Catch Basins as Sources of Mosquitoes and West Nile Virus: An Evaluation of Contractor Compliance, Efficacy of Control, and Alternative Control Strategies for Grated Basins

Final Report

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Final Report

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Executive Summary

In 2006, in response to the threat of West Nile Virus, Seattle Public Utilities (SPU) initiated efforts to evaluate strategies to control mosquito breeding in Seattle Public Utilities' catch basins in the City of Seattle to ensure that they are protective of human health and the environment. Following characterization of mosquito breeding within the City's catch basins and evaluation of the efficacy of different control strategies (larvicides) in 2006 by Landau & Associates and the Washington Cooperative Fish and Wildlife Research Unit (WACFWRU), SPU initiated an operational control program in 2007 to treat all of the City's catch basins once using VectoLex® CG (*Bacillus sphaericus*, VALENT BioSciences Corporation) through a contract with Eden Advanced Pest Technologies. The WACFWRU was contracted to (1) determine the extent to which the pesticide applicator (Eden Advanced Pest Technologies) complied with performance goals of its contract with the City of Seattle, (2) determine the efficacy of the larvicide in reducing the abundance of mosquito pupae in the treated catch basins, (3) determine the fate of the larvicide in treated catch basins, and (4) determine the potential for the larvicide to enter surface waters and adversely affect non-target species within creeks in the City. Results of these studies can be found in the Unit's annual reports to SPU (Grue et al. 2007, 2008).

Based on monitoring in 2007, SPU again contracted with Eden Advanced Pest Technologies in 2008 to treat all of the City's catch basins once with VectoLex® CG beginning the second week of July. SPU contracted with the Washington Cooperative Fish and Wildlife Research Unit (WACFWRU) to (1) determine the extent to which the pesticide applicator (Eden Advanced Pest Technologies) complied with performance goals of its contract with the City of Seattle, (2) determine the efficacy of the larvicide in reducing the abundance of mosquito pupae in round-top catch basins citywide, (3) compare the efficacy of VectoLex® CG and Altosid® XR (methoprene, Wellmark

Key Findings:

- The mosquito-breeding season within catch basins in 2008 was similar to that in 2006 and 2007 with numbers of larvae increasing in the third week of June and sharply declining in the middle of September.
- Precipitation occurred frequently and was associated with dramatic declines in mosquito larvae within basins, particularly control (untreated) basins.
- Compliance, as measured by the percentage of the round top basins treated with VectoLex® CG by the contractor in which the number of mosquito pupae was less than controls, was ≥ 96 percent.
- In most cases, efficacy was realized for at least 5 weeks post-treatment.
- VectoLex® CG was also effective in reducing the number of pupae within treated grated basins compared to controls for at least 4 weeks. Efficacy was comparable to that observed in the closest round-top basins.
- Altosid® XR (methoprene) was also effective in reducing the number of pupae within grated basins. However, results varied among the areas of the City monitored.
- The VectoLex® CG product contained significantly more Bt (100x) than Bs. The Bt detected is believed to be Bti.
- Concentrations of Bs and Bt within base flows entering Thornton Creek before treatment of catch basins within the watershed with VectoLex® CG were very low.
- Concentrations during precipitation events were also low.
- Concentrations detected in outflows were orders of magnitude less than those shown to be non-toxic to juvenile coho salmon.

International) in reducing the abundance of mosquito pupae in grated basins in the city, and (4) determine the potential for the larvicides to enter surface waters and adversely affect non-target species within creeks in the City.

The mosquito-breeding season in 2008 was similar to that in 2006 and 2007 with numbers of larvae increasing in the third week of June and sharply declining in the middle of September. Across the three years, larval abundance peaked between Week 28 (second week of July) and Week 34 (third week of August). In general, peak larval abundance was associated with weekly average maximum ambient air temperatures ≥ 24 C. Unlike 2006, rain was a frequent occurrence in 2007 and 2008 and was associated with dramatic declines in larval counts. The proportion of dry basins was much lower in 2007 (2.3%) and 2008 (2.7%) compared to 2006 (7.4%).

Treatment of round top catch basins with VectoLex® CG reduced the number of mosquito pupae below counts within untreated (control) basins. Compliance, as measured by the percentage of the treated round top basins monitored in which pupae counts were less than controls, was ≥ 96 percent. In most cases, efficacy was realized for

Recommendations:

- With 3 years of comparable data on the abundance of mosquito larvae and environmental factors (2006-2008), a synthesis should be conducted with the primary objective of re-examining the relationships between larval abundance and environmental factors and informing control strategies.
- The relationship between numbers of larvae, numbers of pupae, adult emergence and environmental variables also warrants examination, as does the species composition, sex ratios, and seasonality of adults emerging from basins.
- Should SPU envision treating catch basins long term, the Utility should evaluate an alternative to VectoLex® CG to reduce the potential for the development of resistance.
- A re-examination of the effects of basin cleaning on mosquito reproduction within basins using numbers and emergence of pupae vs numbers of larvae as the end-points may be warranted.
- Toxicity tests with VectoLex® CG using measures (e.g., colony-forming units/ml) that can be applied to water from outflows and invertebrates within urban creeks believed to be vulnerable need to be conducted.
- Comparable tests with methoprene-based products and sensitive non-target invertebrates may also be warranted.

at least 5 weeks. Differences between treated and control basins were likely minimized by precipitation events that reduced the number of larvae and pupae in the catch basins (through flushing), particularly the controls.

VectoLex® CG was effective in reducing the number of pupae within treated grated basins compared to controls for at least 4 weeks post-treatment. During this time period, results were consistent within each of the three regions monitored (Magnolia, and Thornton Creek and Longfellow Creek sub-basins), and efficacy within the grated basins was comparable to that observed in the closest round-top basins.

Altosid® XR (methoprene) was also effective in reducing the number of pupae within treated basins, in most cases fewer than 10 percent of pupae produced adults with maximum efficacy within 3 weeks after treatment. Results varied among regions with the greatest efficacy observed in Magnolia (100% control) and the poorest in the Thornton Creek watershed (≥ 80%).

The VectoLex® CG product contained significantly more Bt (100x) than Bs. The Bt detected is believed to be Bti. Reasons for this are not known, but it raises questions as to the source of the efficacy observed (Bs *vs* Bt or both) within round top and grated basins.

In most cases, concentrations of Bs and Bt in treated grated basins were less than 100 colony-forming units (cfu's) per ml and in many cases not different from pre-treatment concentrations. Concentrations post treatment did not follow the expected pattern of maximum concentration 1-week post treatment followed by decreases with time observed in efficacy trials in 2006. The relatively low concentrations of the bacterial larvicides detected were associated with significant reductions in the numbers of pupae present in treated basins for at least 4 weeks. Either the bacterial larvicides were extremely effective at low concentrations, or the observed efficacy was due to the effects of low temperatures at the time of treatment coupled with precipitation events, or both.

Methoprene was detected in only 2 of the 45 water samples collected (detection limit = 0.14 ppb). Results suggest that methoprene is effective at concentrations less than existing analytical detection limits. Reduced efficacy of Altosid® XR (methoprene) was associated with precipitation events and varied among regions likely due to differences in the magnitude of precipitation and basin storm water inputs (extent of dilution). Efficacy increased after 1 week without precipitation.

Concentrations of Bs and Bt within base flows entering Thornton Creek before and after treatment of catch basins within the watershed with VectoLex® CG were very low. Concentrations during precipitation events were also low and comparable to those detected in the treated catch basins within the watershed. In comparison, concentrations of Bt within outflows pre- and post treatment in 2007 were much greater. Whereas data for 2007 suggested use of Bt (Bti) within the watershed before the treatment of catch basins by SPU, this was not evident in 2008. Concentrations detected were orders of magnitude less than those shown to be non-toxic to juvenile coho salmon (*Oncorhynchus kisutch*).

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Acknowledgments

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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).

Within the US, 59 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 2005, Maine and Washington were the only states within the contiguous US without human cases of WNV. In Washington State, the virus was detected in animals in six counties in 2002. 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 multi-year citywide research effort in summer 2006. The overall goal of this research was to develop effective strategies to monitor and control mosquito breeding in SPU catch basins that are protective of human health and the environment. Specific objectives in 2006 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 and fate of three common larvicides (for results, see Grue et al. 2007). The Washington Cooperative Fish and Wildlife Research Unit (WACFWRU) at the University of Washington in collaboration with Landau & Associates, Inc. conduced the research in 2006. 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).

Based on these incident reports and the results of the 2006 research effort, SPU in 2007 contracted with Eden Advanced Pest Technologies (Eden) to treat all of the City's catch basins once with VectoLex® CG (*Bacillus sphaericus,* 10 g per basin, VALENT BioSciences Corporation) during a period of 4 weeks beginning the second week of July. SPU contracted with the WACFWRU to (1) determine the extent to which the pesticide applicator (Eden) complied with performance goals of its contract with the City of Seattle, (2) determine the efficacy of the larvicide in reducing the abundance of mosquito pupae in the treated catch basins, (3) determine the fate of the larvicide treated catch basins, and (4) determine the potential for the larvicide to enter surface waters and adversely affect non-target species within creeks in the City (Longfellow, Thornton, and Pipers; for results, see Grue et al. 2008).

Based on the efficacy of the 2007 control effort, SPU again contracted with Eden Advanced Pest Technologies to treat all of the City's catch basins once with VectoLex® CG (10 g per basin) beginning the second week of July 2008. SPU contracted with the WACFWRU to (1) determine the extent to which the pesticide applicator (Eden Advanced Pest Technologies) complied with performance goals of its contract with the City of Seattle, (2) determine the efficacy of the larvicide in reducing the abundance of mosquito pupae in round-top catch basins citywide, (3) compare the efficacy of VectoLex® CG and Altosid® XR (methoprene, Wellmark International) in reducing the abundance of mosquito pupae in grated basins in the city, and (4) determine the potential for VectoLex® CG to enter surface waters and adversely affect non-target species within creeks in the City.

Larval Abundance, Compliance, and Efficacy of Larvicide Treatments in Round-top Basins

Synopsis — The mosquito-breeding season in 2008 was similar to that in 2006 and 2007 with numbers of larvae increasing in the third week of June and sharply declining in the middle of September. Across the three years, larval abundance peaked between Week 28 (second week of July) and Week 34 (third week of August). In general, peak larval abundance was associated with weekly average maximum ambient air temperatures ≥ 24 C. Unlike 2006, rain was a frequent occurrence in 2007 and 2008 and was associated with dramatic declines in larval counts. The proportion of dry basins was much lower in 2007 (2.3%) and 2008 (2.7%) compared to 2006 (7.4%).

Treatment of round top catch basins with VectoLex® CG reduced the number of mosquito pupae below counts within untreated (control) basins. Compliance, as measured by the percentage of the treated round top basins monitored in which pupae counts were less than controls, was ≥ 96 percent. In most cases, efficacy was realized for at least 5 weeks. Differences between treated and control basins were likely minimized by precipitation events that reduced the number of larvae and pupae in the catch basins (through flushing), particularly within the controls.

Objective

Our objective was to determine the extent to which the pesticide applicator (contractor, Eden Advanced Pest Technologies) complied with performance goals of its contract with the City of Seattle and the efficacy of the larvicide (VectoLex® CG) in reducing the abundance of mosquito pupae within round-top catch basins. Specific tasks were:

- Monitor larval abundance within untreated round-top catch basins within the city.
- Determine if the contractor complied with the performance goals of the City's contract as measured by treatment efficacy.
- Determine if the larvicide treatment was effective in reducing the abundance of mosquito pupae within the catch basins treated.
- Determine the effects of precipitation on the efficacy of the larvicide treatments.
- Compare the efficacy of the larvicide in 2008 with that observed in 2007.

Methods

Selection of Basins — We randomly selected 50 basins from those treated by the contractor within a given week to determine compliance and efficacy of the larvicide treatment. Only "round top" basins (Fig. 1), one basin per intersection, were selected. We selected 50 basins treated during the first week of treatment (Week 1), the second

Figure 1. Round top (left) and grated (right) basins within the City of Seattle. Round top basins are normally situated within the roadway at one or more corners of intersections and receive stormwater through pipes from inlets (small catch basins) along nearby curbs. In contrast, grated basins are normally located along curbs and primarily receive stormwater directly from the road surface.

week of treatment (Week 2), and the third week of treatment (Week 3) for a total of 150 basins during the specified treatment window (3 weeks beginning 7 July). An additional 50 dedicated control (untreated) basins were also monitored. These control basins were the same basins that served as controls in 2006 and 2007. Fifty represented the minimum number of basins to obtain at least one basin per zone per sector in a design for the controls comparable to that used in 2006.

Monitoring of Basins — All treated basins were monitored weekly beginning 1-week post-treatment (Bs, VectoLex® CG) through the end of September. Control basins were monitored beginning 6 weeks before larvicide application until the end of September. At each visit to the treated and control basins, the number of mosquito pupae per three standard dips was recorded as were composition and extent of surface debris, water and sediment depth (0.5 inches), and water temperature (C), pH, conductivity (us/cm), and dissolved oxygen (mg/L). Additionally, in the control round top basins, we recorded the number of mosquito larvae in each of the three dips so that we could compare counts in 2008 with those in the same basins in 2006 and 2007. Separate sets of all sampling equipment we used for treated and control basins and the equipment and water quality instruments was thoroughly rinsed with dechlorinated water and dried between each use (basin).

Data Analyses — We compared the number of larvae counted per three dips in the untreated (control) round-top basins (n=50) through time in 2006 and 2007 graphically as well as data on ambient air temperature and precipitation. Counts of pupae in treated and control basins were compared graphically through time by treatment week (Weeks 1-3) including frequency distributions (percentage of basins with specific ranges ["bins"] of pupae). We compared the pupae counts between treated and control basins within treatment weeks by week post treatment using a one-sided Mann-Whitney test (alpha = 0.05). Larvicide treatments were considered efficacious if the difference between the counts of pupae within treated and control were statistically significant (i.e., the probability associated with the test statistic was $\leq$ 0.05). Treated basins in which the number of pupae was consistently equal to or greater than the control mean were considered "untreated" and used as a measure of non-compliance by the contractor.

Results

Larval Abundance — The mosquito-breeding season in 2008 in the City of Seattle was generally similar to that in 2006 and 2007 with numbers of larvae increasing in Week 26 (23-29 June) and sharply declining in Week 38 (15-21 September). Across the three years, larval abundance peaked between Week 28 (second week of July) and Week 34 (third week of August) (Fig. 2). In general, peak larval abundance was associated with weekly average maximum ambient air temperatures $\geq$ 24 C (Fig. 3).

Whereas precipitation was largely absent during the breeding season in 2006, rain was a frequent occurrence in 2007 and 2008 (Table 1, Fig. 4). Precipitation events were associated with dramatic declines in larval counts, frequently to zero within individual basins, making statistical comparisons among years difficult (Fig. 5). A comparison between the same 50 round top basins monitored during the 3 years suggests that larval counts outside of the precipitation events were greatest in 2008 with 2008>2007>2006. The proportion of dry basins was also correlated with the amount of precipitation during the breeding seasons with the percentage greatest in 2006 (7.4%) and lowest in 2007 (2.4%) and 2008 (2.7%) (Table 2).

Table 1. Precipitation accumulation (in) for the Pre-monitoring and Monitoring seasons in 2006-2008. For each year, the Pre-monitoring Season includes Weeks 18 through 24 and the Monitoring Season extends from Week 25 through Week 39 (Week 38 in 2008).

	2006	**2007**	**2008**
Pre-monitoring Season	3.48	2.42	2.57
Monitoring Season	0.23	4.81	3.08
Total	3.71	7.23	5.65

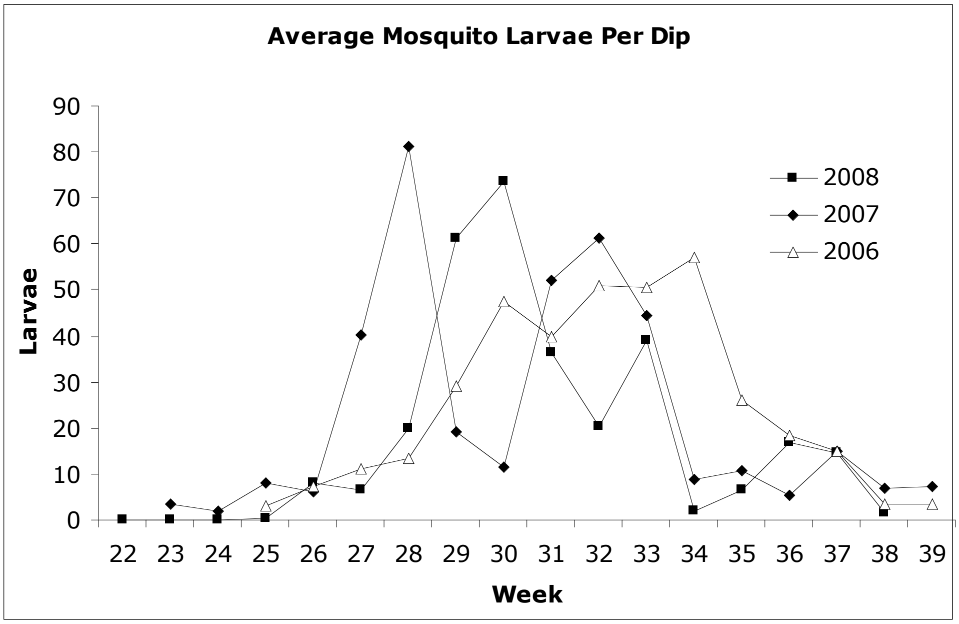

Figure 2. Average number of mosquito larvae per dip within untreated round-top basins in 2006, 2007, and 2008. The same 50 basins were monitored in each year.

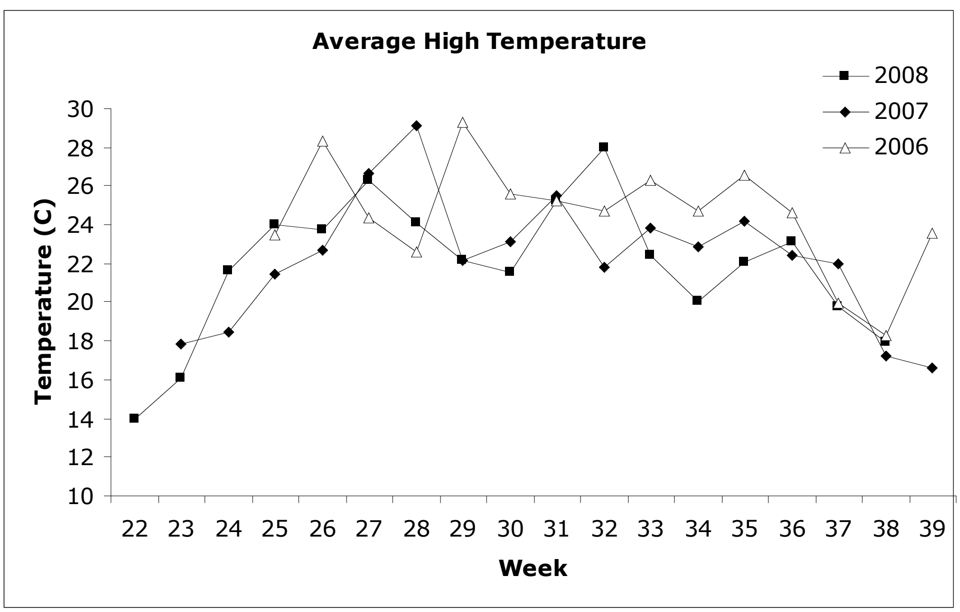

Figure 3. Average high temperature (C) by week in 2006, 2007, and 2008. Temperature data were taken from the weather station at Seattle-Tacoma International Airport.

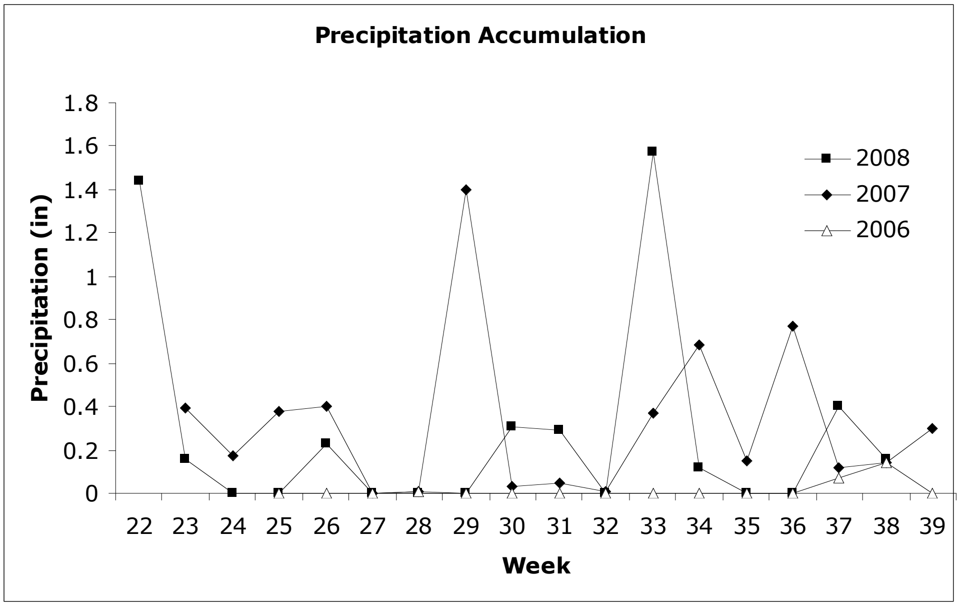

Figure 4. Precipitation accumulation (in) during 2006, 2007, and 2008. Precipitation data were taken from the weather station at Seattle-Tacoma International Airport.

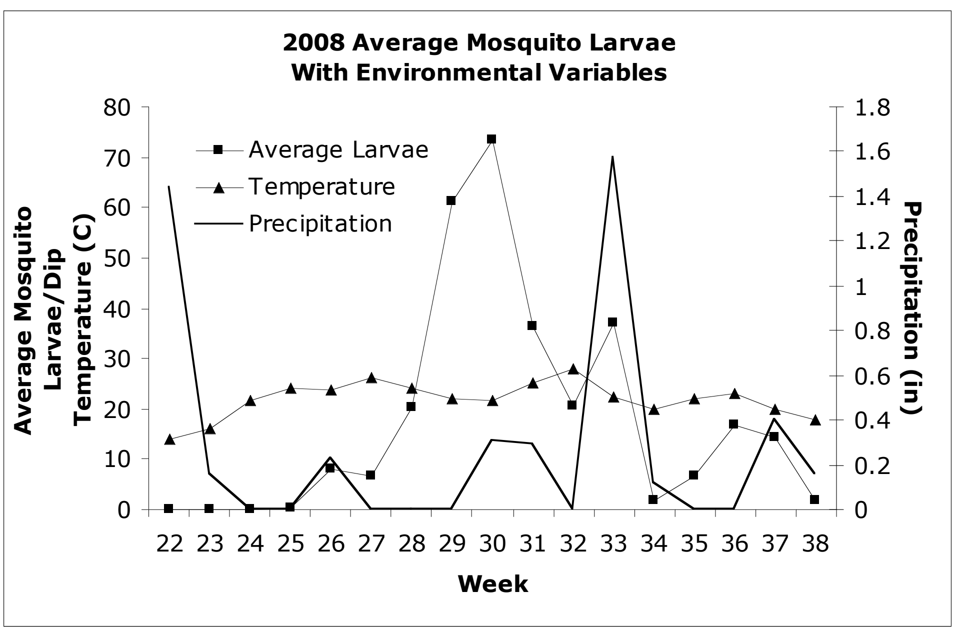

Figure 5. Average number of larvae per dip within untreated round-top basins in 2008 from 2 June through 26 September. Temperatures are the average daily high air temperatures per week recorded from Seattle-Tacoma International Airport and precipitation is the total accumulated precipitation per week as recorded from the same location. N = 50 basins.

Table 2. Percentage of dry basins among the same 50 control round-top basins in 2006, 2007, and 2008.

Week	2006	2007	2008
22	N/A	N/A	4
23	N/A	0	0
24	N/A	0	4
25	2	2	6
26	4	6	1
27	4	0	2
28	8	4	1
29	11	0	8
30	15	0	8
31	9	6	4
32	13	6	2
33	14	8	2
34	14	0	0
35	10	2	2
36	6	0	1
37	4	2	1
38	0	0	0
39	0	4	N/A
AVERAGE	7.6	2.4	2.7

Treatment Compliance — We evaluated compliance based on the percentage of "treated" basins for which pupae counts were consistently (across weeks) less than the corresponding mean of the controls (Table 3). Compliance was based on data for round top basins only, and by this measure average compliance was 96 percent (94-100% among treatment weeks) 1 week after treatment. By 2 weeks post treatment, 97% of treated basins met the criteria, and by 4 weeks after treatment, 99% of the basins met the criteria. We note that this is likely a very conservative estimate of non-compliance as the criteria for compliance may represent an unacceptable level of efficacy, and precipitation events may have artificially increased the proportion of basins that met the criteria for compliance.

Treatment Efficacy — We evaluated the efficacy of the larvicide treatments for each treatment week through time (9-11 weeks) by comparing the average number of pupae per three dips between the treated (n=50) and control (n=50) round top basins. Results for each of the three treatment weeks are presented in Figure 6. Statistical comparisons were made at 1, 3, 5, 7, 9 and if possible 11 weeks post-treatment. With the exception of Treatment Week 3, pupae counts in the treated basins were statistically lower ($P \leq 0.05$) than those in the control basins for a period of at least 5 weeks (Table 4).

Table 3. Percentage of basins with pupae counts consistently equal to or greater than control means for each week. After 5 weeks post-treatment, none of the basins that had been high in the previous weeks continued to be high. Treatment Group 1 in 2007 was not sampled 2 weeks post treatment. Treatment Group = week of treatment with N = 50 for each group.

Weeks Post-Treatment	**Treatment Group 1**		**Treatment Group 2**		**Treatment Group 3**	
	2007	2008	2007	2008	2007	2008
1	4	6	4	6	8	0
2	N/A	4	0	6	2	0
3	2	4	0	2	0	0
4	0	2	0	2	0	0
5	0	2	0	0	0	0

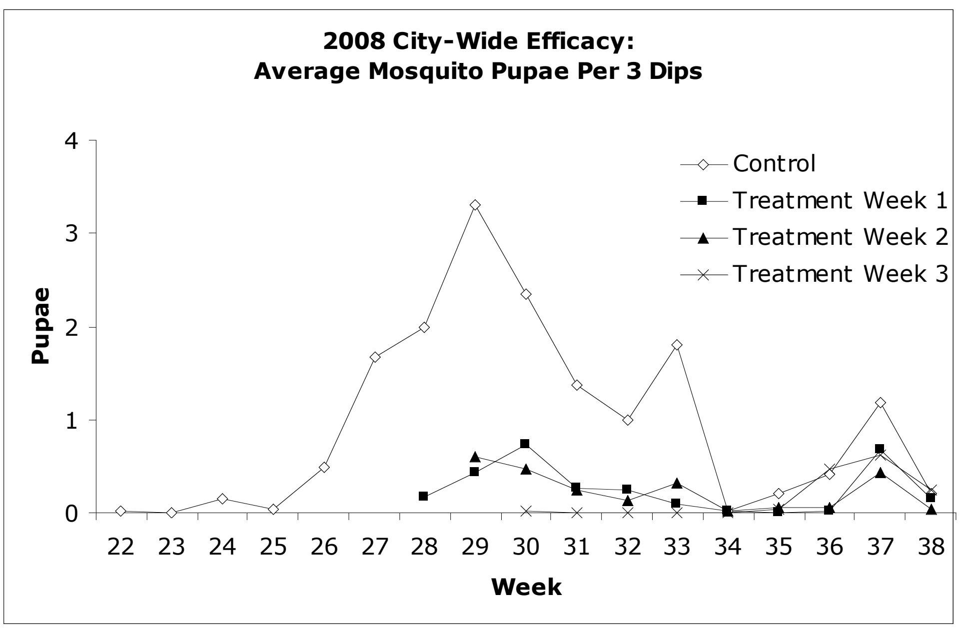

Figure 6. Average number of mosquito pupae per 3 dips for each group of 50 citywide round-top basins treated during the first, second, and third week of treatment. Treatment Week 1 basins were treated in Week 27, Treatment Week 2 basins were treated in Week 28, and Treatment Week 3 basins were treated in Week 29.

Table 4. Statistical analysis using one-sided Mann-Whitney test (alpha=0.05) on 2008 efficacy data for mosquito pupae within citywide round top basins. The analyses compared pupae counts between control basins and those treated with VectoLex® CG for three groups (treatment weeks) at 1, 3, 5, 7 and 9 weeks post-treatment. Comparisons were also made for Treatment Group 1 through Week 11 post-treatment. *Differences were considered significant when P≤0.05. A lack of statistical significance does not necessarily mean a loss of efficacy as precipitation events significantly reduced control counts making them more similar to those in treated basins.

Treatment Group	Week	Week Post Treatment	Significance
1	28	1	0.0045*
1	30	3	0.0005*
1	32	5	0.0005*
1	34	7	0.4945
1	36	9	0.0440*
1	38	11	0.3510
2	29	1	0.0000*
2	31	3	0.0000*
2	33	5	0.0010*
2	35	7	0.2930
2	37	9	0.0005*
3	30	1	0.0000*
3	32	3	0.0000*
3	34	5	0.1585
3	36	7	0.4865
3	38	9	0.1915

We also evaluated efficacy by comparing the frequency distributions of pupae counts within the treated and control round top basins. This provided a direct comparison of the number of treated and control basins with very low counts. Data for Treatment Week 1 are given in the frequency distributions shown in Figure 7a-f. Similar comparisons for the other two treatment weeks are given in Appendix 1. Differences between treated and control basins were potentially minimized by frequent precipitation (flushing of basins) as indicated by the relatively high proportion of untreated (control) basins with no pupae counted in the three dips. Pupae were not collected in greater than 50 percent of the untreated basins across the 11 weeks of monitoring (Figs. 7a-f). For example, precipitation effects likely explain the lack of statistical significance between treated and control round top basins 7 and 11 weeks after Treatment Week 1 (Figure 7d, f). In both cases, the proportion of treated basins with 0 pupae was high (ca. 95%), but comparable values for control basins were also high (>90%).

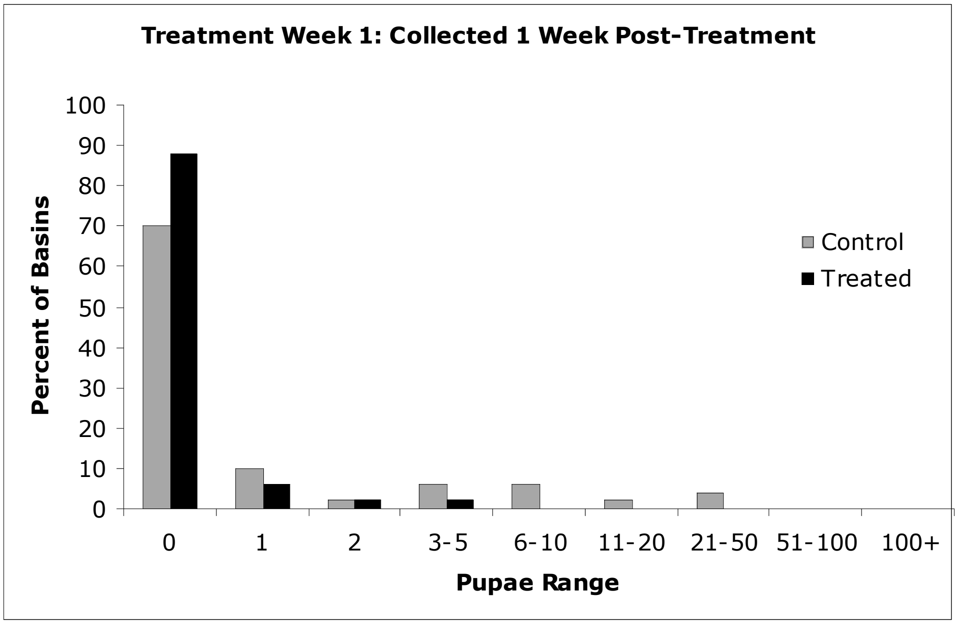

Figure 7a. Percentage of basins with specified ranges ("bins") of mosquito pupae for round top basins monitored following the first week of treatment (Treatment Week 1). Data collected 1 week post-treatment (Week 28).

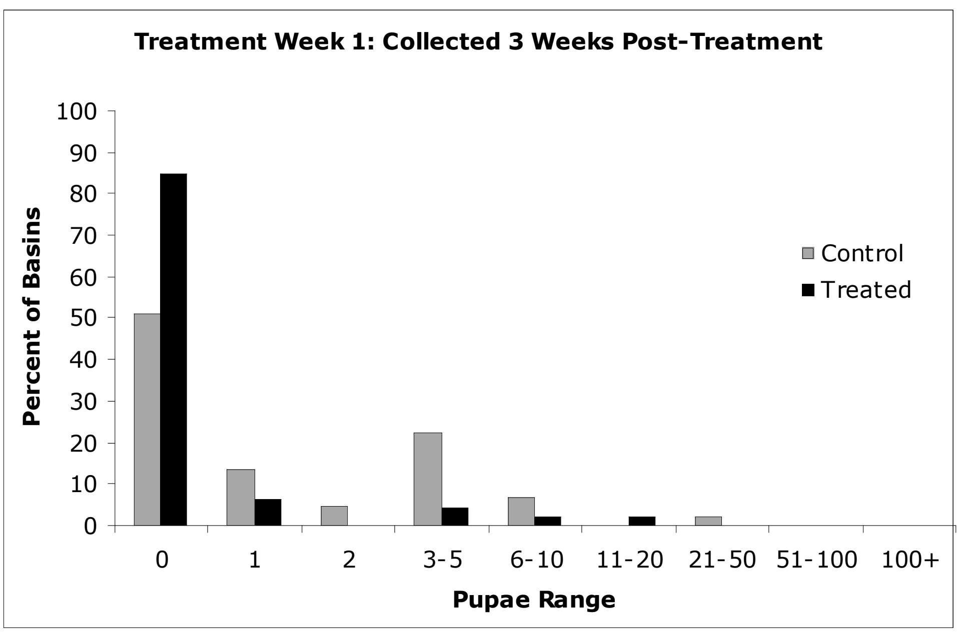

Figure 7b. Percentage of basins with specified ranges ("bins") of mosquito pupae for round top basins monitored following the first week of treatment (Treatment Week 1). Data collected 3 weeks post-treatment (Week 30).

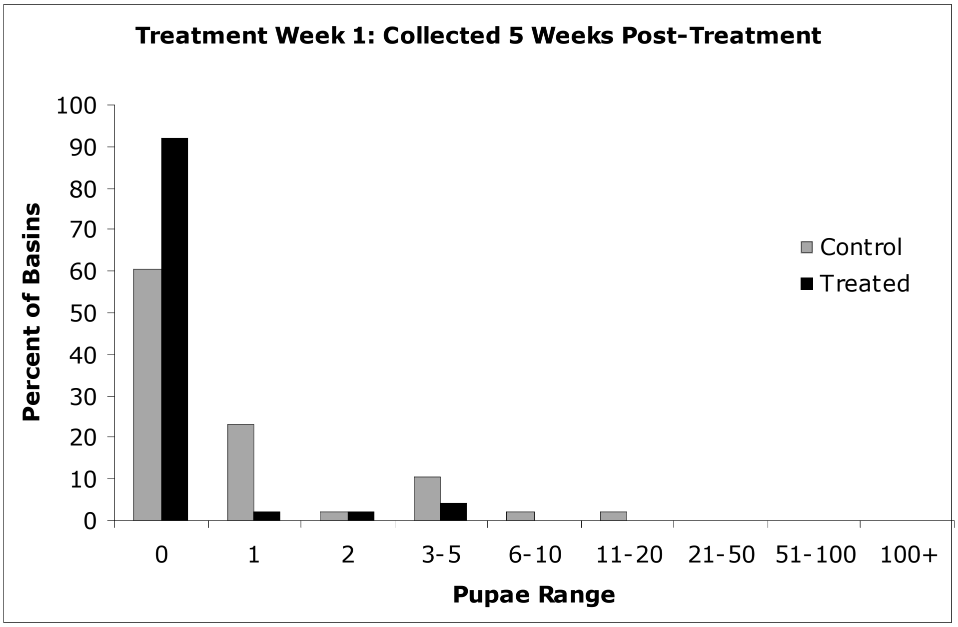

Figure 7c. Percentage of basins with specified ranges ("bins") of mosquito pupae for round top basins monitored following the first week of treatment (Treatment Week 1). Data collected 5 weeks post-treatment (Week 32).

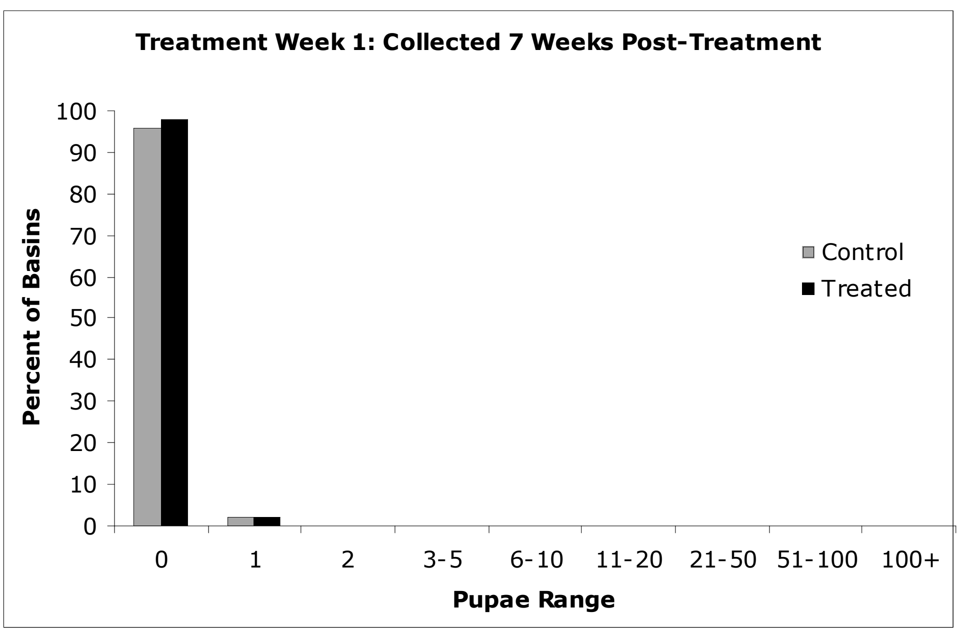

Figure 7d. Percentage of basins with specified ranges ("bins") of mosquito pupae for round top basins monitored following the first week of treatment (Treatment Week 1). Data collected 7 weeks post-treatment (Week 34).

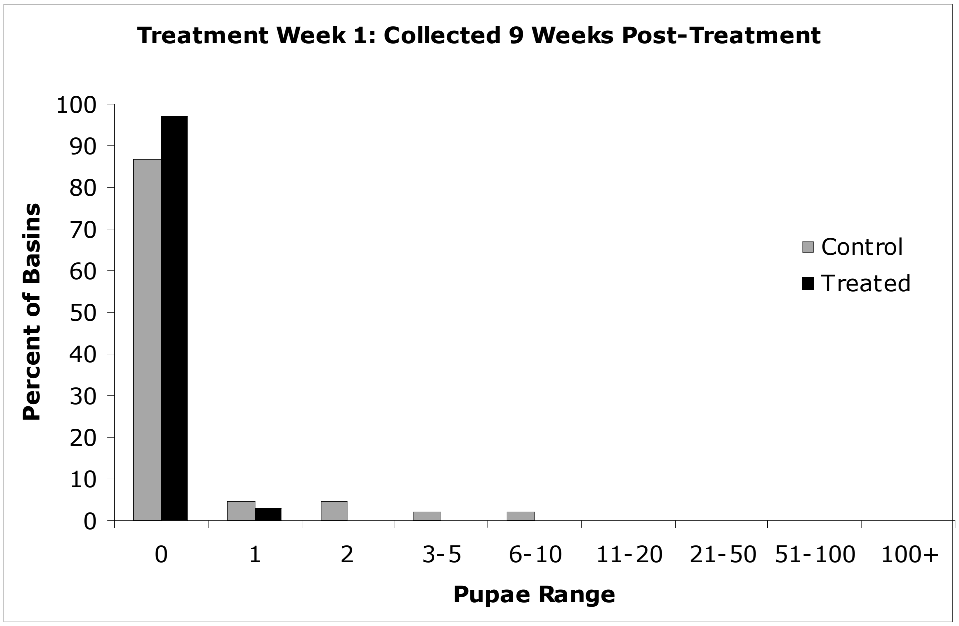

Figure 7e. Percentage of basins with specified ranges ("bins") of mosquito pupae for round top basins monitored following the first week of treatment (Treatment Week 1). Data collected 9 weeks post-treatment (Week 36).

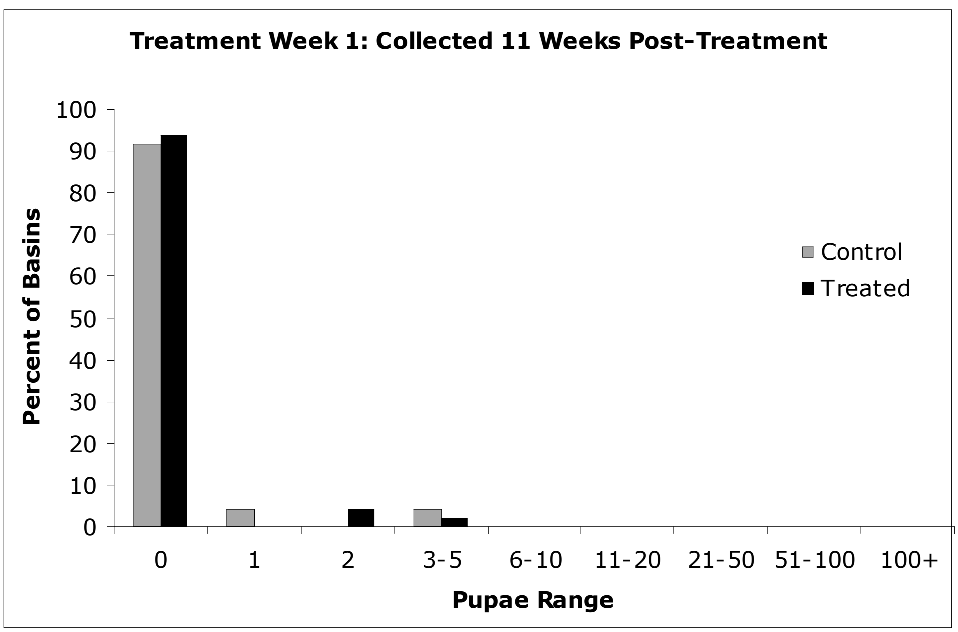

Figure 7f. Percentage of basins with specified ranges ("bins") of mosquito pupae for round top basins monitored following the first week of treatment (Treatment Week 1). Data collected 11 weeks post-treatment (Week 38).

Conclusions

The mosquito-breeding season in 2008 was similar to that in 2006 and 2007 with numbers of larvae increasing in the third week of June and sharply declining in the middle of September. Across the three years, larval abundance peaked between Week 28 (second week of July) and Week 34 (third week of August). In general, peak larval abundance was associated with weekly average maximum ambient air temperatures ≥ 24 C. Unlike 2006, rain was a frequent occurrence in 2007 and 2008 and was associated with dramatic declines in larval counts. The proportion of dry basins was much lower in 2007 (2.3%) and 2008 (2.7%) compared to 2006 (7.4%).

Treatment of round top catch basins with VectoLex® CG reduced the number of mosquito pupae below counts within untreated (control) basins. Compliance, as measured by the percentage of the treated round top basins monitored in which pupae counts were less than controls, was ≥ 96 percent. In most cases, efficacy was realized for at least 5 weeks. Differences between treated and control basins were likely minimized by precipitation events that reduced the number of larvae and pupae in the catch basins (through flushing) within each treatment group, particularly the controls.

Research Needs

With 3 years of comparable data on the abundance of larvae and environmental factors we recommend that a synthesis be conducted with the following objectives: (1) re-examine the relationship between larval abundance and environmental factors, (2) evaluate spatial distributions of larval abundance, and (3) summarize maintenance needs (i.e., basins exceeding criteria for cleaning). The utility may wish to also evaluate the possibility of targeting control efforts and establishing an annual index based on that used over the last 3 years. As noted in the results, greater than 50% and during several weeks greater than 90% of control basins did not contain measurable numbers of pupae. Emergence was lower in control basins in 2008 compared to 2006, and the reductions appear to be related to cooler temperatures, precipitation events, and reductions in available nutrients. The relationship between numbers of larvae, numbers of pupae, emergence and environmental variables needs to be examined. And finally, SPU should evaluate an alternative to VectoLex® CG to reduce the potential for the development of resistance. This is complicated by the fact that the Valent BioSciences product appears to be primarily, if not entirely, *Bacillus thuregiensis*, and not *B. sphaericus*.

Efficacy and Fate of Larvicides within Grated Catch Basins

Synopsis — VectoLex® CG was effective in reducing the number of pupae within treated grated basins compared to controls for at least 4 weeks post-treatment. During this time period, results were consistent within each of the three regions monitored and efficacy within the grated basins was comparable to that observed in the closest round-top basins. Altosid® XR (methoprene) was also effective in reducing the number of pupae within treated basins, in most cases fewer than 10 percent of pupae produced adults with maximum efficacy within 3 weeks after treatment. Results varied among regions with the greatest efficacy observed in Magnolia (100% control) and the poorest in the Thornton Creek watershed ($\geq$ 80%).

Untreated grated basins contained ca. 3-times more pupae than round-top basins. The contribution of grated basins in terms of potential adult WNV carriers is likely greater than that of round top basins on a per basin basis.

The VectoLex® CG product contained significantly more Bt (100x) than Bs; the Bt detected is believed to be Bti. Reasons for this are not known, but it raises questions as to the source of the efficacy observed (Bs *vs* Bt or both) within round-top and grated basins.

In most cases, concentrations of Bs and Bt were less than 100 colony-forming units (cfu's) per ml and in many cases not different from pre-treatment concentrations. Concentrations post treatment did not follow the expected pattern of maximum concentration 1-week post treatment followed by decreases with time. The relatively low concentrations of the bacterial larvicides detected were associated with significant reductions in the numbers of pupae present in treated basins for at least 4 weeks. Either the bacterial larvicides were extremely effective at low concentrations, or the observed efficacy was due to the effects of low temperatures at the time of treatment coupled with precipitation events, or both.

Methoprene was detected in only 2 of the 45 water samples collected (detection limit = 0.14 ppb). Results suggest that methoprene is effective at concentrations less than existing analytical detection limits. Reduced efficacy of Altosid® XR (methoprene) was associated with precipitation events and varied among regions likely due to differences in the magnitude of precipitation and basin storm water inputs (extent of dilution). Efficacy increased after 1 week without precipitation.

Objectives

Our objective was to compare the efficacies of the larvicides VectoLex® CG and growth regulator, Altosid® XR (methoprene, Wellmark International), within grated catch basins in the City of Seattle and determine the fate of the larvicides within the basins. Specific tasks were:

- Compare the efficacies of Bs and methoprene within three regions of the city containing high numbers of grated basins: Magnolia, and Thornton and Longfellow Creek sub-basins.
- Determine the effect of precipitation events on the efficacy of the two larvicides
- Determine the fate of the larvicides within the catch basins

Methods

Selection of Basins — Prior to the first week of treatment by the contractor, we randomly selected a subset of 45 grated top catch basins, 15 within each of the regions of the city with the greatest proportion of grated basins: Magnolia, Thornton and Longfellow Creek sub-basins (Appendix 2). Five of the basins in each region were randomly selected for treatment with Altosid® XR and five with VectoLex® CG (BS, 10 g) according to label recommendations during the first week of citywide treatment by a licensed pesticide applicator on the staff of the Washington Cooperative Fish and Wildlife Research Unit (J.M. Grassley). The five remaining basins within each region served as untreated controls.

Basin Monitoring — Monitoring began 1 week before treatment and coincided with the monitoring of control and BS-treated round-top basins citywide. At each basin, the number of mosquito pupae per three standard dips was determined each week of sampling, in addition to composition and extent of surface debris, water and sediment depth, and water temperature, pH, conductivity, and dissolved oxygen. Water samples were collected from treated basins (depth integrated, DIPSTIK 84 PP, Norwell Environmental Products) for either bacterial analysis (Bs, colony-forming units/ml; 1, 3, 5 and 7 weeks post-treatment; Institute of Environmental Health [IEH], Lake City, WA) or methoprene ($\geq$ 0.14 ppb; 1, 6 and 10 weeks post-treatment; Warren Analytical Laboratories [WAL], Colorado Springs, CO). Samples for bacterial analysis were delivered to IEH on the day of collection; samples for methoprene analysis were shipped without preservative (methylene chloride) overnight on the day collection to WAL. Samples were either extracted on the day of arrival to WAL or 75 ml of methylene chloride was added to each sample. In the case of the latter, samples were refrigerated at 4 C until analyses were conducted.

For Bs, efficacy was determined by counting the number of pupae within three standard larvae dips immediately before treatment and weekly through September. 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 (Fig. 8, Grue et al. 2007). 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 dips were taken from each basin to obtain the pupae. 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 16 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 fabric band-aid. The cup with the pupae was then placed in a covered cooler and secured in a cardboard cup carrier. Cups, lids and carriers were provided by Starbucks Corporation, Seattle, WA. Upon return to the University, any adults that had emerged were counted, removed, and the racks with cups placed in an incubator set at 24-26 C (ca. 80° F, Fig. 8) with a 16 L:8 D light cycle. Adults that successfully emerged in each cup each day were counted and removed. The total number of pupae from which adults emerged after 4 days was expressed as a percentage of the original number of pupae. Separate sets of all sampling equipment were used for each larvicide and water quality instruments were thoroughly rinsed with dechlorinated water and dried between each use (basin).

Data Analyses — Treatments were considered efficacious as long as the number of pupae per three dips (Bs) or the percent emergence (methoprene) remained less than the concurrent mean for the 15 control basins. Differences between the counts of pupae within Bs-treated and control basins were also tested statistically using a one-sided Mann Whitney test (alpha = 0.05). Pupae counts (Bs-treated and controls) or percent emergence (methoprene-treated and controls) were plotted each week during the monitoring period and compared among and across the three regions. If detected, average concentrations of the two larvicides were plotted by week among and across regions.

Results

Efficacy — VectoLex® CG (10 g per basin) was effective in reducing the number of pupae within treated grated basins compared to controls (Fig. 9). VectoLex® CG was effective for at least 4 weeks post-treatment (Table 5, Figs. 10-12). During this time period, results were consistent within each of the three regions monitored: Magnolia, Thornton and Longfellow Creek sub-basins (Figs. 10-12); efficacy was comparable to that observed in the closest round-top basins (Tables 6a-d, pages 37-39). Precipitation during Week 33 significantly reduced the number of pupae within controls such that there was no difference between treated and control basins. Within the Magnolia township and Longfellow Creek sub-basin, differences in pupae counts between treated and control basins were again statistically significant 9 weeks post-treatment (Table 5), although counts of pupae within treated basins had steadily increased beginning 1-2 weeks after the precipitation event. A similar pattern occurred within the Thornton Creek sub-basin,

Figure 8. Incubation cups and chamber for assessing the efficacy of methoprene for controlling the production of mosquitoes in catch basins in Seattle. Pupae were incubated for 4 days at 24-26 C to determine percent emergence.

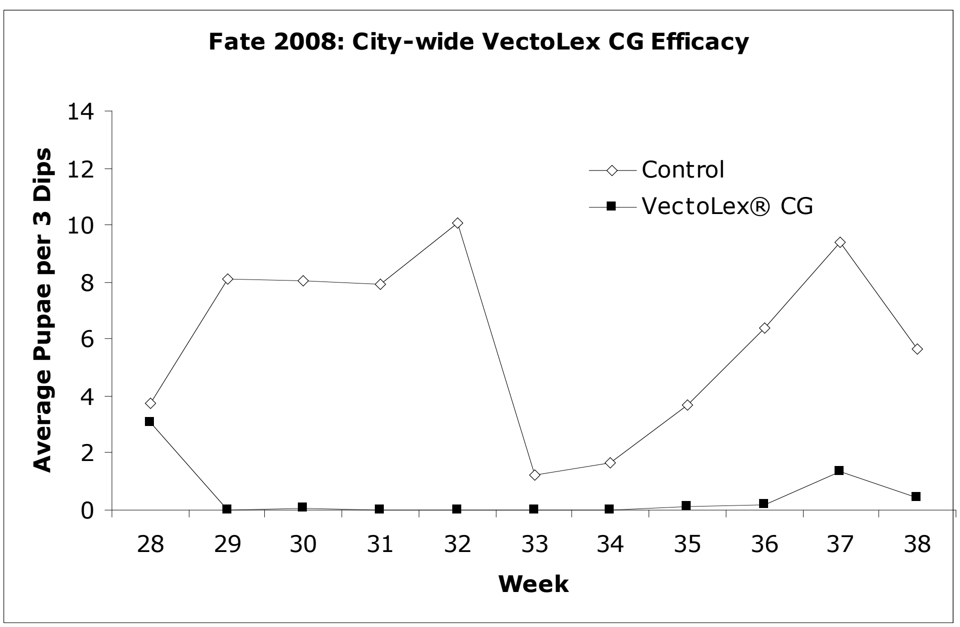

Figure 9. Average number of mosquito pupae per three dips for grated-top basins, citywide (Magnolia, Thornton and Longfellow Creek sub-basins) treated with VectoLex® CG in 2008. Graph depicts both control and treated basins. Week 28 represents the pre-treatment data. (Control N = 15, Treated N = 15)

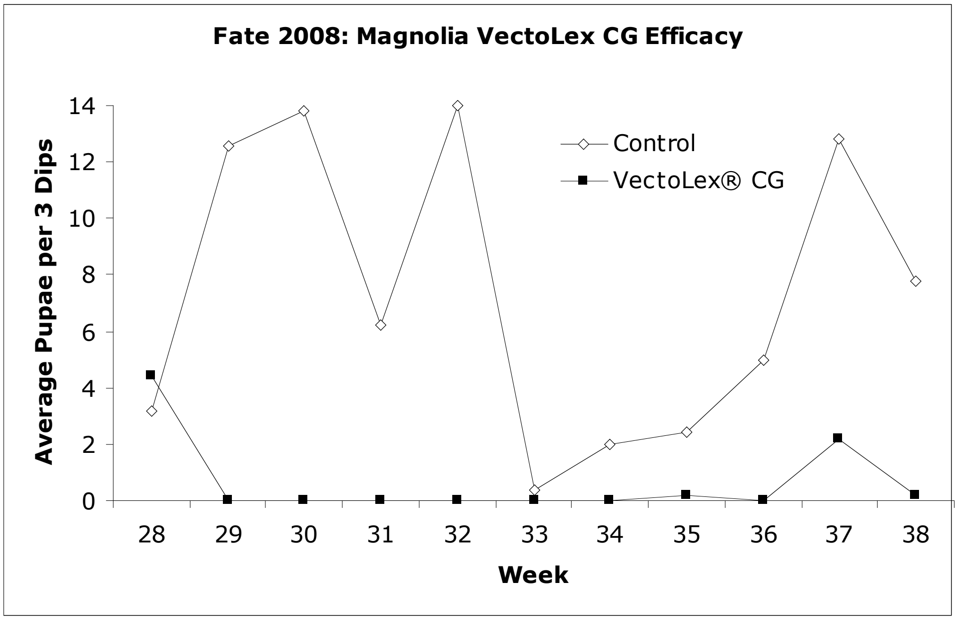

Figure 10. Average number of mosquito pupae per three dips for grated-top basins in Magnolia treated with VectoLex® CG in 2008. Graph depicts both control and treated basins. Week 28 represents the pre-treatment data. (Control N = 5, Treated N = 5).

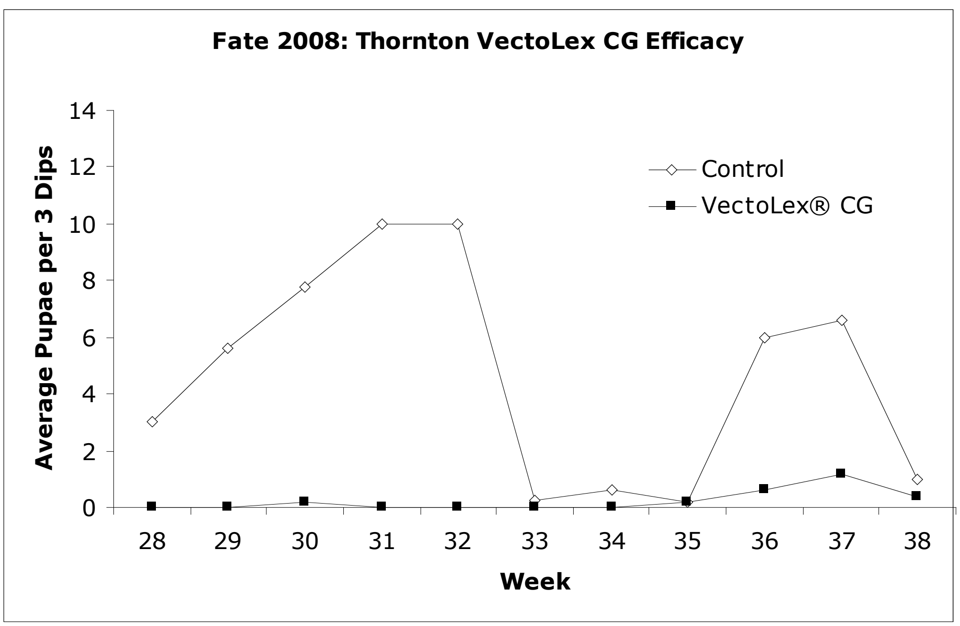

Figure 11. Average number of mosquito pupae per three dips for grated-top basins in the Thornton Creek sub-basin treated with VectoLex® CG in 2008. Graph depicts both control and treated basins. Week 28 represents the pre-treatment data. (Control N = 5, Treated N = 5).

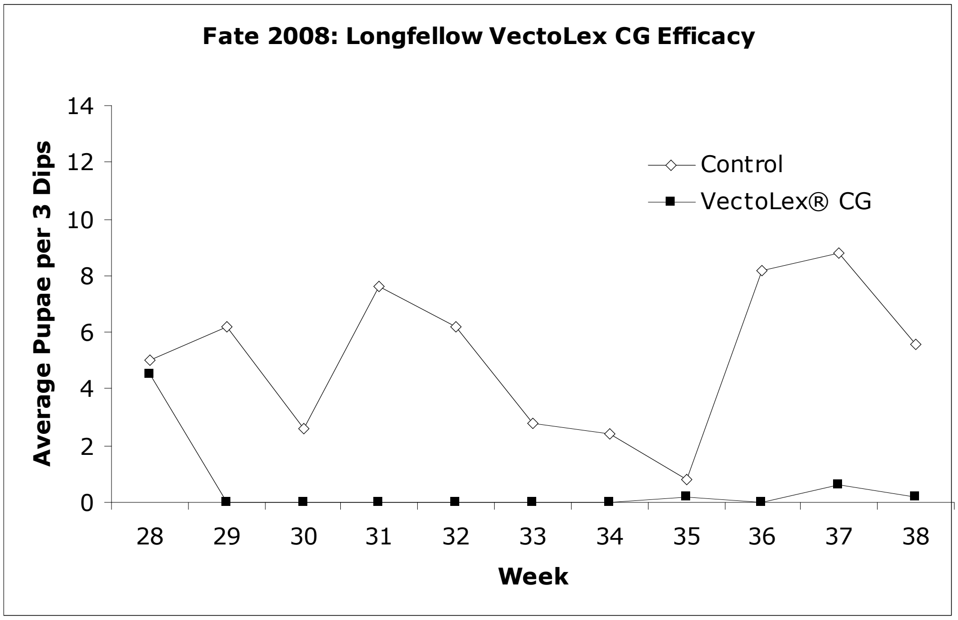

Figure 12. Average number of mosquito pupae per three dips for grated-top basins in the Longfellow Creek sub-basin treated with VectoLex® CG in 2008. Graph depicts both control and treated basins. Week 28 represents pre-treatment data. (Control N = 5, Treated N = 5).

but differences never reached statistical significance and numbers of pupae exceeded (2X) those in adjacent round-top basins after 7 weeks post-treatment (Table 6d). The slower recovery of efficacy within the Thornton creek sub-basin may have been related to the lower basin water temperatures within the sub-basin compared to the other two regions in 2008 (Appendix 3). In all three regions, precipitation in Week 37 likely masked a significant decrease in efficacy after 9 weeks.

It is not clear why the efficacy of VectoLex® CG was greater in grated basins in 2008 compared to 2007. In 2007, efficacy within the grated basins citywide and in the Thornton Creek watershed lasted only 3 weeks and was less than that in round top basins (≥ 5 weeks, Grue et al. 2008), whereas efficacy was realized for at least 4 weeks within the three regions in 2008 and was more comparable to that in round tops (≥ 5 weeks). Differences between years in the timing of significant precipitation events post-treatment are one explanation. Precipitation events occurred post treatment each year, but in 2007, a significant precipitation event (ca. 1.4 in) occurred 1 week after treatment, whereas in 2008, the first rainfall of similar magnitude (ca. 1.6 in) occurred 5 weeks post treatment. The flushing of the basins in 2007 may have occurred before the bacteria became fully established.

Nor do we know why pre-treatment counts within the treated basins in the Thornton Creek sub-basin in 2008 were much lower (no pupae detected) than those within comparable controls (Fig. 11). Observations suggest that many of the treated basins were

Table 5. Statistical analysis (one-sided Mann-Whitney test, alpha=0.05) comparing the numbers of pupae within grated basins (n=5) within the Magnolia township, and Longfellow and Thornton Creek sub-basins treated with VectoLex® CG and a comparable number of untreated controls 1, 3, 5, 7, and 9 weeks post-treatment. *Differences were considered statistically significant when $P \leq 0.05$ using the “exact significance value”.

Treatment Area	Week	Week Post-Treatment	Significance
Longfellow	29	1	0.0755
Longfellow	31	3	0.0160*
Longfellow	33	5	0.1550
Longfellow	35	7	0.3450
Longfellow	37	9	0.0475*
Magnolia	29	1	0.0160*
Magnolia	31	3	0.0755
Magnolia	33	5	0.1550
Magnolia	35	7	0.1110
Magnolia	37	9	0.0280*
Thornton	29	1	0.0040*
Thornton	31	3	0.0040*
Thornton	33	5	0.3450
Thornton	35	7	0.1550
Thornton	37	9	0.1550

Table 6a. Average number of pupae per three dips for VectoLex® CG treated grated top basins (N = 15) and the closest VectoLex® CG treated round top basins (N = 15) citywide beginning 1-2 weeks post-treatment. Round top basins were treated in Weeks 27-29, grated basins in Week 28.

Week	Treated Round-Tops	Treated Grated-Tops
30	0.2	0.07
31	0	0
32	0	0
33	0.07	0
34	0	0
35	0	0.13
36	0.07	0.2
37	1.4	1.33
38	0	0.4
AVERAGE	0.19	0.24

Table 6b. Average number of pupae per three dips for VectoLex® CG treated grated basins (N = 5) and the closest VectoLex® CG treated round top basins (N = 5) within the Longfellow Creek sub-basin. Round top basins were treated during Treatment Week 1 (Week 27).

Week	Treated Round-Tops	Treated Grated-Tops
29	0	0
30	0	0
31	0	0
32	0	0
33	0	0
34	0	0
35	0	0
36	0.2	0
37	0.8	0.6
38	0	0.2
AVERAGE	0.10	0.08

cleaner (less floating debris, clear water) than basins treated in 2007. The watershed was a focus for basin cleaning by the utility between the two years (S. Kelleher, personal communication) and basin water temperatures were not only lower in the sub-basin compared to the other study areas in 2008, but water temperatures in VectoLex® treated basins in the sub-basin were lower than untreated controls (Appendix 3). These observations coupled with the low concentrations of bacterial larvicide detected in the basins (Figs. 18a-d, pages 44-45) make it difficult to identify the cause of the apparent increase in efficacy within the sub-basin.

Altosid® XR (methoprene) was also effective in reducing the emergence of adults within treated basins compared to controls. In most cases, fewer than 10 percent of the pupae

Table 6c. Average number of pupae per three dips for VectoLex® CG treated grated basins (N = 5) and the closest VectoLex® CG treated round top basins (N = 5) within the Magnolia township. Round-top basins were treated in either Treatment Week 2 or Treatment Week 3 (Weeks 28 or 29).

Week	Treated Round-Tops	Treated Grated-Tops
30	0.6	0
31	0	0
32	0	0
33	0.2	0
34	0	0
35	0	0.4
36	0	0
37	2.2	2.2
38	0	0.2
AVERAGE	0.33	0.31

Table 6d. Average number of pupae per three dips for VectoLex® CG treated grated basins (N = 5) and the closest VectoLex® CG treated round top basins (N = 5) within the Thornton Creek sub-basin. Round top basins were treated in Treatment Week 1 (Week 27).

Week	Treated Round-Tops	Treated Grated-Tops
29	0	0
30	0	0.2
31	0	0
32	0	0
33	0	0
34	0	0
35	0	0
36	0	0.6
37	1.2	1.2
38	0	0.8
AVERAGE	0.12	0.28

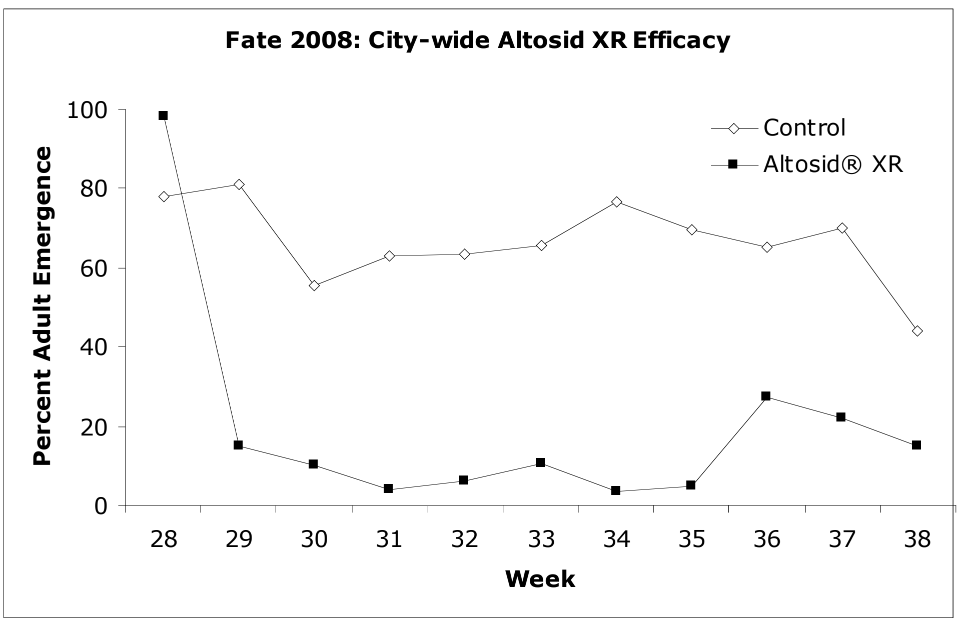

Figure 13. Average percent adult mosquito emergence for grated-top basins, citywide (Magnolia, Longfellow and Thornton Creek sub-basins) treated with Altosid® XR in 2008. Graph depicts both control and treated basins. Week 28 represents pre-treatment data. (Control N = 15, Treated N = 15).

collected produced adults with maximum efficacy within 3 weeks post treatment (Fig. 13). Efficacy was good until the first major precipitation event 5 weeks post-treatment (Week 33). Results varied among the three regions monitored: Magnolia, and Thornton and Longfellow Creek sub-basins (Figs. 14-16) with the greatest efficacy observed in Magnolia (100% control) and the poorest in the Thornton Creek watershed (≥ 80%).

Precipitation during Week 33 significantly reduced the number of pupae within control and treated basins due to flushing and subsequently reduced the efficacy of the larvicide due to dilution. Efficacy in the Longfellow and Thornton Creek sub-basins was regained within 2-3 weeks. In contrast, efficacy within the Magnolia township did not recover. Because the product is effective for 90 days according to the manufacturer, we believe the reductions in efficacy observed were due to precipitation events or other water inputs into the basins. The role of decreasing basin temperatures (Fig. 17) in observed reductions in efficacy are not known, although reductions in emergence of adults from control pupae appears correlated with decreasing basin water temperatures. In addition, because we averaged the emergence success across basins within regions by week, each basin contributed equal weight irrespective of the number or pupae collected (maximum = 10). As such, the successful emergence of a lone pupae within an incubation cup was weighted the same as a single cup in which none of 10 pupae hatched. We are currently developing a mathematical approach to address this bias, particularly when sample sizes are low.

Grated basins contained ca. 3-times more pupae than round-top basins (Figs. 6, 9-12). Therefore, assuming similar species composition and sex ratios in emerging adults, the contribution of grated basins in terms of potential adult WNV carriers is likely greater than that of round top basins on a per basin basis.

Fate of VectoLex® CG and Altosid® XR within Grated Basins — As in previous years, the VectoLex® CG product contained significantly more (100x) Bt than Bs (Table 7). The Bt detected was Bti and identical to that found in Mosquito Dunks® (Summit Chemical Company, Baltimore, MD; Ma, personal communication). Reasons for this are not known, but it raises questions as to the source of the efficacy observed (Bs *vs* Bt or both) within round top and grated basins. The label for the product does not list Bt as an active ingredient. Species identification was confirmed with DNA fingerprinting (G. Ma, Institute of Environmental Health [IEH], Lake City, WA, 5 December 2008).

Table 7. Pooled counts of *Bacillus sphaericus* and *Bacillus thuringiensis* (cfu/g product) from four samples of the VectoLex® CG product used by Eden Advanced Pest Technologies to treat the catch basins in the City of Seattle in 2008. The same product was used by WACFWRU to treat the grated study basins within the three regions of the city.

Sample Numbers	*Bacillus sphaericus*	*Bacillus thuringiensis*
1A, 1B, 2A, 2B	300,000	20,000,000

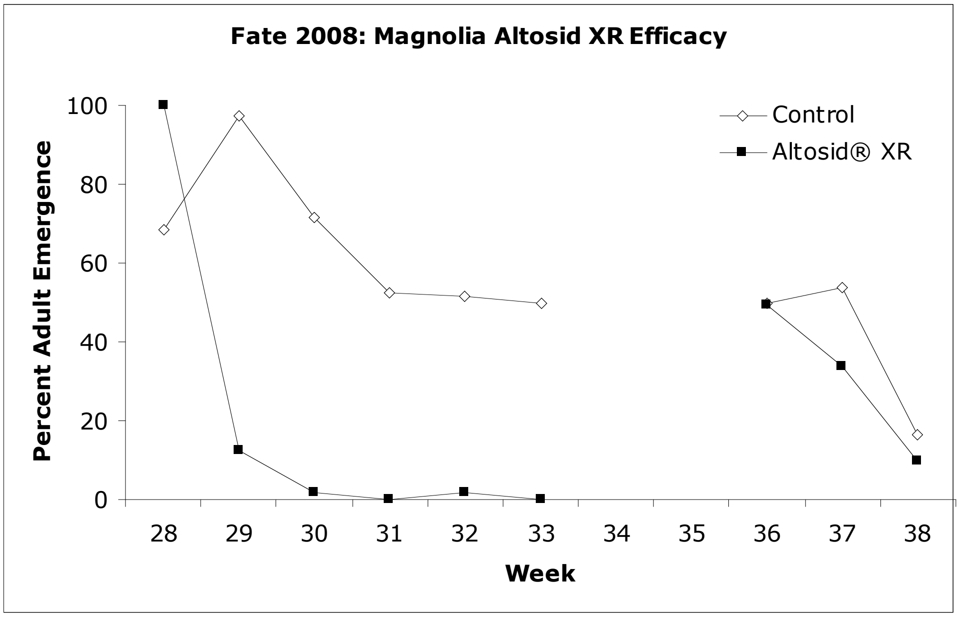

Figure 14. Average percent adult mosquito emergence for grated-top basins within Magnolia treated with Altosid® XR in 2008. Graph depicts both control and treated basins. Week 28 represents pre-treatment data. (Control N = 5, Treated N = 5). Lack of data for Weeks 34 and 35 is the result of an absence of pupae within the basins following precipitation during Week 33.

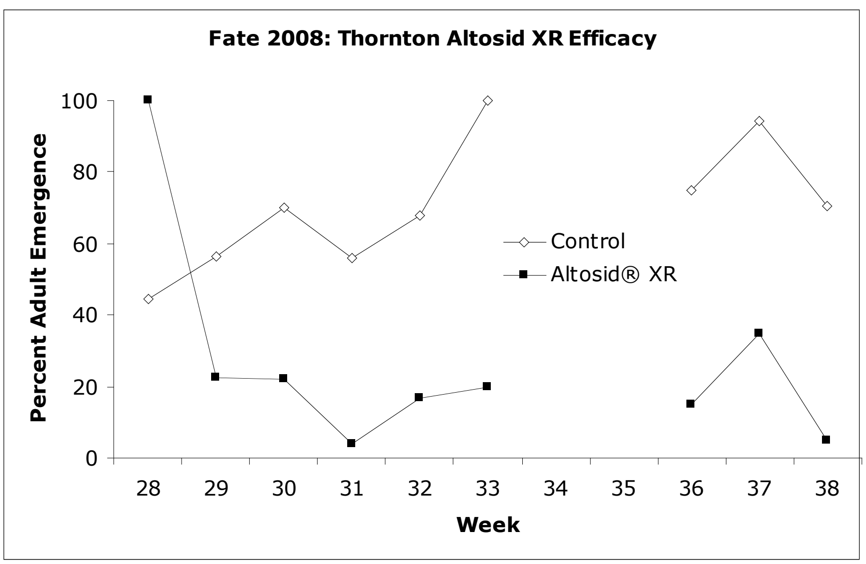

Figure 15. Average percent adult mosquito emergence for grated-top basins within the Thornton Creek sub-basin treated with Altosid® XR in 2008. Graph depicts both control and treated basins. Week 28 represents pre-treatment data. (Control N = 5, Treated N = 5). Lack of data for Weeks 34 and 35 is the result of an absence of pupae within the basins following precipitation during Week 33.

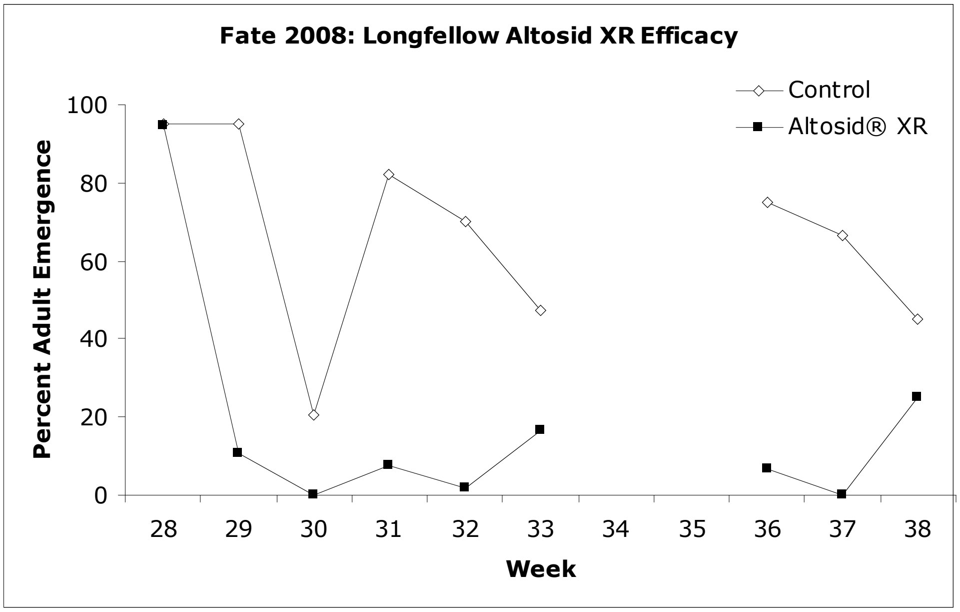

Figure 16. Average percent adult mosquito emergence for grated-top basins within the Longfellow Creek sub-basin treated with Altosid® XR in 2008. Graph depicts both control and treated basins. Week 28 represents pre-treatment data. (Control N = 5, Treated N = 5). Lack of data for Weeks 34 and 35 is the result of an absence of pupae within the basins following precipitation during Week 33.

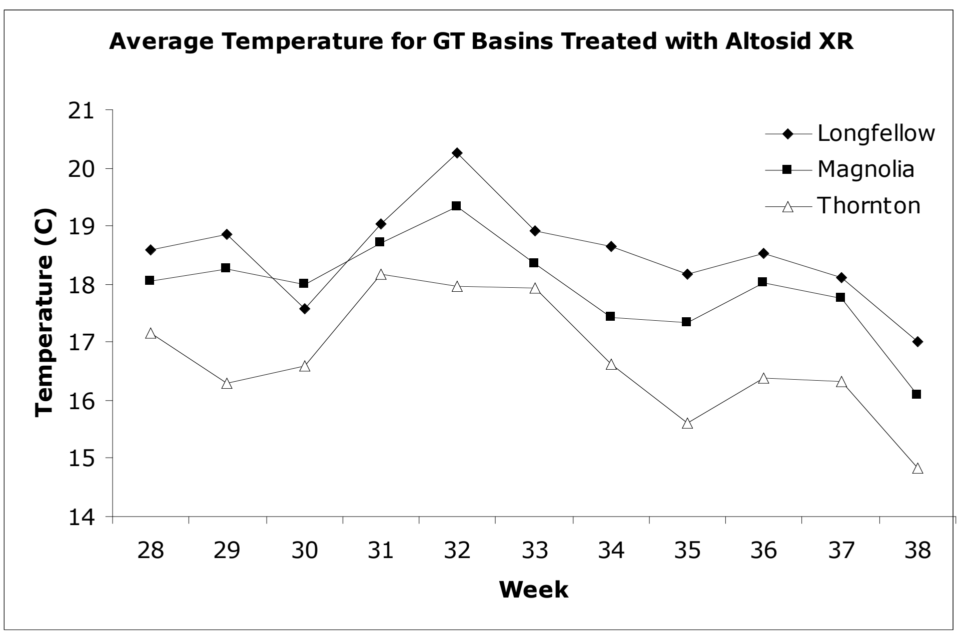

Figure 17. Average water temperature (C) in grated-top basins treated with Altosid® XR within the Magnolia township and Longfellow and Thornton Creek sub-basins in 2008. N = 5/area/week.

In most cases (3 exceptions), concentrations of Bs and Bt were less than 100 cfu's (colony-forming units) per ml and in many cases not different from pre-treatment concentrations (Figs. 18a-d). Concentrations post treatment did not follow the expected pattern of maximum concentration 1-week post treatment followed by decreases with time, comparable to that observed in 2006 for round top basins when precipitation was absent (Grue et al. 2007). Concentrations in round top and grated basins studied in 2007 were greatest 1 week after treatment, but were then essentially absent during subsequent weeks with only Bt detected. Concentrations in 2007 1-week post treatment were 10-100x those detected in 2008, and those in 2006 were 10-100x those observed in 2007 (Grue et al. 2007, 2008). The relatively low concentrations of the bacterial larvicides detected in 2007 and 2008 were still associated with significant reductions in the numbers of pupae present in treated basins for up to 5 weeks and at least 4 weeks, respectively, with the exception of grated basins in the Thornton Creek watershed in 2007 (Grue et al. 2008). Either the bacterial larvicides were extremely effective at low concentrations, or the observed efficacy was due to low temperatures at the time of treatment (Fig. 3) coupled with precipitation events (Fig. 4), or both. As noted previously, pupae were not collected in greater than 50 percent of the untreated basins across the 11 weeks of citywide monitoring in 2008 (Figs. 7a-f).

Chemical analyses of water samples collected from methoprene-treated basins yielded results similar to those in efficacy trials in 2006 and laboratory experiments in 2007 (Grue et al. 2007, 2008). Methoprene was detected in only 2 of the 45 water samples collected (detection limit = 0.14 ppb). Both detections occurred 1-week post-treatment. One sample taken from a basin in the Thornton Creek watershed had 11.4 ppb methoprene and one sample taken from a basin in the Longfellow Creek watershed basin had 3.1 ppb methoprene. Results from 2006 and 2008 suggest that methoprene is effective at concentrations less than existing analytical detection limits. As expected, reduced efficacy of Altosid® XR (methoprene) was associated with precipitation events and varied among regions likely due to differences in the magnitude of precipitation and basin stormwater inputs (extent of dilution). In some weeks (Weeks 34 and 35), efficacy could not be adequately assessed because of the low numbers of pupae within treated and control basins following precipitation events due to flushing of the basins. Efficacy increased after 1 week without precipitation (Figs. 13-16).

Conclusions

VectoLex® CG (10 g per basin) was effective in reducing the number of pupae within treated grated basins compared to controls. VectoLex® CG was effective for at least 4 weeks post-treatment. During this time period, results were consistent within each of the three regions monitored and efficacy was comparable to that observed in the closest round-top basins. Precipitation during Week 33 significantly reduced the number of pupae within controls such that there was no difference between treated and control basins. Within the Magnolia township and Longfellow Creek sub-basin, differences in pupae counts between treated and control basins were again statistically significant 9

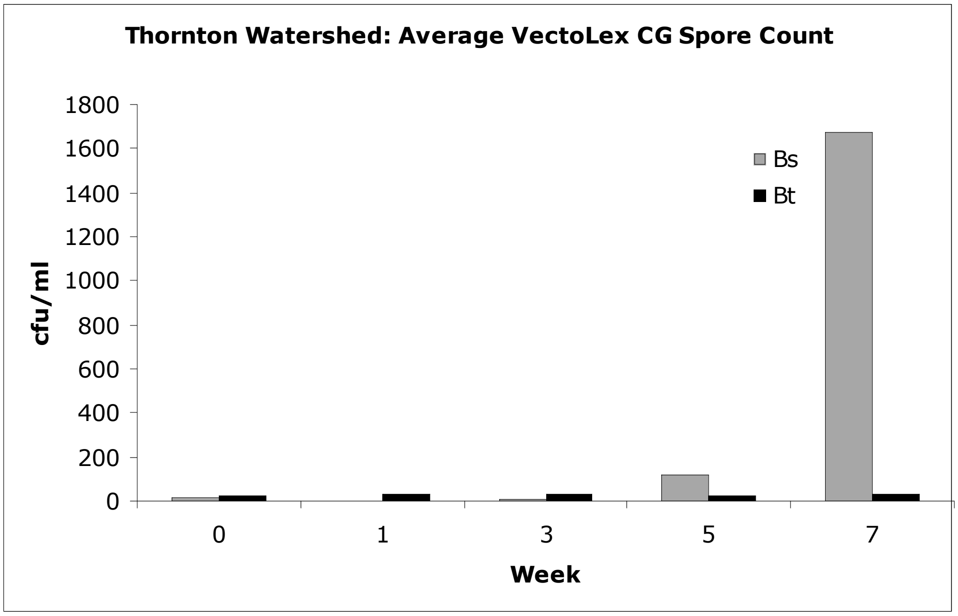

Figure 18a. Average counts (colony forming units/ml) of *B. sphaericus* (Bs) and *B. thuringiensis* (Bt) within grated catch basins (N=5) within the Thornton Creek watershed before and after treatment with VectoLex® CG (Week 0) in 2008.

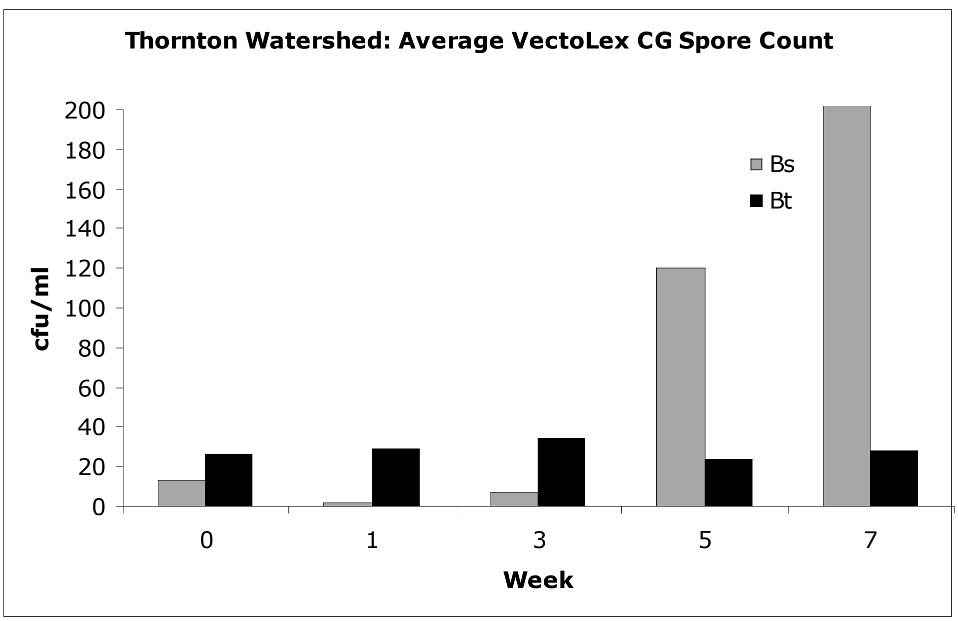

Figure 18b. Average counts (colony forming units/ml) of *B. sphaericus* (Bs) and *B. thuringiensis* (Bt) within grated catch basins (N=5) within the Thornton Creek watershed before and after treatment with VectoLex® CG (Week 0) in 2008. Y-axis has been adjusted to a maximum of 200 cfu/ml. Bs concentrations in Week 7 > 1,600 cfu/ml (Fig. 18a).

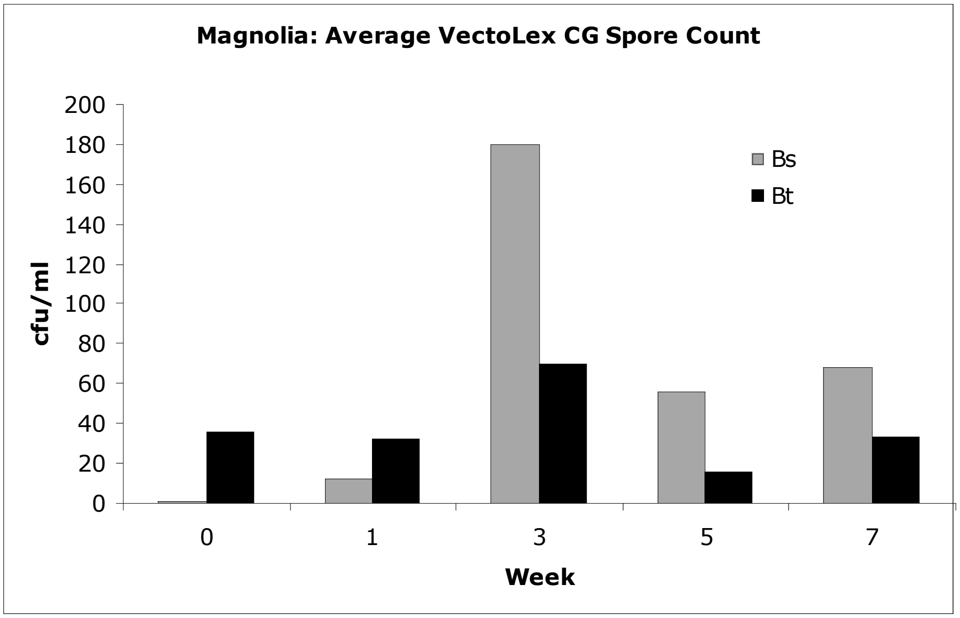

Figure 18c. Average counts (colony forming units/ml) of *B. sphaericus* (Bt) and *B. thuringiensis* (Bs) within catch basins (N=5) within Magnolia before and after treatment with VectoLex® CG (Week 0) in 2008.

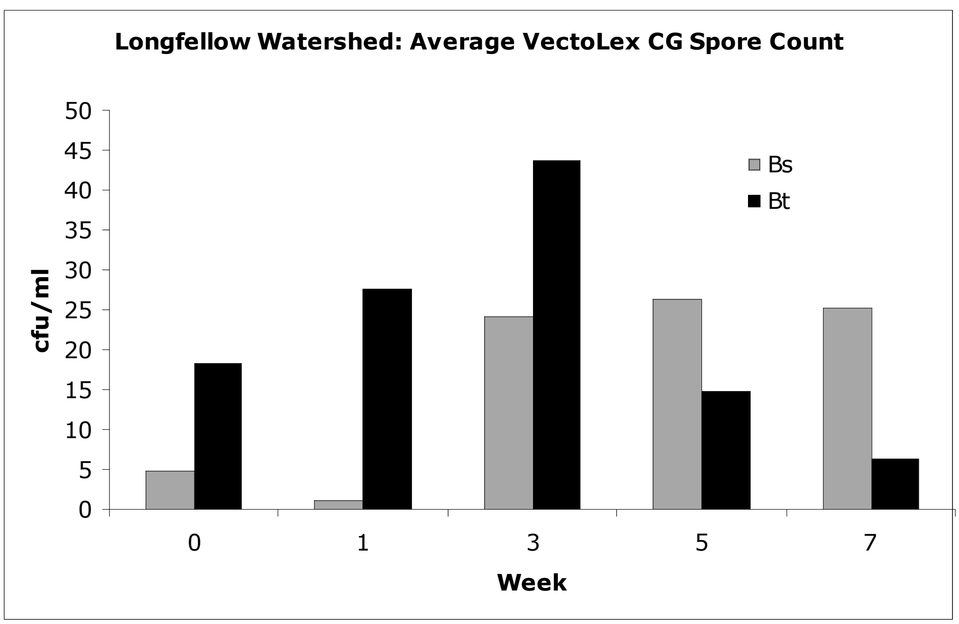

Figure 18d. Average counts (colony forming units/ml) of *B. sphaericus* (Bt) and *B. thuringiensis* (Bs) within catch basins (N=5) within the Longfellow Creek watershed before and after treatment with VectoLex® CG (Week 0) in 2008.

weeks post-treatment, although counts of pupae within treated basins had steadily increased beginning 1-2 weeks after the precipitation event. A similar pattern occurred within the Thornton Creek sub-basin, but differences never reached statistical significance and numbers of pupae exceeded those in adjacent round-top basins after 7 weeks post-treatment.

Altosid® XR (methoprene) was also effective in reducing the emergence of adults within treated basins compared to controls; in most cases fewer than 10 percent of pupae produced adults with maximum efficacy within 2-3 weeks post treatment. Results varied among the three regions monitored (Magnolia, Longfellow and Thornton Creek sub-basins), with the greatest efficacy observed in Magnolia (100% control) and the poorest in the Thornton Creek watershed (≥ 80%).

Grated basins contained ca. 3-times more pupae than round-top basins (Figs. 6, 9-12). Therefore, assuming similar species composition and sex ratios in emerging adults, the contribution of grated basins in terms of potential adult WNV carriers is likely greater than that of round top basins on a per basin basis.

The VectoLex® CG product contained significantly more Bt (100x) than Bs. The Bt detected was Bti. Reasons for this are not known, but it raises questions as to the source of the efficacy observed (Bs *vs* Bt or both) within round top and grated basins.

In most cases concentrations of Bs and Bt were less than 100 cfu's (colony forming units) per ml and in many cases not different from pre-treatment concentrations. Concentrations post treatment did not follow the expected pattern of maximum concentration 1-week post treatment followed by decreases with time, comparable to that observed in 2006 in round top basins when precipitation was absent. The relatively low concentrations of the bacterial larvicides detected were still associated with significant reductions in the numbers of pupae present in treated basins for at least 4 weeks. Either the bacterial larvicides were extremely effective at low concentrations, or the observed efficacy was due to the effects of low temperatures at the time of treatment coupled with precipitation events, or both.

Methoprene was detected in only 2 of the 45 water samples collected (detection limit = 0.14 ppb). Both detections occurred 1-week post-treatment: 3.1 and 11.4 ppb. Results suggest that methoprene is effective at concentrations less than existing analytical detection limits. Reduced efficacy of Altosid® XR (methoprene) was associated with precipitation events and varied among regions likely due to differences in the magnitude of precipitation and basin stormwater inputs (extent of dilution). Efficacy increased after 1 week without precipitation.

Research Needs

Repeated bacterial analyses of the VectoLex® products (WSP and CG) suggest that Bt (believed to be Bti) is the primary constituent, with Bs being an extremely small percentage (1.5% in 2008). This raises questions as to the benefits of using VectoLex® vs a Bti product. Since Bs is particularly effective in static aquatic environments with high organic content, flushing of basins due to precipitation events resulting in a decrease or absence of organic matter may reduce the effectiveness of Bs. It also raises concerns about strategies to combat resistance to the larvicide(s) as part of a long-term mosquito control strategy, as mosquitoes are exposed to two larvicides instead of one, reducing options for alternative controls, particularly the use of a Bti product. Should SPU envision treating catch basins long term, the utility should evaluate an alternative to VectoLex® CG to prevent the development of resistance.

The efficacy of VectoLex® CG within grated basins within the Thornton Creek watershed differed between 2007 and 2008. In 2007, efficacy was realized for 3 weeks, whereas in 2008, the larvicide was still effective 4 weeks post treatment, and possibly longer. Reasons for this difference are not known. Precipitation events occurred post treatment each year, but in 2007, a significant precipitation event occurred 1 week after treatment, whereas in 2008, the first significant rainfall occurred 2 weeks post treatment. The flushing of the basins in 2007 before the bacteria became established is one hypothesis. We note that a comparable decrease in efficacy was not observed within the Longfellow and Pipers Creek watersheds, suggesting something unique about the grated basins in the Thornton Creek watershed, or differences in magnitude of precipitation or stormwater flow. Another factor may be differences in the administration/effectiveness of the delivery of the larvicide. In 2007, the basins were treated operationally by Eden staff, whereas in 2008, basins were treated individually by WACFWRU staff at the same application rate (10 g product per basin) and using product provided by Eden. Differences associated with the Thornton grated basins were also evident in the effectiveness of methoprene compared to other regions of the city in 2008, with efficacy within the Thornton watershed also lower for this product. Reasons for these differences need to be determined, and could be addressed during a more extensive trial with methoprene within the watershed, as well as new briquette formulation of Bs + Bti, (e.g., Four Star Microbial®, VALENT BioSciences Corp.) should it enter the market in 2009.

In 2006, we evaluated the effects of street sweeping and cleaning of basins on the abundance of mosquito larvae. Mosquito abundance was not statistically lower in cleaned basins *vs* uncleaned controls (Grue et al. 2007). A comparison of the emergence of adults within untreated round top and grated basins in 2008 in comparison to data for untreated round top basins in 2006 indicates that emergence was lower in 2008. One hypothesis is that frequent flushing of basins reduced the amount of organic matter within the basins leading to a reduction in the viability of pupae due to a shortage of nutrients. If so, the same effect may be realized through the cleaning of basins. Unfortunately, in 2006, we did not assess the abundance and viability (percent emergence) of pupae. The

City may want to support a subsequent study of the effects of basin cleaning on these endpoints.

And finally, a citywide assessment of the production of adult mosquitoes that are known or potential WNV carriers within catch basins would provide a better understanding of species composition and sex ratios of emerging adults and establish a baseline for comparing the contribution of other sources (e.g., gutters, storm water detention ponds) as well as between basin types (round top *vs* grated).

Presence of VectoLex® CG within Outflows to Thornton Creek

Synopsis — Concentrations of Bs and Bt within baseflows entering Thornton Creek before treatment of catch basins within the watershed with VectoLex® CG were very low. Concentrations during precipitation events were also low and more comparable to those detected within the treated catch basins within the watershed. In comparison, concentrations of Bt within outflows pre- and post treatment in 2007 were much greater. Whereas data for 2007 suggested use of Bt (Bti) within two watersheds before the treatment of catch basins by SPU, this was not evident in 2008. Concentrations detected were orders of magnitude less than those shown to be non-toxic to juvenile coho salmon.

Objective

Our objective was to determine the extent to which the larvicide VectoLex® CG entered surface waters within the Thornton Creek sub-basin following application to catch basins in the City of Seattle in 2008. Specific tasks were:

- Determine the extent to which the larvicide entered surface waters within Thornton Creek.
- Compare concentrations of the larvicide within the creek during base flows and precipitation events before and after application.
- Compare concentrations of the larvicide within the creeks with those known to be toxic to aquatic invertebrates and salmonids

Methods

Selection and Monitoring of Outflows — Water samples (250 ml grab samples) were collected from the same five outflows (drain pipes) to Thornton Creek monitored in 2007 (see Grue et al. 2008). To characterize the concentrations of the VectoLex® CG in outflows before treatment, we collected one water sample from each outflow during a precipitation event and one outside of precipitation events (baseline). Water flows (height in outflow pipe) were measured in each outflow pipe at each visit to establish a baseline and facilitate the identification of "precipitation events". Precipitation events were defined using the same criteria applied in 2007 (Grue et al. 2008): an increase in height of the water in the outflow pipes above baseline and rainfall ≥ 0.03 inches in the vicinity of Thornton Creek. Following treatment of the basins within the watershed by Eden or WACFWRU, a baseline water sample was collected from each outflow pipe at random times during the 1st, 3rd, 5th, and 7th weeks post-treatment for bacterial analysis (Bs and Bt, colony-forming units/ml, Institute of Environmental Health [IEH], Lake City, WA). [Quantification was requested for both Bs and Bt because analyses of the product (VectoLex® CG) used in 2007 and 2008 (Table 7) indicated the product contained both Bs and Bt.] Water samples were also collected from each outflow pipe during three distinct precipitation events post-treatment. Samples were placed on ice in the field and maintained on ice until delivery to IEH in most cases, the day of collection, but in <15 h, given that some samples were collected between 1700 and 0800 the next day.

Data Analyses — Concentrations of Bs and Bt (colony forming units/ml) within the water samples from the outflow pipes were compared graphically through time. Unfortunately, the contract laboratory lost the water samples collected during the third precipitation event post-treatment. Bacterial composition of VectoLex® CG is presented in Table 7 (page 40).

Results

Concentrations of Bs and Bt within baseflows entering Thornton Creek before treatment of catch basins within the watershed with VectoLex® CG were very low with values less than 9 colony forming units (Fig. 19). Concentrations during precipitation events were low and more comparable to those detected within the treated catch basins within the watershed. Maximum values post treatment were < 35 cfu's/ml with concentrations of Bt much greater than those of Bs (Fig. 20). Concentrations of Bt post treatment were only about 2x the pre-treatment value (Fig. 20). In comparison, concentrations of Bt within outflows pre- and post treatment in 2007 were much greater (maximum = 8,000 cfu's/ml, Figs. 21-22). The timing of precipitation events relative to the application of the larvicide likely explains the difference in concentrations in the outflows between years. In 2007, 1.4 inches of rain fell shortly within 1 week of application. In 2008, significant rainfall did not occur until 2 weeks after treatment with ca 0.7 inches falling over the subsequent 2 weeks. Whereas data for 2007 suggested use of Bt (Bti) within the watershed before the treatment of catch basins by SPU, this was not evident in 2008.

Unfortunately, non-target toxicity data in which concentrations of the larvicides are expressed as colony forming units per ml are lacking, making a direct comparison to the concentrations we detected within the outflows and creeks difficult. In an effort to address this data gap relative to juvenile salmonids, we conducted toxicity tests with several larvicides, singly and in combination, and juvenile coho salmon (*Oncorhynchus kisutch*; ca. 18 g) in 2007 (Grue et al. 2008; M. Sternberg, unpublished data). Results suggest that the concentrations we detected in each of the creeks pose little direct hazard to juvenile salmonids. In all cases, they were orders of magnitude less than those to which we exposed juvenile coho for 96 h without the occurrence of overt effects (Grue et al. 2008: Tables 11 and 12, p. 40).

Conclusions

Concentrations of Bs and Bt within baseflows entering Thornton Creek before treatment of catch basins within the watershed with VectoLex® CG were very low. Concentrations during precipitation events were also low and more comparable to those detected within

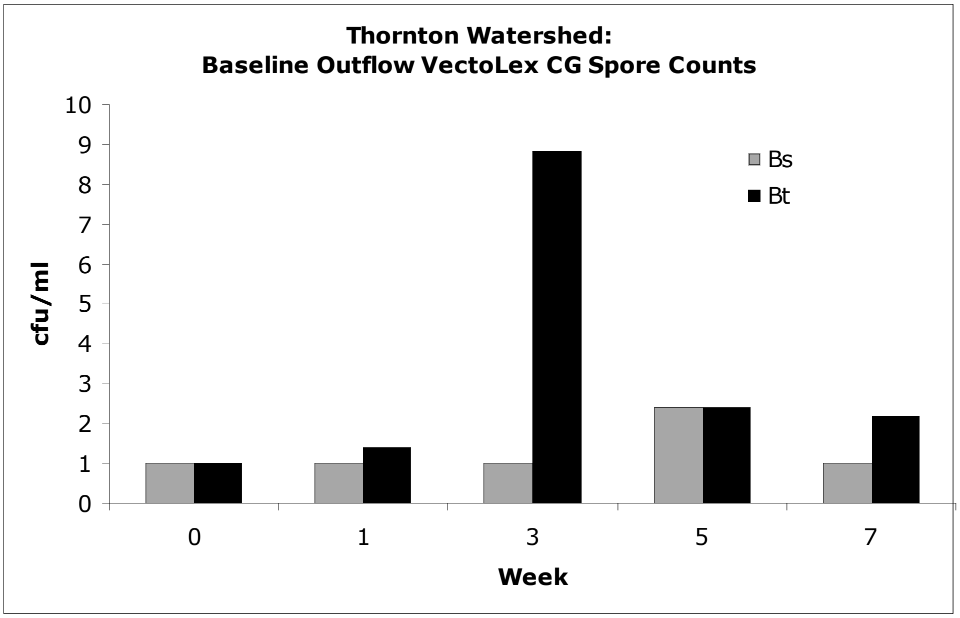

Figure 19. Counts (colony-forming units/ml) of *B. sphaericus* (Bs) and *B. thuringiensis* (Bt) in outflows (N=5) to Thornton Creek before and after treatment of catch basins with VectoLex® CG (Week 0) in 2008. Samples were taken from base flows at randomly selected times within each week post-treatment.

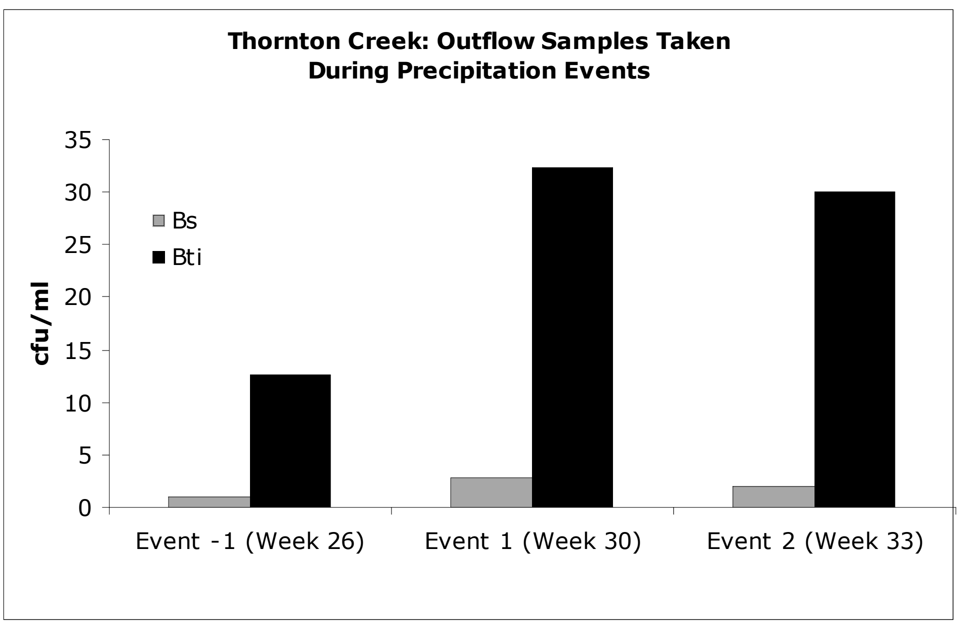

Figure 20. Average Bs and Bt counts (colony-forming units/ml) within outflows (N=5) to Thornton Creek collected during precipitation events before and after treatment of catch basins with VectoLex® CG (Week 28) in 2008.

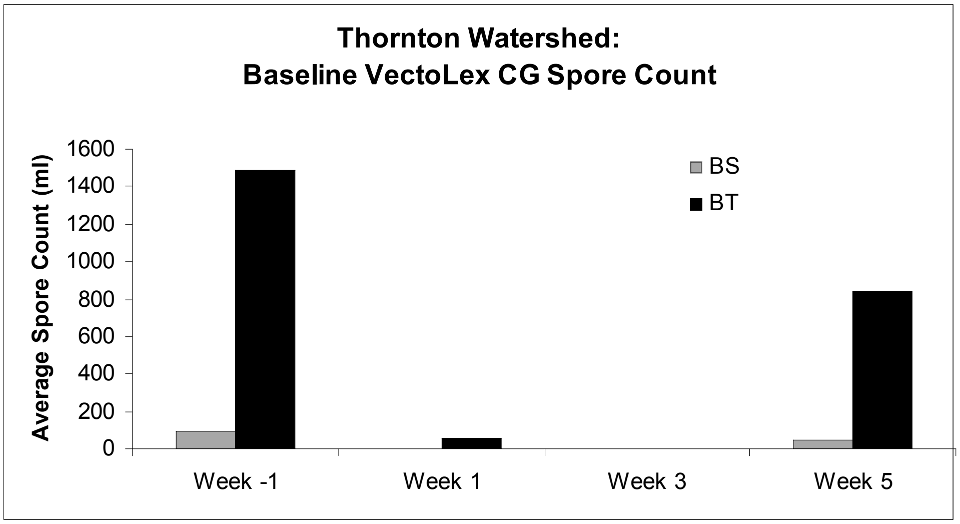

Figure 21. Counts (colony-forming units/ml) of *B. sphaericus* (Bs) and *B. thuringiensis* (Bt) within outflow pipes (N=5) to Thornton Creek measured during base flows before and after treatment of basins within the watershed with VectoLex® CG (Week 0) in 2007.

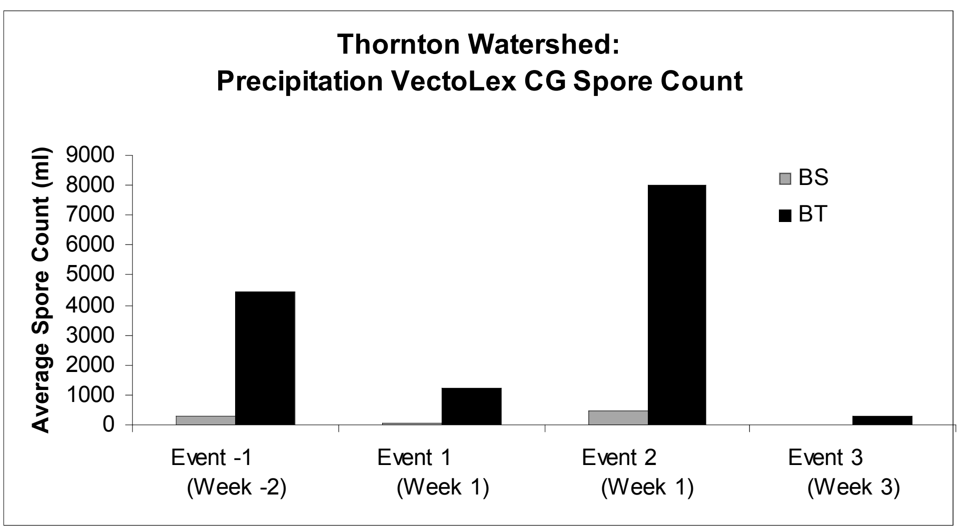

Figure 22. Counts (colony-forming units/ml) of *B. sphaericus* (Bs) and *B. thuringiensis* (Bt) within outflow pipes (N=5) to Thornton Creek measured during precipitation events before and after treatment of basins within the watershed with VectoLex® CG (Week 0) in 2007.

the treated catch basins within the watershed. In comparison, concentrations of Bt within outflows pre- and post treatment in 2007 were much greater. Whereas data for 2007 suggested use of Bt (Bti) within the watershed before the treatment of catch basins by SPU, this was not evident in 2008. Concentrations detected were orders of magnitude less than those shown to be non-toxic to juvenile coho salmon.

Research Needs

Whereas concentrations of Bs and Bti expressed as colony-forming units per ml within outflows to the three urban creeks previously studied do not appear to present a direct toxic hazard to juvenile salmonids, comparable data for non-target invertebrates that may be sensitive to the larvicides are lacking. Available toxicity data have been expressed as units of product per unit of volume (e.g., mg/L) or as an application rate based on nominal values, and not actual concentrations expressed as measures of bacteria per unit volume (e.g., colony-forming units per ml). Toxicity tests using measures that can be applied to natural waters and taxa believed to be vulnerable (e.g., dipterans and chironomids) need to be conducted.

References

Bost, H.L. 2004. A comparison of West Nile virus vector mosquito populations in sites with and without storm water drainage ponds. MS thesis, University of Washington, Seattle, WA. 74 pp.

Grue, C.E., M.R. Sternberg, J.M. Grassley, K.A. King and L.L. Conquest. 2007. 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 submitted to Seattle Public Utilities by the Washington Cooperative Fish and Wildlife Research Unit, School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA. 64 pp.

Grue, C.E., M.R. Sternberg, J.M. Grassley, K.A. King and L.L. Conquest. 2008. Catch Basins as Sources of Mosquitoes and West Nile Virus: An Evaluation of Control Strategies and Potential for Non-target Effects — Final Report submitted to Seattle Public Utilities by the Washington Cooperative Fish and Wildlife Research Unit, School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA. 62 pp.

Appendix 1. Frequency distributions of the numbers of mosquito pupae within catch basins treated with VectoLex® CG during Treatment Weeks 2 and 3 and control (untreated) basins within the City of Seattle in 2008.

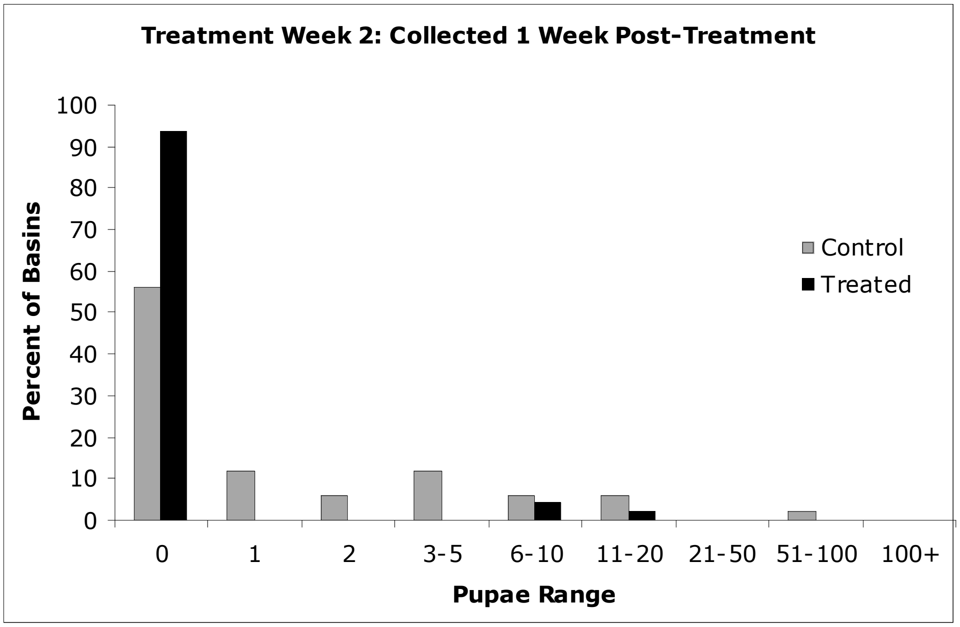

Figure A1-1. Percentage of basins with specified ranges of mosquito pupae for round-top basins selected during the second week of treatment. Data collected 1 week post-treatment (Week 29).

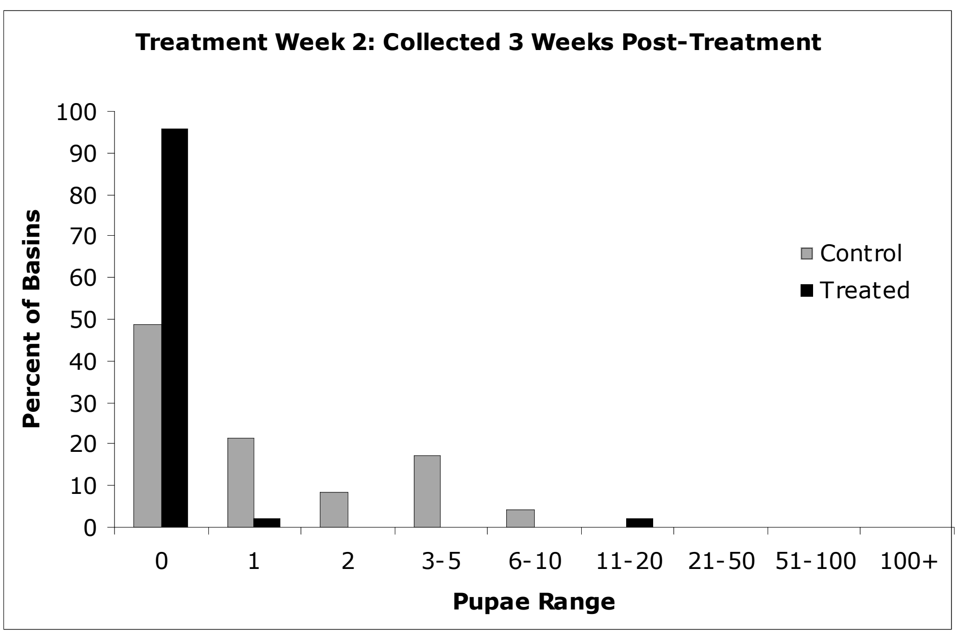

Figure A1-2. Percentage of basins with specified ranges of mosquito pupae for round-top basins selected during the second week of treatment. Data collected 3 weeks post-treatment (Week 31).

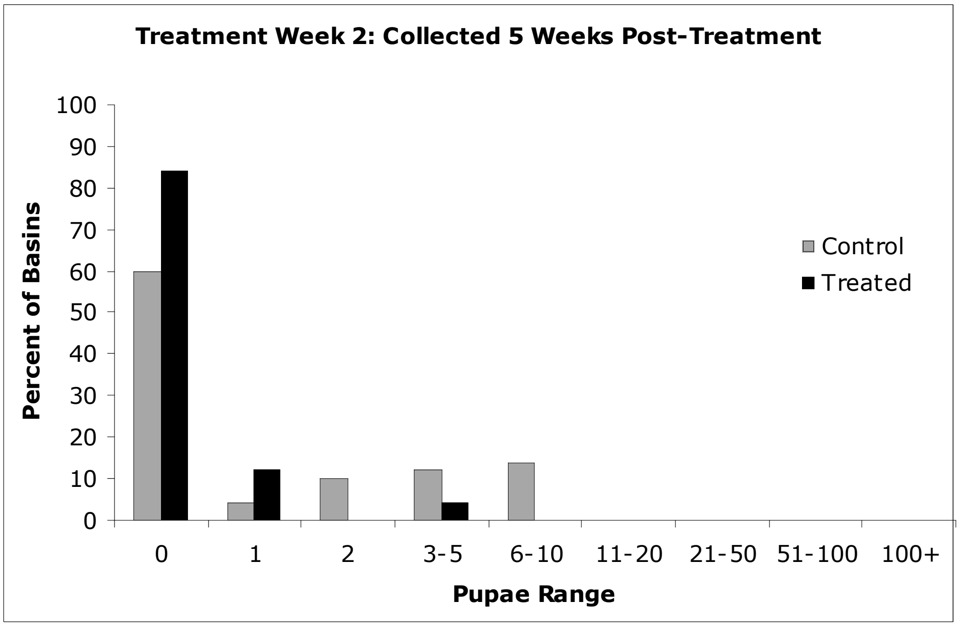

Figure A1-3. Percentage of basins with specified ranges of mosquito pupae for round-top basins selected during the second week of treatment. Data collected 5 weeks post-treatment (Week 33).

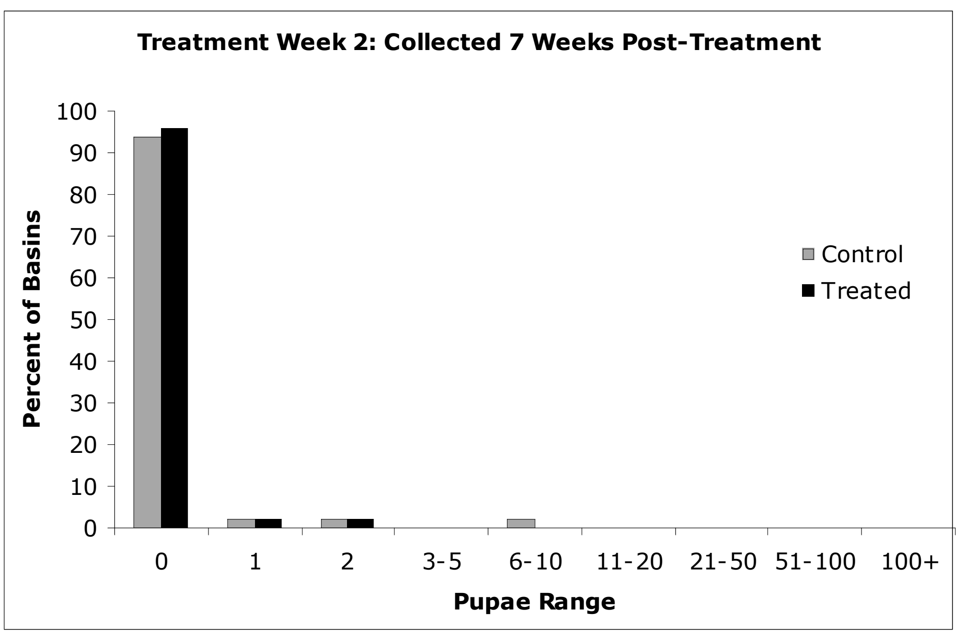

Figure A1-4. Percentage of basins with specified ranges of mosquito pupae for round-top basins selected during the second week of treatment. Data collected 7 weeks post-treatment (Week 35).

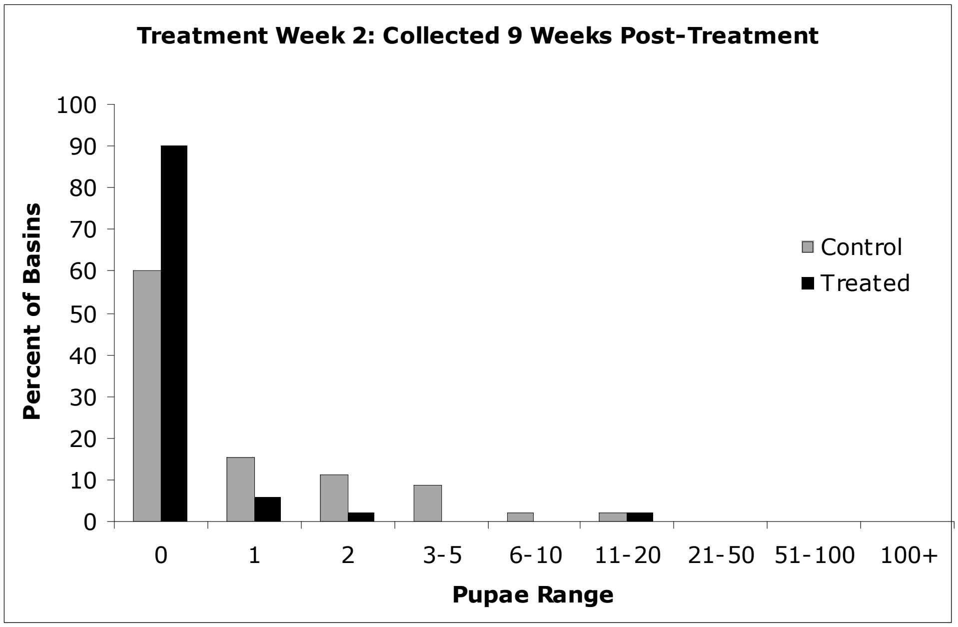

Figure A1-5. Percentage of basins with specified ranges of mosquito pupae for round-top basins selected during the second week of treatment. Data collected 9 weeks post-treatment (Week 37).

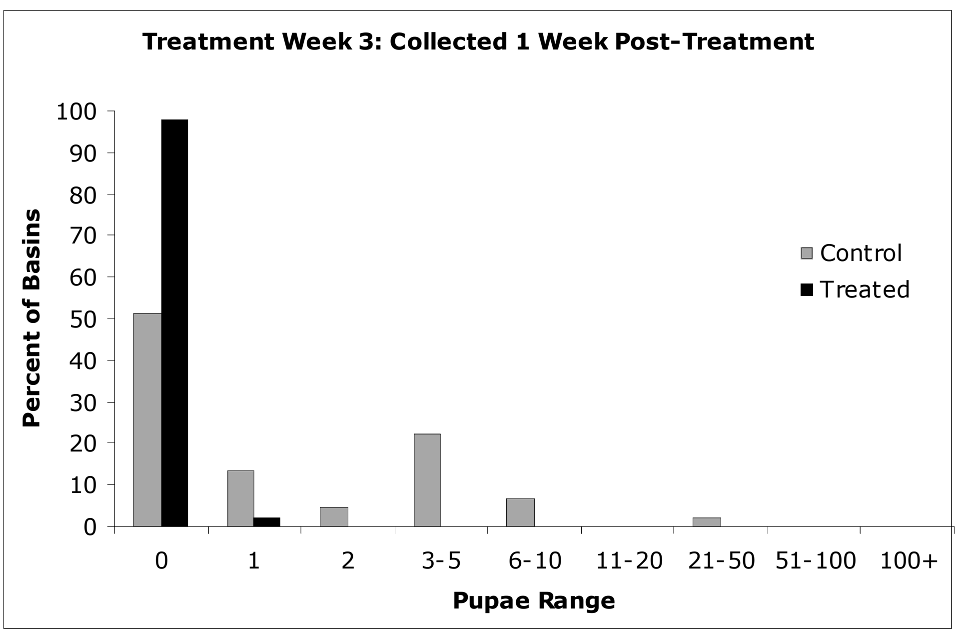

Figure A1-6. Percentage of basins with specified ranges of mosquito pupae for round-top basins selected during the third week of treatment. Data collected 1 week post-treatment (Week 30).

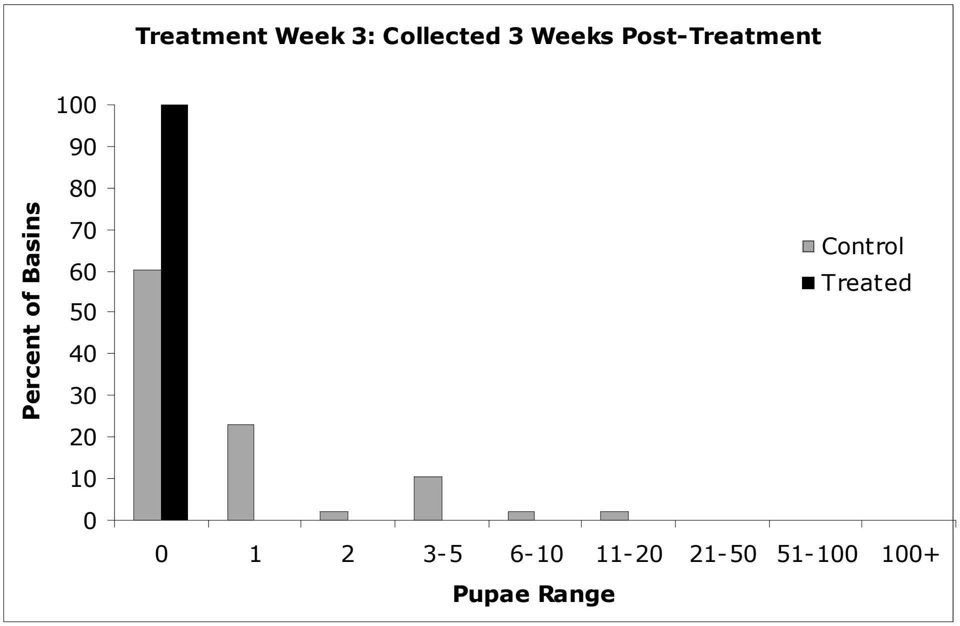

Figure A1-7. Percentage of basins with specified ranges of mosquito pupae for round-top basins selected during the third week of treatment. Data collected 3 weeks post-treatment (Week 32).

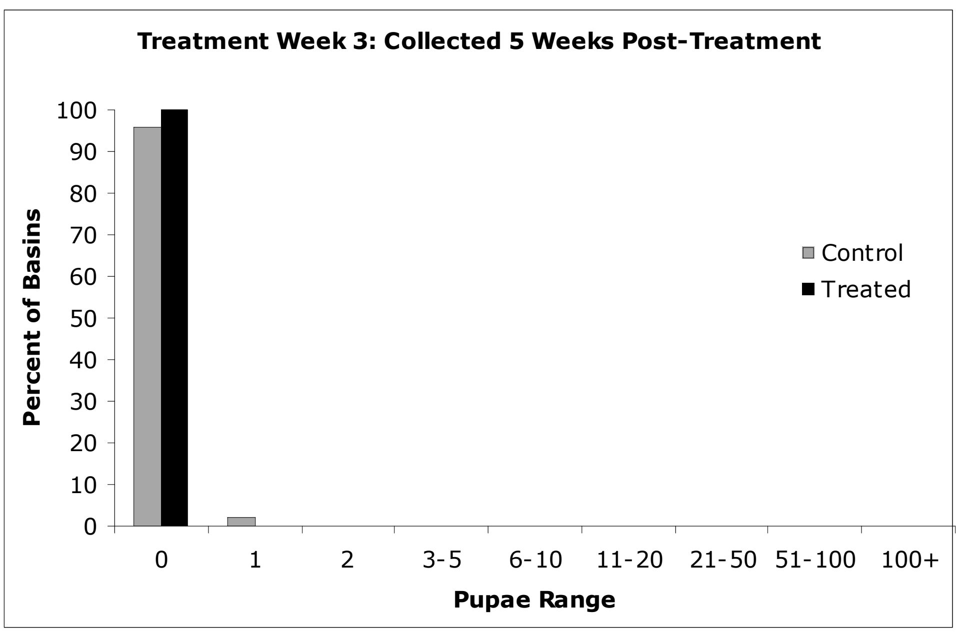

Figure A1-8. Percentage of basins with specified ranges of mosquito pupae for round-top basins selected during the third week of treatment. Data collected 5 weeks post-treatment (Week 34).

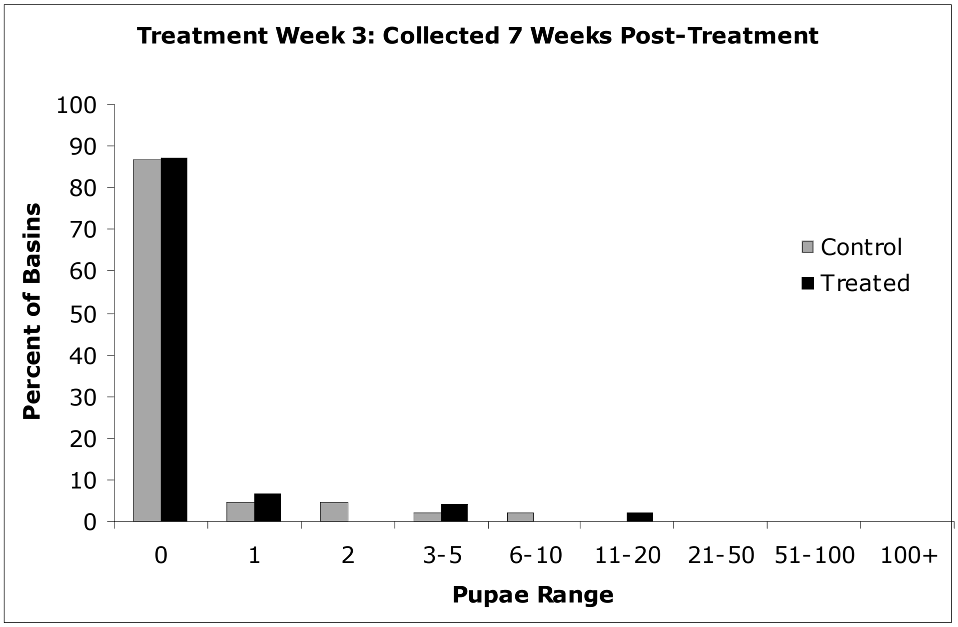

Figure A1-9. Percentage of basins with specified ranges of mosquito pupae for round-top basins selected during the third week of treatment. Data collected 7 weeks post-treatment (Week 36).

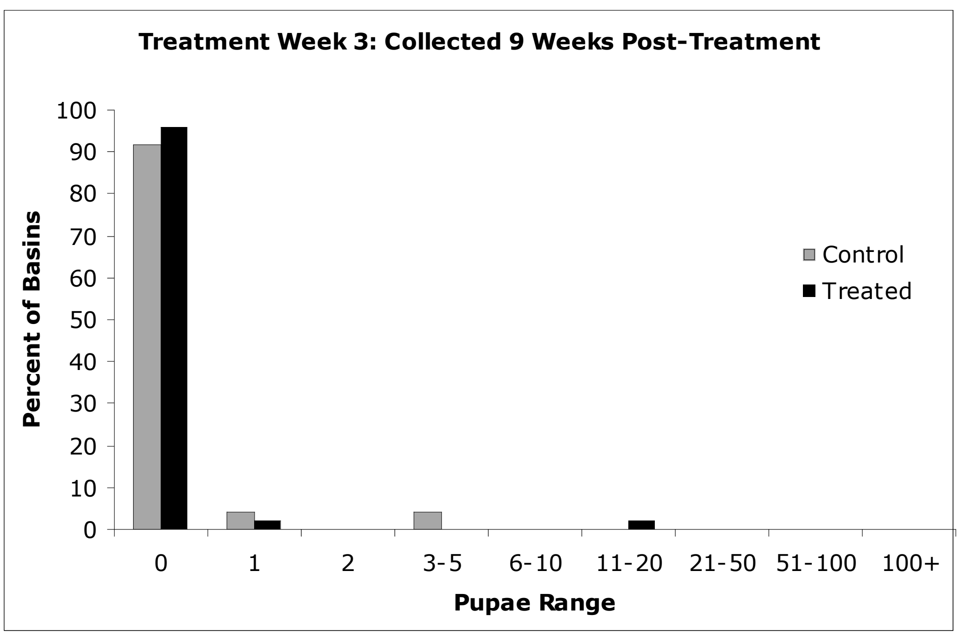

Figure A1-10. Percentage of basins with specified ranges of mosquito pupae for round-top basins selected during the third week of treatment. Data collected 9 weeks post-treatment (Week 38).

Appendix 2. Locations of catch basins within the three regions of the City in which the efficacy and fate of VectoLex® CG and Altosid® XR were monitored in 2008.

Basin Number*	Cross Street	Corner Direction	Basin Type
01B	NE 88th and 35th NE	Southwest	Grated
02B	Mid-block between 42nd NE and dead-end on NE 89th St	South side	Grated
03C	NE 93rd and 35th NE	Southeast	Grated
04M	NE 98th and 35th NE	Southwest	Grated
05B	NE 115th and 35th NE	Northwest	Grated
06C	NE 117th and 35th NE	Southeast	Grated
07C	NE 108th Pl and 27th NE	East side (only one at corner)	Grated
08C	NE Northgate and 20th NE	Northwest	Grated
09M	Roosevelt NE and 8th NE	Southeast	Grated
10B	NE 105th and Meridian N	Southwest	Grated
11M	Mid-block between NE 113th and NE 110th on 35th NE	Northwest	Grated
13M	NE 103rd and 35th NE	Southwest	Grated
14M	NE 100th and 36th NE	Northeast	Grated
15B	NE 92nd and 35th NE	Northeast	Grated
16C	Mid-block between W Dravus and W Bertona on 26th W	West	Grated
17B	Mid-block between 25th W and 24th W on W Ruffner	South	Grated
18M	W Emerson and 31st W	Southwest	Grated
19M	W Raye and Perkins Ln W	East	Grated
20C	W Raye and Viewmont W	West	Grated
21C	Mid-block between Montevista W and W Howe on Magnolia Blvd	South	Grated
22B	W Raye and 27th W	Southwest	Grated
23B	W Barton Pl and 25th W	Southwest (on gravel)	Grated
24C	Dead-end between W Dravus and W	North	Grated

	Bertona on 40th W		
25M	W Manor Pl and Gilman W	East	Grated
26C	W Commodore and 26th W	Southwest	Grated
27M	W Commodore and W Harley	North (only basin next to park)	Grated
28M	W Government and 29th W	North (only basin on that side)	Grated
29B	Mid-block between W Elmore and W Thurman on 30th W	West	Grated
30B	W Elmore and 31st W	North (at dead-end)	Grated
31C	SW Findlay and 31st SW	Northeast	Grated
32B	SW Willow and 23rd SW	Northwest	
33C	SW Elmgrove and 28th SW	Southwest	Grated
34M	SW Kenyon and 27th SW	Southeast	Grated
35C	SW Trenton and 29th SW	Northwest	Grated
36B	SW Trenton and 28th SW	Southeast	Grated
37B	Mid-block between SW Henderson and SW Barton on 25th SW	East	Grated
38M	SW Barton and 29th SW	Northwest	Grated
39C	Mid-block between SW Henderson and SW Barton on 30th SW	West	Grated
40B	SW Cambridge and 30th SW	Northwest	Grated
41C	SW Webster and 12th	Southwest	Grated
42M	SW Webster and 14th SW	North	Grated
43M	SW Myrtle and 17th SW	Southeast	Grated
44M	SW Thistle and 29th SW	Southeast	Grated
45B	SW Othello and 16th SW	Northeast	Grated

* B = Vectolex® CG, C = Control (untreated), M = Altosid® XR

Appendix 3. Water temperatures in grated basins within the three regions of the City in which the efficacy and fate of VectoLex® CG were monitored in 2008.

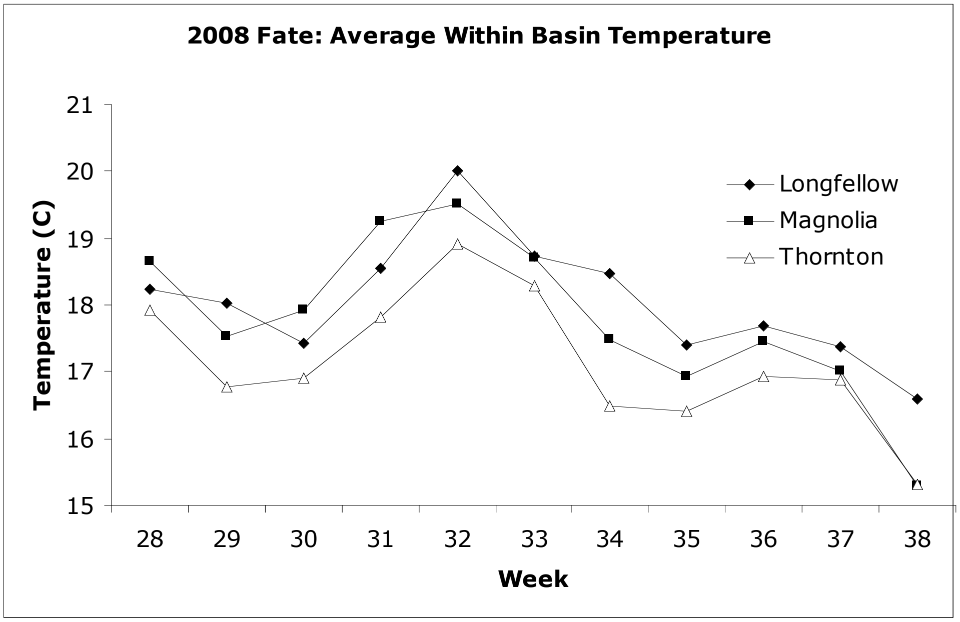

Figure A3-1. Average basin water temperature (C) for grated basins treated with VectoLex® CG in the Longfellow Creek watershed, Magnolia township, and the Thornton Creek watershed. N=5 per area.

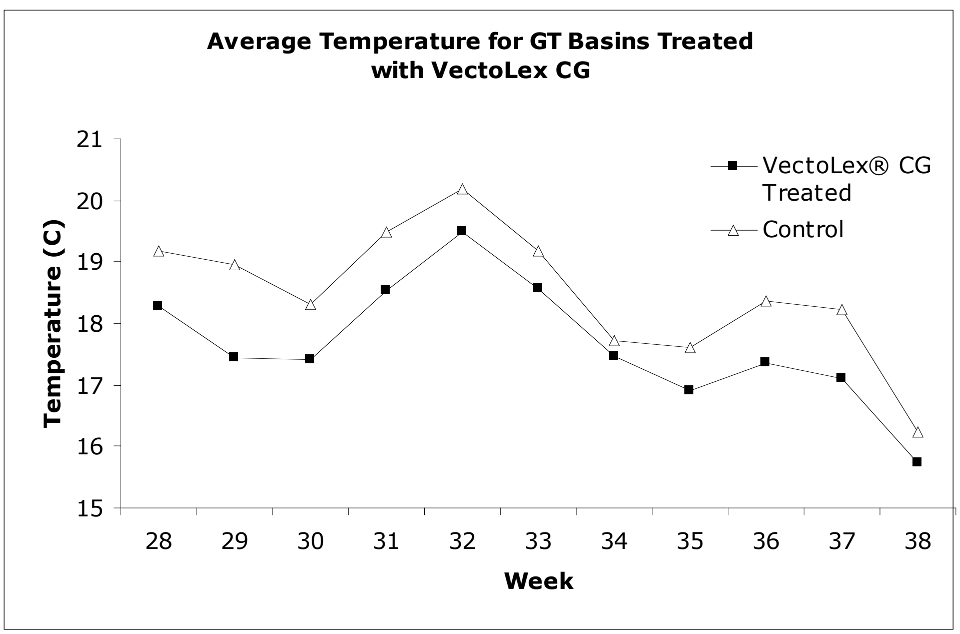

Figure A3-2. Average within-basin temperature for grated-top (GT) basins in the Thornton sub-basin treated with VectoLex® CG and untreated controls. N=5 per group.