated in Weeks 25-37 (13 weeks, 19 Jun to 15 Sep) of the 15-week survey season.

Low basins were selected within each zone by ranking the number of surveys (max = 13) wherein the basin was found to have water and zero mosquito larvae per dip. The 5 basins within each zone that were most frequently wet with zero larvae per dip were selected. Selected basins had zero larvae per dip 6-10 weeks of the 13 total surveys, resulting in overall 13-week average number of larvae per dip for the 15 "low" basins between 0 and 2.1 larvae per dip.

High basins were selected within each zone by ranking the number of surveys (max = 13) wherein the basin was found to have water and 30 or more mosquito larvae per dip. The 5 basins within each zone that were most frequently wet and had 30 or more larvae per dip were selected. Selected basins had 30 or more larvae per dip 4-10 weeks of the 13 total surveys, resulting in an overall 13-week average number of larvae per dip for the 15 "high" basins of between 31 and 299 larvae per dip.

Appendix 8. Larvicide selection for efficacy and fate study.

Following review of the mosquito control strategies of other municipalities in the Northwest, review of product labels, and discussions with manufacturers and their marketing representatives, we chose the products listed below for the efficacy and fate component of our contract with Seattle Public Utilities. The three products tested were BTI, BS and Methoprene as the active ingredients. Maximum label rates were used.

BTI (*Bacillus thuringiensis israelensis*)
Mosquito Dunks®
Summit
235 South Kresson Street
Baltimore, MD 21224
(410) 522-0661

Pre-treatment with Mosquito Bits®
Summit Chemical Co.
235 South Kresson Street
Baltimore, MD 21224
(410) 522-0661
Contact: John Cohen

The application rates were the maximum label rate that translates to 2 "dunks" per basin with the addition of ½ teaspoon of the bits at the time the dunks were applied. The addition of the "bits" should have resulted in rapid control followed by 30+ days of control from the "dunks". The product was provided by Summit.

BS (*Bacillus sphaericus*)
VectoLex® WSP (water soluble pouches)
VALENT BioSciences Corp
870 Technology Way
Libertyville, IL 60048
(800) 323-9597
Northwest Technical Representative: Stephanie Whitman (307) 721-4335
Products distributed in the Northwest by Clarke Mosquito Control Products, Inc.
(406) 396-3093

We chose this product because the maximum label rate (g of product) is greater than that allowed under the label for VectoLex® CG, the product selected by the SPU Contractor for the SPU pilot study. According to VALENT, the larvicide is the same just packaged and labeled differently. Control was expected for 30+ days. Product was provided by Clarke Mosquito Control Products, Inc.

Methoprene
Altosid® Briquettes
Wellmark International
1501 E. Woodfield Rd.
Suite 200 West
Schaumburg, IL 60173
(800) 877-6374

Distributor: ADAPCO, INC.
Contact: Janice Stroud
(866) 557-5813

The maximum label rate is one briquette per basin per 2 feet of water depth. Therefore basins received 1 or 2 briquettes. Control was expected for 30+ days. We note that an extended release (XR) formulation is available and is expected to provide control for 150 days. Product was purchased from ADAPCO, INC.

Mosquito larvicides studied in 2006. Photo: C. Grue

This report includes results of analyses from contracted analytical laboratories received by 14 March 2007. Data on the fate of Methoprene in the basins we treated are still outstanding. Because of this and because this research was conducted in partial fulfillment of the requirements for an MS degree by one of the authors, the data included will be subject to additional analysis and interpretation.

Appendix 9. Strategy for future research.*

The goal of the research outlined below (Fig. 22, page 63) is to help the City of Seattle identify and implement strategies that will control mosquito breeding in its catch basins and that will be protective of human health and the environment. Future research should build on the results and conclusions of studies funded by the City in 2005 and 2006. Toward this end, future research should focus on fulfilling the following four objectives:

- Determining the influence of environmental conditions external to basins on mosquito larval abundance.
- Determining the fate of larvicides within catch basins.
- Determining the potential for non-target effects of treatments within receiving surface waters
- Incorporating results into a mosquito control strategy that minimizes treatments, minimizes costs, addresses concerns associated with the development of resistance by mosquitoes to the larvicides, minimizes the potential for non-target effects, and ensures public safety.

Influence of environmental conditions external to basins on larval abundance — Regression analyses reinforced the importance of sector and zone in influencing the abundance of mosquito larvae within catch basins. Additional efforts to identify those environmental factors contributing to differences among sectors and zones are warranted. In addition to the pending analyses of the digital photographs characterizing the habitat surrounding each of the basins by the Unit, analyses of conventional and/or digital aerial photographs or satellite imagery may also be helpful. Initial efforts should be focused on those basins that consistently supported high or low larval counts. If urban habitat features consistently associated with basins supporting high larval counts could be identified and applied to maps of basin locations and adjacent habitat, then control efforts could be targeted toward "hotspots" within the City. Furthermore, this information would facilitate the identification of the most appropriate control agent(s), and the timing and number of applications.

Fate of larvicides within catch basins — Both bacterial larvicides were efficacious and above background concentrations 7 weeks after application. How long levels would have remained effective and elevated is not known and should be determined in the context of efforts to assess the potential for non-target effects in surface waters receiving storm water from treated basins. Fate data for the Methoprene product tested (Altosid® Briquettes, Wellmark International) have not been received from the analytical laboratory

*Details to be provided pending the development of a contractual agreement between SPU and the WACFWRU.

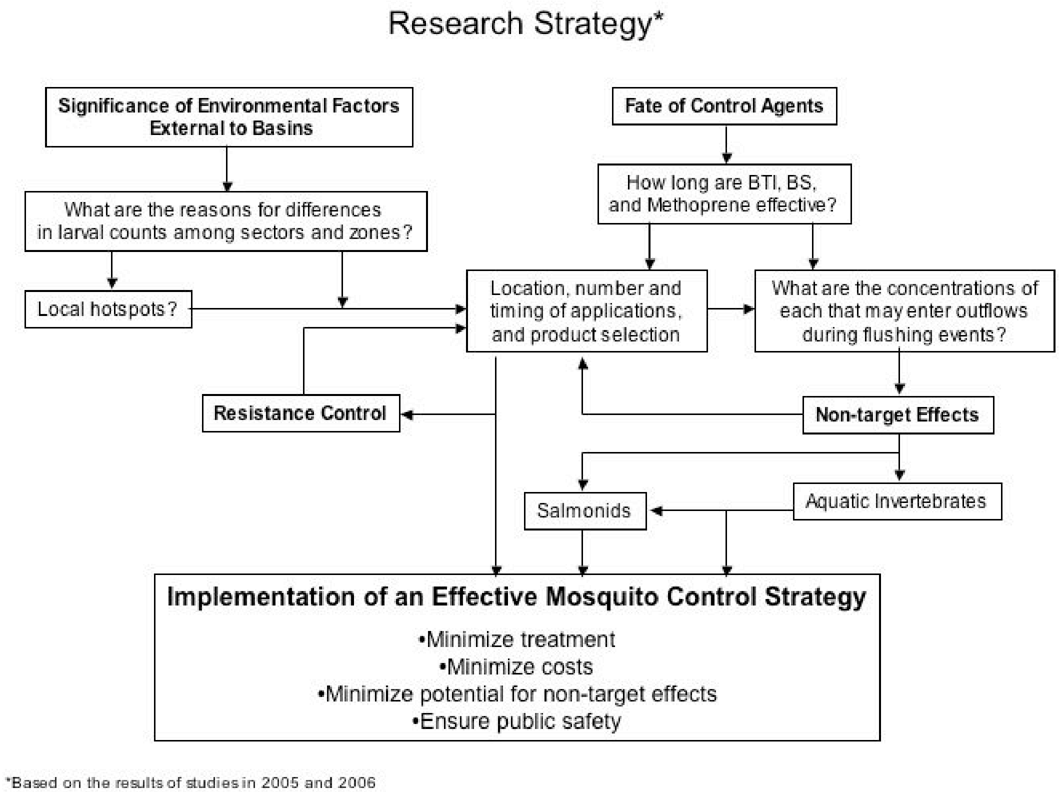

Figure 22. Strategy for future research.

contracted for the analyses. However, the briquettes were still effective 7 weeks after application, though, as expected, efficacy was reduced temporarily by precipitation events. Longer-term studies of the fate of the standard and extended release formulations of Methoprene are warranted to determine if one or both of these products are suitable for the weather conditions in Seattle, and to determine their potential for non-target effects.

Potential for non-target effects — In view of the City of Seattle's efforts to enhance and restore salmon populations and their habitats, we reviewed the available literature on non-target effects of mosquito larvicides and on-going research efforts by other municipalities on the West Coast (Appendix 1). In particular, we were interested in knowing if data exist on the toxicity of formulations of the three larvicides to salmon. In the absence of these data, we were asked by SPU to develop a proposal to conduct the studies necessary to evaluate the direct and indirect effects of larvicides on salmon and the surface waters they occupy within the City's jurisdiction (to be submitted as a separate document).

A recent review/discussion of the environmental safety of the three larvicides relative to their use in Washington State can be found in WADOE (2004). Formulations of BTI, BS and Methoprene are approved for use in fish bearing waters and considered safe when applied according to label directions. Based on our review, however, data on the toxicity of the three larvicides to salmonids (trout [rainbow trout, *Oncorhynchus mykiss*] and salmon) are few, and data for salmon appear to be lacking. The 96-hour median lethal concentration (LC50) of BTI to juvenile rainbow trout (that which results in 50% mortality) has been reported to be >10 ppm when fish were exposed to a 100% commercial formulation (Mayer and Ellersieck 1986). How this threshold compares to concentrations of the bacteria (spores/ml water) we found in our treated basins is not clear. We could not find comparable data for BS. Johnson and Finley (1980 *in* CDC 2005) reported a comparable LC50 for methoprene and juvenile rainbow trout to be 1.55 ppm. Mayer and Ellersieck (1986) reported a 96-hour LC50 for juvenile rainbow trout of 1.60 ppm when fish were exposed to a formulation of methoprene containing 69 percent active ingredient. The efficacy threshold for methoprene has been reported to be 0.20 ppb (Doug VanGundy, Wellmark International; personal communication), 4 orders of magnitude less than the reported LC50 for juvenile rainbow trout. How these toxicity values compare to the actual concentrations of methoprene in the basins cannot be determined until we receive the results of the chemical analyses from the basins we treated in 2006. Irrespective, dose response curves are lacking, as are data on endpoints other than mortality.

Concerns have been expressed over the possibility that the use of Methoprene is associated with malformations in amphibians inhabiting treated waters; however, the results of studies conducted to date have been inconclusive. Dr. Sharon Lawler (UC-Davis) is currently investigating the relationship between amphibian malformations and the breakdown product of Methoprene, retinoic acid. Depending on the results of Dr. Lawler's studies, comparable studies with salmon embryos may be warranted.

The extent to which overflows from treated basins from precipitation events may impact aquatic invertebrate communities within receiving surface waters is also not known. Initial steps to address these concerns include the conduct of the fate studies mentioned above as well as measurements of concentrations of the larvicides within outflows entering surface waters. Should these concentrations exceed those known to adversely affect aquatic invertebrate communities, field studies within receiving waters may be warranted. It should be noted that concentrations entering receiving waters will depend on the spatial extent (number of catch basins) within the watershed serving each outflow, the extent and magnitude of the precipitation or other event leading to basin overflows, and the extent to which storm water is diverted to sewage treatment facilities.

Development of an effective control strategy — We believe the results of the 2005-06 studies as well as those identified above for 2007-08 will provide the City of Seattle with the information needed to implement strategies that will effectively control mosquito breeding in its catch basins and will be protective of human health and the environment. In addition, we believe that it is important that the City evaluate the efficacy and environmental safety of all of the available (Federal and State permitted) control agents so that the control strategy adopted incorporates resistance control management.