Catch Basins as Sources of Mosquitoes and West Nile Virus: An Evaluation of Control Strategies and Potential for Non-target Effects

Final Report

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Catch Basins as Sources of Mosquitoes and West Nile Virus: An Evaluation of Control Strategies and Potential for Non-target Effects

Final Report

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Executive Summary

In anticipation of the spread of West Nile Virus (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 effort was to develop strategies to monitor and control mosquito breeding in SPU catch basins that would be 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 of three common larvicides. 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.

Based on these incident reports and the results of the 2006 research effort, Seattle Public Utilities (SPU) contracted with Eden Advanced Pest Technologies to treat all of the City's catch basins once with VectoLex® CG (*Bacillus sphaericus* [BS] and *Bacillus thuringiensis* [BT], 10 grams of product 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 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 larvae in the treated catch basins, and (3) determine the potential for the larvicide to enter surface waters and adversely affect non-target species especially within creeks within the City (Longfellow, Pipers, and Thornton).

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Highlights — The following are the key findings and conclusions of the research described in this report:

- Contractor compliance was greater than or equal to 98 percent.
- Treatment of the catch basins with VectoLex® CG citywide significantly reduced the number of mosquito pupae compared to untreated (control) basins.
- Efficacy was greater in round top *vs* grated basins: 5-7 weeks post treatment within round top basins, but less than 3 weeks within grated basins.
- Differences between treated and control basins were minimized by frequent precipitation events making determinations of efficacy difficult.
- Concentrations of the larvicide detected in the water samples collected from Longfellow, Pipers, and Thornton creeks during base flows, before and after treatment of the catch basins, were at background levels.
- Elevated concentrations of the larvicide were detected in the creeks during precipitation events prior to the start of the City's control program and after treatment; concentrations were low relative to the maximum concentrations detected in the basins.
- Results suggest that bacterial larvicides also entered the creeks from sources other than the City's control program.
- Recent toxicity tests with several larvicides, singly and in combination, suggest that the concentrations detected in each of the creeks pose little direct hazard to juvenile salmonids.
- Additional studies are needed to further examine the effects of precipitation on efficacy, determine the reasons for the reduced efficacy in grated basins, test an alternative control strategy for grated basins, and develop additional data on non-target toxicity using a common metric for quantifying exposure.

Additional details are provided in the Executive Summary below and within the report as a whole.

Larval Abundance, Compliance, and Efficacy of Citywide Larvicide Treatments — Fifty basins from those treated by the contractor within each week of treatment were monitored (150 total) to determine compliance and efficacy of the larvicide treatment. Only "round top" basins, were selected. Prior to treatment, an additional 50 dedicated control basins were randomly selected from those monitored in 2006. In order to determine the efficacy of the larvicide treatments within "grated" catch basins (grated basins represent a small proportion of the overall population of basins), we selected a subset of 10 untreated grated catch basins (controls) and 10 grated basins that were treated during the second week of treatment. All treated basins were monitored weekly beginning 1-week post-treatment through the end of September. Control basins were monitored beginning 5 weeks before the onset of 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

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was recorded as were water and sediment depth and water quality. Additionally, in the control round top and grated basins, we recorded the number of mosquito larvae in each of the three dips so that we could compare counts in 2007 with those in the same basins in 2006.

Treatment of the catch basins within the City of Seattle with VectoLex® CG reduced the number of mosquito pupae below counts within untreated (control) basins. Contractor compliance, as measured by the percentage of the treated round top basins monitored in which pupae counts after the second week post treatment were less than controls, was 98 percent; 100 percent by the fourth week post treatment. Efficacy was greater in round top *vs* grated basins. Efficacy was realized for at least 5 weeks, and in most cases 7 weeks post treatment within round top basins and for less than 3 weeks within grated basins. Differences between treated and control basins were likely minimized by frequent precipitation events that reduced the number of pupae (and larvae) in the catch basins within each treatment group, particularly the controls. In all comparisons, pupae were not detected in the three standard dips within at least 40 percent (in most cases $\geq$60%) of the control round top basins.

Efficacy and Fate of Larvicides within Catch Basins in the Longfellow, Thornton, and Pipers Creek Watersheds — To determine the fate of the larvicide and the potential for inputs into surface waters, 30 basins were randomly selected within the watersheds (sub-basins) associated with the outflows to each of the three urban creeks: Longfellow, Pipers and Thornton. The distribution of the basins per creek sub-basin was proportional to the number and type of catch basins within each sub-basin. During the first week of treatment, the contractor treated the catch basins within the sub-basins selected for study with VectoLex® CG. Efficacy was determined following treatment by counting the number of pupae per three dips weekly for 13 weeks beginning 2 weeks pre-treatment, and resuming 1-week post treatment. Fate of the larvicide was quantified by determining the concentration of the larvicide (spores/ml) in each basin 1 week before treatment, and 1, 3, 5 and 7 weeks after treatment, or until the treatment was no longer efficacious. Treatments were considered efficaceous if the counts of pupae per three standard dips within individual basins or the mean for the sub-basin were less than the mean of the 50 control round top basins monitored in the overall city evaluation. Water and sediment depths and water quality were also measured weekly. Outflows (drain pipes) carrying water from the City's catch basins were identified within each of the three creeks and a total of 10 outflows selected with the number of basins selected proportional to the size (length) of the creek systems. Water samples were collected during base flows and during precipitation before and after treatment. To characterize the concentrations of BS and BTI before treatment, we collected one sample from each outflow during a precipitation event prior to treatment, and one outside of precipitation events (baseline) when water was flowing from the outflow. One water sample within each creek was also collected pre-treatment at a site determined by SPU during base flows and one during a precipitation event. Following treatment of the basins within the watersheds each outflow was visited once during Weeks 1, 3, 5 and 7 post treatment (days and time randomly selected) outside of precipitation events to collect water samples within the outflows. One water sample within each creek was also collected during each of these visits. Because of a lack of efficacy in catch basins within the Thornton Creek watershed, water samples from the outflows or creek were not collected after Week 5. Water samples from each of the outflows and creek were also collected during three precipitation events post treatment.

With the exception of catch basins within the Thornton Creek watershed, treatment of the basins with VectoLex® CG reduced the number of pupae detected to zero. The lack of efficacy in the Thornton Creek watershed appeared to be associated with grated basins. Efficacy in the grated basins monitored in the citywide assessment was also short-lived in comparison to round tops. BT was present in concentrations above background in the basins within the Longfellow and Pipers Creek watersheds after treatment, but not before. In comparison, BT was detected in catch basins within the Thornton Creek watershed before and after treatment, but at much lower concentrations. Little BS was detected in any of the samples.

Neither BS nor BT was detected above background concentrations in the majority of the water samples collected from the three creeks during base flows before or after treatment of the catch basins within the watersheds. Elevated concentrations of both larvicides were detected in the creeks during precipitation events prior to the start of the City's control program, however concentrations were low. During precipitation events following treatment, concentrations of the larvicides were frequently greater than pre-treatment levels.

Data on non-target toxicity in which concentrations of the larvicides expressed as spores per ml are lacking, making a direct comparison of the results of existing studies of non-target effects to the concentrations we detected within the outflows and creeks difficult. However, results of recent toxicity tests with several larvicides, singly and in combination, and juvenile coho suggest that the concentrations detected in each of the creeks (spores/ml) pose little hazard to juvenile salmonids.

Research Needs — Efficacy in the round top basins differed from that in grated catch basins. A similar difference was observed in the grated basins within the Thornton Creek watershed. Although reasons for the reduced efficacy are not known, the differences observed are likely due to differences in the effects of precipitation on the levels and/or persistence of the larvicide within the basins. The City may want to test the efficacy of the extended release formulation of Aquaprene® XL (active ingredient = methoprene) as an alternative to bacterial larvicides as the briquettes are less likely to be flushed from the basins, efficacy is regained rapidly after flushing, and recent toxicity tests with the product and juvenile coho salmon indicate little potential for hazard to salmonids within surface waters that may receive product from treated catch basins following precipitation events.

Frequent precipitation events likely contributed to the observed efficacy of BS (VectoLex® CG) in the round top basins citywide. BS concentrations in the basins

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treated in 2007 were lower than those reported in 2006 (basins treated with VectoLex® WSP) when precipitation was not a factor. Water temperatures were lower in 2007 and there appeared to be less organic matter as a result of frequent flushing; conditions that may not be the most favorable for BS. The effects of precipitation (water temperature and organic matter) on the efficacy of VectoLex® CG need to be investigated further.

The elevated concentrations of BT within catch basins and outflows before the start of the City's treatment program suggests that there are other sources of BT within the watersheds monitored in 2007. The City may wish to consider having some of the samples further analyzed to identify the specific strain of BT within the samples, although it is likely BTI, as the larvicide is available to the general public through retail stores. Additionally, it would be helpful to know the extent to which these "other" inputs are occurring within the basins, outflows and creeks, in order to more effectively address questions of non-target toxicity.

Toxicity tests with the most frequently used larvicides and juvenile salomonids suggest little potential for direct toxicity. However, comparable data for younger salmonids and sensitive invertebrates using a common metric for quantifying exposure of bacterial larvicides are lacking. Should methoprene be considered as an alternative for controlling mosquito larvae in grated basins, comparisons of methoprene concentrations in outflows to those associated with toxicity to sensitive invertebrate taxa would also be warranted. These data would enhance our ability to evaluate the potential for non-target effects associated with inputs of these larvicides to surface waters following treatment of catch basins.

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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, 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 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, Seattle Public Utilities (SPU) contracted with Eden Advanced Pest Technologies 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 months beginning the second week of July. 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 larvae 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).

Larval Abundance, Compliance, and Efficacy of Larvicide Treatments

Synopsis — The mosquito-breeding season in 2007 was similar to that in 2006 with numbers increasing in the third week of June and sharply declining in the middle of September. Unlike 2006, rain was a frequent occurrence in 2007 and was associated with dramatic declines in larval counts. Early in the 2007 season, larval counts outside of the precipitation events were greater than those in 2006, likely due to differences in ambient temperatures relative to precipitation events.

Treatment of 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 ≥98 percent. Efficacy was greater in round top *vs* grated basins. Efficacy was realized for at least 5 weeks, and in most cases 7 weeks post treatment within round top basins, but <3 weeks within grated basins. Differences between treated and control basins were potentially minimized by precipitation events that reduced the number of larvae and pupae in the catch basins within each treatment group, particularly 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 larvae within the treated catch basins. Specific tasks were:

- 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 larvae within the catch basins treated.
- Determine the effects of precipitation on the efficacy of the larvicide treatments.
- Compare the efficacy of the larvicide with that observed in efficacy trials in 2006.

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 week of treatment (Week 2), and the third week of treatment (Week 3) for a total of 150 basins during the specified treatment window (4 weeks: 9 July – 3 Aug). Due to precipitation, the planned second week of treatment was delayed a week such that the

WACFWRU

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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 receive stormwater directly from the road surface.

actual weeks sampled within the treatment window were Weeks 1, 3, and 4. (For the purpose of this report, we refer to these weeks as Weeks 1-3). Prior to treatment, an additional 50 dedicated control basins were randomly selected (with stratification by sector and zone) from the 250 untreated basins monitored in 2006 using the same selection criteria. 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.

In order to determine the efficacy of the larvicide treatments within "grated" catch basins (Fig. 1) that represent a small proportion of the overall population of basins, we selected a subset of 10 untreated grated catch basins (controls) and 10 grated basins that were treated during the second week of treatment (Week 2). Basins were selected from those that were proximate to the round tops already selected for study.

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 5 weeks before the onset of larvicide application until the end of September. At each visit to the treated and control basins, the number of mosquito pupae

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per three standard dips was recorded as were water and sediment depth (0.5 inches), temperature (C), pH, conductivity (us/cm), and dissolved oxygen (mg/L). Additionally, in the control round top and grated basins, we recorded the number of mosquito larvae in each of the three dips so that we could compare counts in 2007 with those in the same basins in 2006.

Data Analyses — We compared the number of larvae counted per three dips in the untreated (control) basins through time in 2006 (n=250) and 2007 (n=50) graphically as well as data on ambient air temperature and precipitation in 2007. In addition, we compared larval counts for the 50 untreated (control) basins monitored in both 2006 and 2007. Counts of pupae in treated and control basins were compared graphically through time by treatment week (Weeks 1-3) using 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 2007 in the City of Seattle was generally similar to that in 2006 with numbers increasing in Week 25 (18-24 June) and sharply declining in Week 38 (17-23 September). Whereas precipitation was largely absent from this time period in 2006, rain was a frequent occurrence in 2007 (Table 1, Fig. 2).

Precipitation events were associated with dramatic declines in larval counts, frequently to zero within individual basins, making statistical comparisons between 2006 and 2007 difficult (Fig. 2). A comparison between the same 50 round top basins monitored in 2006 and 2007 suggests that larval counts outside of the precipitation events were greater in

Table 1. Precipitation accumulation (in) for the Pre-monitoring and Monitoring seasons in 2006 and 2007. For both 2006 and 2007, the Pre-monitoring Season includes Weeks 18 through 24 and the Monitoring Season extends from Week 25 through Week 39.

	2006	**2007**
Pre-monitoring Season	3.48	2.42
Monitoring Season	0.23	4.81
Total	3.71	7.23

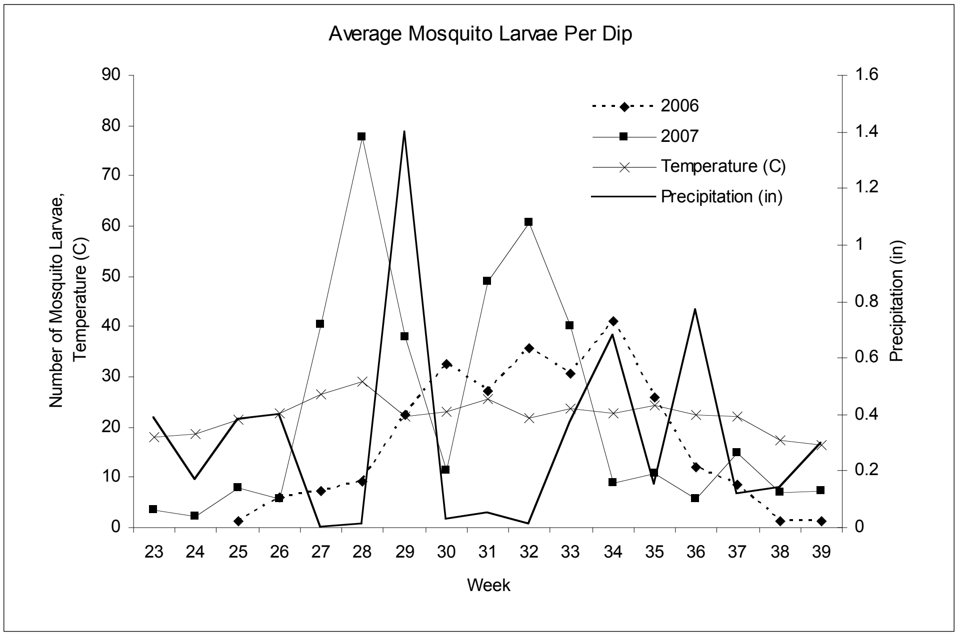

Figure 2. Average number of mosquito larvae per dip for round top basins for 2006 (N = 250) and for 2007 (N = 50). Averages are calculated across sectors and zones. Cumulative precipitation data per week (inches) as well as average high temperature data per week (C) are included.

2007 than those in 2006 early in the season (Fig. 2). This difference was not associated with differences in the sampling fraction or proportion of dry basins between the two years (Table 2, Figs. 3 and 4). Differences in ambient temperatures relative to precipitation events may account for the greater abundance of larvae in 2007 early in the season (Fig. 5). There were no consistent differences between larval counts within control round top and control grated basins in 2007 (Table 3, p. 24).

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 4, p. 24). Compliance was based on data for round top basins only, and by this measure compliance exceeded 98 percent. By 2 weeks post treatment ≤ 2 percent of the treated basins did not meet this criteria, and by 4 weeks after treatment, all of the basins met criteria. We note that this is likely a very conservative estimate of non-compliance as the criteria for non-compliance clearly represent an unacceptable level of efficacy. 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 (8-10 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 Figures 6-8 (pp. 25-26). Statistical

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Table 2. Percentage of dry basins within the same 50 control basins used in 2006 and 2007.

Week	2006 Percentage of Dry Basins	2007 Percentage of Dry Basins
23	N/A	0
24	N/A	0
25	2	2
26	4	6
27	4	0
28	8	4
29	11	0
30	15	0
31	9	6
32	13	6
33	14	8
34	14	0
35	10	2
36	6	0
37	4	2
38	0	0
39	0	4

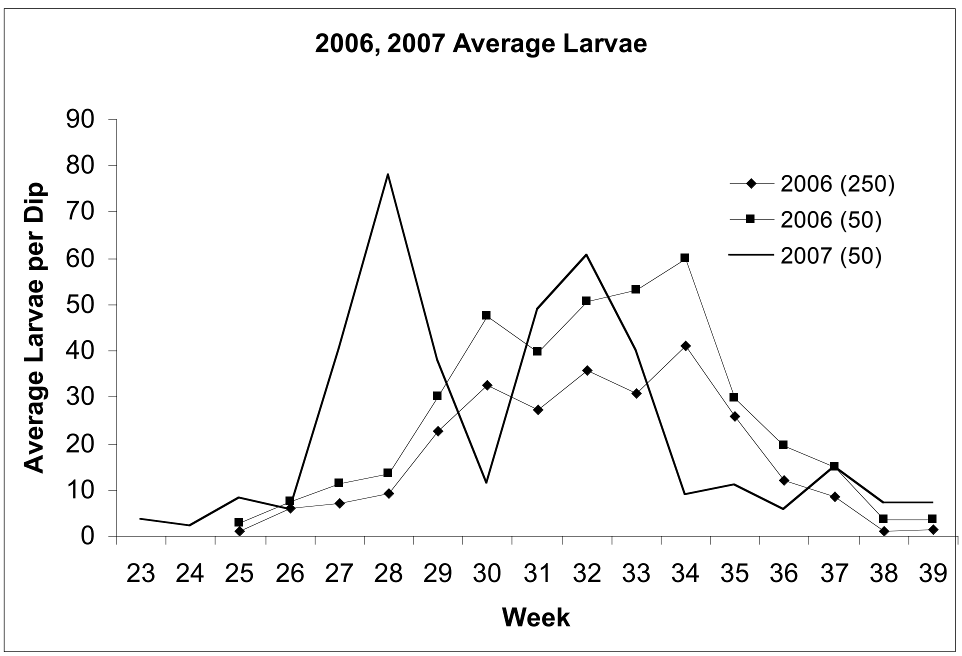

Figure 3. Average number of mosquito larvae per dip for untreated (control) round top basins monitored in 2006 (N = 250) and the subset monitored in 2007 (N = 50).

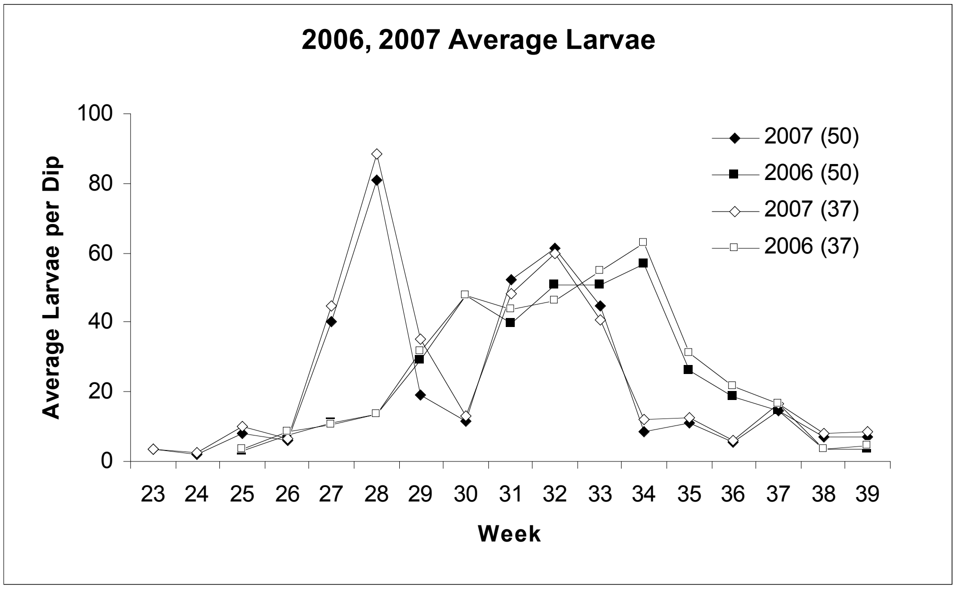

Figure 4. Average mosquito larvae per dip for the same set of control basins in 2006 and 2007 (N = 50). Also shown are the averages for the set of control basins (N = 37) that were never dry in either the 2006 or 2007 monitoring seasons.

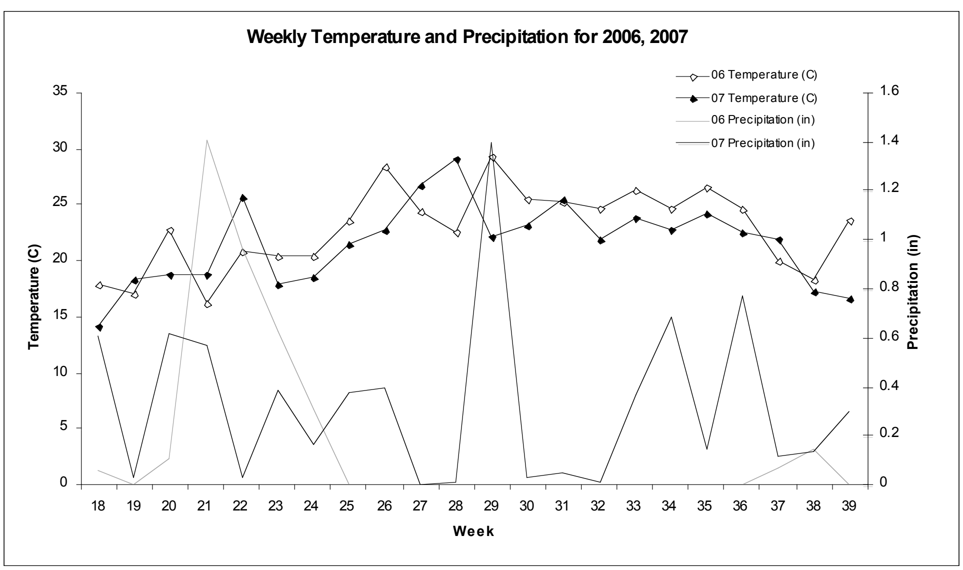

Figure 5. Weekly precipitation accumulation (in) and weekly average high temperature (C) from the first week in May through the last week in September in 2006 and 2007.

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Table 3. Average number of mosquito larvae per dip and pupae per three dips for round top (RT) and grated top (GT) control basins for the Weeks 23 through 39 in 2007.

Week	RT Average Pupae	GT Average Pupae	RT Average Larvae	GT Average Larvae
23	0.59	—	3.44	—
24	0.56	0.60	2.11	4.10
25	0.76	2.11	7.99	8.00
26	1.06	1.78	5.63	2.93
27	2.78	0.75	40.39	14.25
28	2.41	5.25	77.83	107.45
29	5.14	2.40	37.97	72.67
30	1.14	0.70	11.47	15.27
31	1.58	1.00	49.07	50.67
32	4.34	5.75	60.61	56.33
33	3.33	3.22	40.07	51.59
34	0.52	0.22	8.79	4.74
35	1.38	0.78	10.86	5.52
36	0.42	0.33	5.71	4.85
37	3.10	0.67	14.72	12.30
38	0.40	0.67	7.04	3.74
39	2.26	1.00	7.19	3.52

comparisons were made at weeks 1, 3, 5 and 7 post-treatment. With the exception of Week 3 in Treatment Week 1 and Week 7 in Treatment Week 3, pupae counts in the treated basins were statistically lower ($P \leq 0.05$) than those in the control basins (Table 5).

We also evaluated efficacy by comparing the frequency distributions of pupae counts within the treated and control round top basins. This comparison provided a direct comparison of the number of treated and control basins with very low counts. The comparisons are presented in Appendix 1. An example is given in Figure 9. Differences between treated and control basins were potentially minimized by frequent precipitation

Table 4. Percentage of basins with pupae counts consistently equal to or greater than control means for each week. At 4 weeks post-treatment, none of the basins that had been high in the previous weeks continued to be high. Treatment Group 1 was not sampled 2 weeks post treatment. Treatment Group = week of treatment with N = 50 for each group.

Treatment Group	1 Week Post Treatment	2 Weeks Post Treatment	3 Weeks Post Treatment
1	4%	—	2%
2	4%	0%	0%
3	8%	2%	0%

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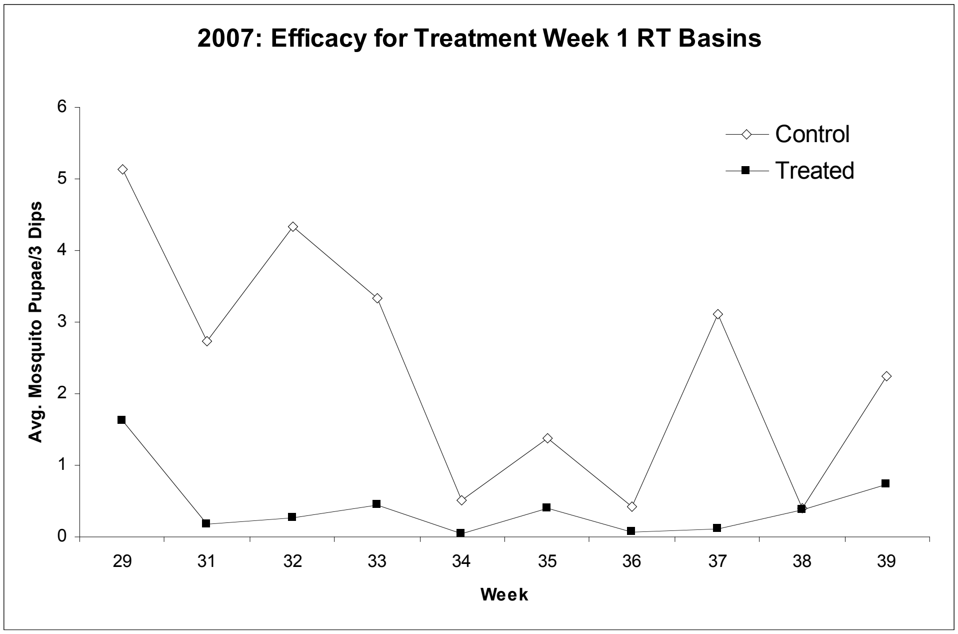

Figure 6. Average number of mosquito pupae per three dips for round top basins selected during the first week of treatment and monitored for 10 weeks (Weeks 29-39) beginning 1 week after treatment. Samples sizes for treated and control basins = 50.

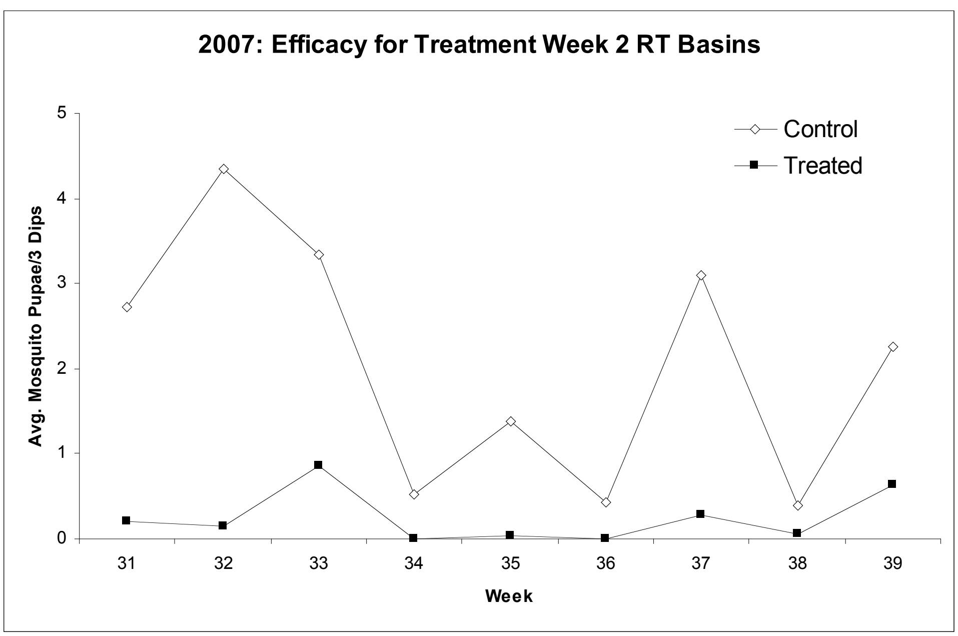

Figure 7. Average number of mosquito pupae per three dips for round top basins selected during the second week of treatment and monitored for 9 weeks (Weeks 31-39) beginning 1 week after treatment. Samples sizes for treated and control basins = 50.

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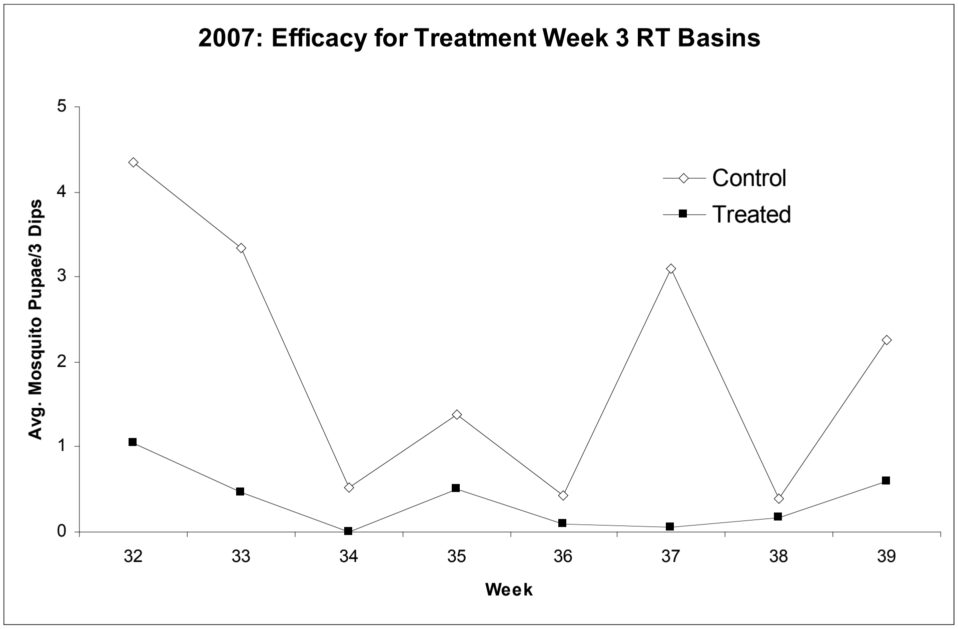

Figure 8. Average number of mosquito pupae per three dips for round top basins selected during the third week of treatment and monitored for 8 weeks (Weeks 32-39) beginning 1 week after treatment. Samples sizes for treated and control basins = 50.

Table 5. Statistical analysis using one-sided Mann-Whitney test (alpha=0.05) on 2007 efficacy data for mosquito pupae within round top basins. Analysis compared pupae counts between control basins and those treated with VectoLex®CG for three groups (treatment weeks) at 1, 3, 5, and 7 weeks post-treatment. *Differences were considered significant when P≤0.05.

Treatment Group	Week	Week Post Treatment	Significance
1	29	1	0.0005*
1	31	3	0.0745
1	33	5	0.0000*
1	35	7	0.0070*
2	31	1	0.0005*
2	33	3	0.0000*
2	35	5	0.0000*
2	37	7	0.0000*
3	32	1	0.0000*
3	34	3	0.0005*
3	36	5	0.0125*
3	38	7	0.0940

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events that reduced the number of pupae (and larvae) in the catch basins within each treatment group. In all comparisons, pupae were not detected in the three standard dips within at least 40 percent (in most cases ≥60%) of the control round top basins (Appendix 1). Precipitation effects likely explain the lack of statistical significance between treated and control round top basins 3 and 7 weeks after Treatment Week 1 (Appendix 1: Figures A1-4, A1-10). In both cases, the proportion of treated basins with 0 pupae was high (95 and 85%, respectively), but comparable values for control basins were also high (85 and 70%).

Within the 20 grated top basins monitored after application of the larvicide in Treatment Week 2, pupae counts were lower than controls for a period of 3 weeks, but only the second week post-treatment was statistically different from the controls (Table 6). By the fourth week post treatment, pupae counts in the treated grated top basins were equal to or exceeded those for the controls (Fig. 10). Reasons for the reduced efficacy in grated compared to round top basins are not known, but the associated with differences in the effects of precipitation on the levels and/or persistence of the larvicide within the basins. Data on the efficacy within treated basins (nearly all grated) in the Thorton Creek watershed in which we also monitored fate of the larvicide support this hypothesis (see next section of this report).

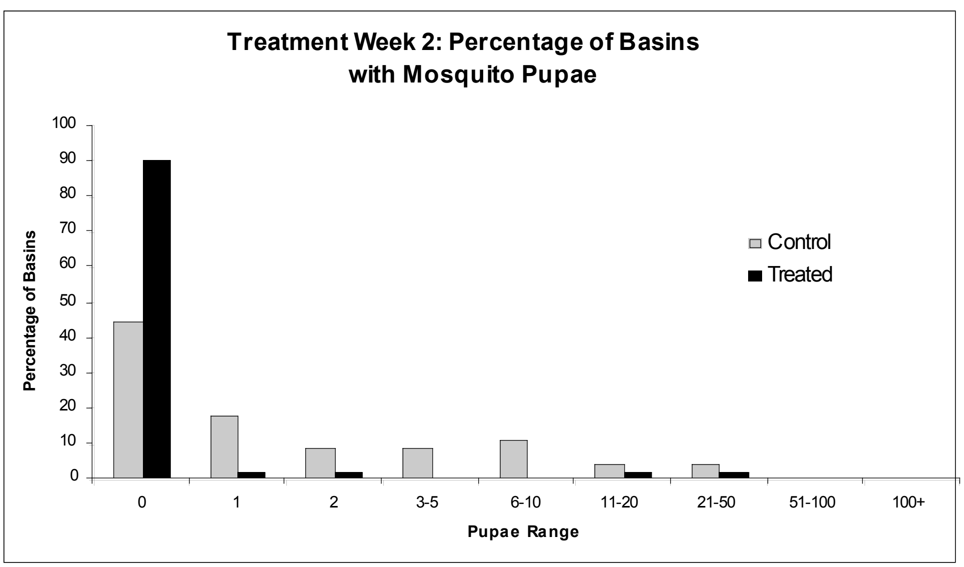

Figure 9. Percentage of basins within specified ranges ("bins") of mosquito pupae for round top basins selected during the second week of treatment. Data collected three weeks post-treatment. Sample size for both treated and control basins = 50.

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Table 6. Statistical analysis using one-sided Mann-Whitney test (alpha=0.05) on 2007 efficacy data for mosquito pupae within grated basins. Analysis compared pupae counts between control basins (N=10) and those treated with VectoLex® CG (N = 10) from 1-9 weeks post-treatment. *Differences were considered significant when P≤0.05.

Week	Week Post-Treatment	Significance
31	1	0.086
32	2	0.025*
33	3	0.128
34	4	0.116
35	5	0.325
36	6	0.293
37	7	0.266
38	8	0.478
39	9	0.219

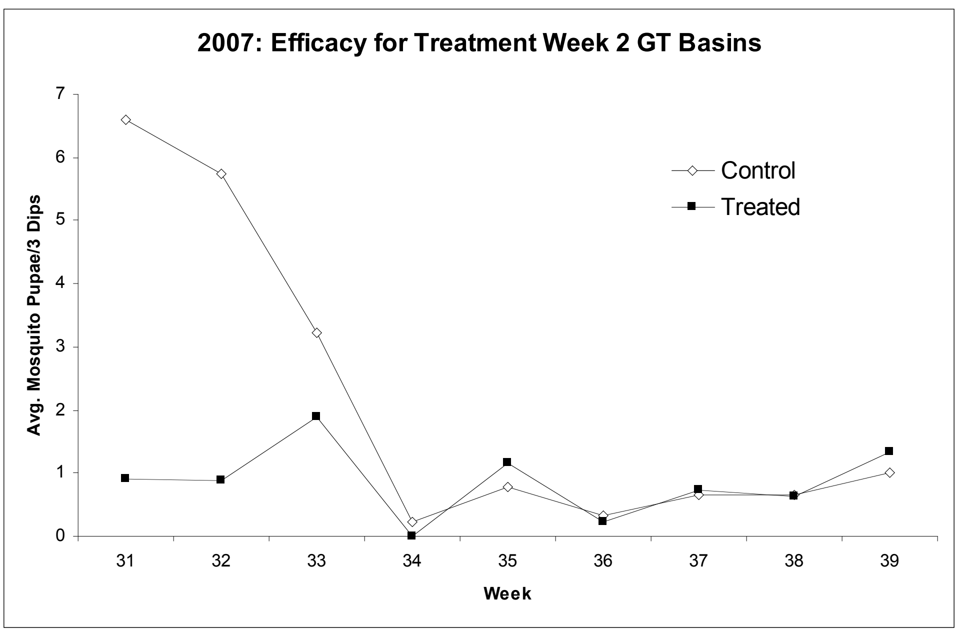

Figure 10. Average number of mosquito pupae per three dips for grated top basins selected during the second week of treatment and monitored for 9 weeks (Weeks 31-39) beginning 1 week after treatment. Samples sizes for treated and control basins = 10.

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Conclusions

The mosquito-breeding season in 2007 was generally similar to that in 2006 with numbers increasing in the third week of June and sharply declining in the middle of September. Whereas precipitation was largely absent from this time period in 2006, rain was a frequent occurrence in 2007 and was associated with dramatic declines in larval counts. A comparison between the same 50 round top basins monitored in 2006 and 2007 suggests that larval counts outside of the precipitation events were greater than those in 2006 early in the season. Differences in ambient temperatures relative to precipitation events likely explain the difference.

Treatment of the 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 after the second week post treatment were less than controls, was ≥98 percent. Efficacy was greater in round top *vs* grated basins. Efficacy was realized for at least 5 weeks, and in most cases 7 weeks, post treatment within round top basins. but <3 weeks within grated basins. Differences between treated and control basins were potentially minimized by frequent precipitation events that reduced the number of pupae (and larvae) in the catch basins within each treatment group, particularly the controls.

Research Needs

Efficacy in the round top basins differed from that in grated catch basins. A similar difference was observed in the grated basins within the Thornton Creek watershed described in the next section of this report. Although reasons for the reduced efficacy are not known, the differences observed are likely due to differences in the effects of precipitation on the levels and/or persistence of the larvicide within the basins. The City may want to test the efficacy of the extended release formulation of Aquaprene® (XL, active ingredient = methoprene) as an alternative to bacterial larvicides as the briquettes are less likely to be flushed from the basins, efficacy is regained rapidly after flushing, and recent toxicity tests with the product and juvenile coho salmon indicate little potential for hazard to salmonids within surface waters that may receive product from treated catch basins following precipitation events.

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Efficacy and Fate of Larvicides within Catch Basins in the Longfellow, Thornton, and Pipers Creek Watersheds

Synopsis — With the exception of catch basins within the Thornton Creek watershed, treatment of the basins with VectoLex® CG significantly reduced the number of pupae present. The lack of efficacy in the Thornton Creek watershed appeared to be associated with grated basins. Efficacy in the grated basins monitored as part of the citywide assessment was also short-lived in comparison to round tops. BT was present in concentrations above background in the basins within the Longfellow and Pipers Creek watersheds after treatment, but not before. In comparison, BT was detected in catch basins within the Thornton Creek watershed before and after treatment, but at much lower concentrations.

Concentrations of BS and BT detected in the water samples collected from Longfellow, Thornton, and Pipers creeks during base flows, before and after treatment of the catch basins, were at background levels. Elevated concentrations of the larvicide were detected in the creeks during precipitation events prior to the start of the City's control program and after treatment, but concentrations were low relative to the maximum concentrations detected in the basins.

Data on non-target toxicity in which concentrations of the larvicides expressed as spores per ml are lacking, making a direct comparison of the results of existing studies of non-target effects to the concentrations we detected within the outflows and creeks difficult. Results of recent toxicity tests with several larvicides, singly and in combination, and juvenile coho suggest that the concentrations detected in each of the creeks pose little hazard to juvenile salmonids.

Objective

Our objective was to determine the fate of the larvicide VectoLex® CG within catch basins in the City of Seattle in 2007 and the potential for the larvicide to enter surface waters and adversely affect non-target species. Specific tasks were:

- Determine the fate of the larvicide within catch basins within the watersheds of three urban creeks: Longfellow, Pipers and Thornton Creeks.
- Determine the extent to which the larvicide enters surface waters within the three creeks.
- Compare concentrations of the larvicide within the creeks with those known to be toxic to aquatic invertebrates and salmonids

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Methods

Selection and Monitoring of Basins — We randomly selected 30 basins within the watersheds (sub-basins) associated with the outflows to each of the three urban creeks: Longfellow, Pipers and Thornton. The distribution of the basins per creek sub-basin was proportional to the number and type of catch basins within each sub-basin: Longfellow = 8 basins selected [1 grated], Pipers = 9 basins [1 grated], and Thornton = 13 basins [11 grated] (Appendix 2). During the first week of treatment (Week 1; week of 9 July), the contractor treated the catch basins within the sub-basins selected for study with BS (VectoLex® CG). Efficacy was determined following treatment by counting the number of pupae per three dips weekly for 13 weeks (end of September) beginning 2 weeks pre-treatment, and resuming 1-week post treatment. Fate of the larvicide was quantified by determining the concentration of the larvicide (spores/ml) in each basin 2 weeks before treatment, and 1, 3, 5 and 7 weeks after treatment, or until the treatment was no longer efficacious. Treatments were considered efficaceous (for the purpose of decisions to continue monitoring) as long as the counts of pupae per three standard dips within individual basins or the mean for the sub-basin did not equal or exceed the mean of the 50 control round top basins monitored in the overall city evaluation described in the previous section of this report. Water samples were collected from each basin (integrated water column, 200 ml) for larvicide analysis after pupae counts, as were measurements of water quality (temperature [C], pH, dissolved oxygen [mg/L], conductivity [us/cm]), and water and sediment depths (0.5 inches]. Methods used to determine water quality and water and sediment depths were the same as those used in the citywide assessment. Water samples were kept on ice in the field and delivered to the laboratory (Institute of Environmental Health, IEH) for analysis by the end of the day they were collected. Quantification (spores/ml) was requested for both BS and BTI (Table 7), as analyses of the product (VectoLex® WSP) used in efficacy trials in 2006 indicated the product contained both BS and BTI (Grue et al. 2006).

Selection and Monitoring of Outflows — Outflows (drain pipes) carrying water from the City's catch basins were identified within each of the three creeks and a total of 10 outflows selected with the number of basins selected proportional to the size (length) of the creek systems: Longfellow = 3 outflows, Pipers = 2 outflows, Thornton = 5 outflows. To characterize the concentrations of BS and BTI before treatment, we collected one sample (200 ml grab sample) from each outflow during a precipitation event (rainfall > 0.03 inches + measurable increase in flow) within the 2 weeks prior to larvicide treatment, and one outside of precipitation events (baseline) when water was flowing from the outflow. Outflows were checked 3-4 times at randomly selected days and times per week outside of precipitation events to collect the one water sample pre-treatment outside of precipitation events and to determine base flows. Water flows were measured in each outflow pipe (depth and height) at each visit to establish a baseline and facilitate the identification of "precipitation events". One water sample within each creek was also collected pre-treatment at a site determined by SPU during base flows and one during a precipitation event. Following treatment of the basins within the watersheds by the contractor (week of 9 July), each outflow was visited once during Weeks 1, 3, 5 and 7

Table 7. Bacterial composition of VectoLex® CG (BS and BT) used in citywide treatment of catch basins in 2007. Product samples were taken from two different lots of VectoLex® CG. Composition of Mosquito Dunks® obtained in 2007 for toxicity tests with salmonids is provided for comparison. The strain of BT among products was the same.

Product	BS Composition (colony forming spores/g product)	BT Composition (colony forming spores/g product)
VectoLex® CG (Lot 1)	4,100,000	400,000
VectoLex® CG (Lot 2)	1,800,000	11,000,000
Mosquito Dunks®	0	4,000,000,000

post treatment (days and time randomly selected) outside of precipitation events to collect water samples if water was flowing within the outflows. One water sample within each creek was also collected during each of these visits. Because of a lack of efficacy in catch basins within the Thornton Creek watershed, water samples from the outflows or creek were not collected after Week 5. Water samples from each of the outflows and creek were also collected during three precipitation events post treatment. Samples were kept on ice in the field and delivered to the laboratory (IEH) for analysis by the end of the day they were collected. Quantification (spores/ml) was requested for both BS and BTI.

In the event the City (SPU) wanted to confirm the identification of any BS or BTI identified within the outflows and creek samples, samples were frozen such that the strain of the *Bacillus sphaericus* within the VectoLex® CG could be isolated in the laboratory and compared to that within the two lots of product used by the contractor. These analyses have not been conducted because BS is not available to the general public through retail outlets or the Internet, and therefore we believed it was safe to assume that any elevated levels of BS in outflows were the result of the city's treatment program.

Data Analyses — Pupae counts within the treated basins were compared to the 50 control basins monitored as part of the citywide assessment of efficacy. Average counts for each watershed were compared to average counts for the control basins through time by treatment week through 11 weeks post treatment using line graphs and frequency distributions (percentage of basins with specific ranges ["bins"] of pupae). We also compared the pupae counts between treated and control basins by week post treatment using a one-sided Mann-Whitney test (alpha = 0.05). Larvicide treatments were considered efficacious if the counts of pupae within treated basins were statistically lower than the counts within the control basins (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. Concentrations of BS and BTI within the water samples from the treated basins, outflow pipes, and creeks were compared graphically through time.

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Results

Efficacy within Basins in the Three Watersheds — With the exception of catch basins within the Thornton Creek watershed, treatment of the basins with VectoLex® CG reduced the number of pupae to, in most cases, zero, i.e., no pupae were detected in the three standard dips per visit (Fig. 11). The lack of efficacy in the Thornton Creek watershed appeared to be primarily associated with grated basins as average pupae counts for round top basins remained below the control average (Fig. 12). By Week 5 post treatment, pupae counts within the watershed were no longer statistically lower than those within grated control basins (Table 8), in fact, they were significantly greater. In comparison, counts within the Longfellow and Pipers Creek watersheds were both statistically lower than round top controls through Week 5; Pipers through Week 7 (Table 9). Even by 11 weeks post treatment, counts within more than 85% of the basins within the Longfellow Creek watershed were 0; within the Pipers Creek watershed, 100% were 0 (Figs. 13 and 14, pp. 35-36).

Reasons for the reduced efficacy within the Thornton Creek watershed are not known. Run-off associated with precipitation events may have a greater flushing effect within grated catch basins because water is entering from street level *vs* lower in the basin with less force in the case of round top basins. Similarly, efficacy in the grated basins monitored as part of the citywide assessment was also short-lived in comparison to round

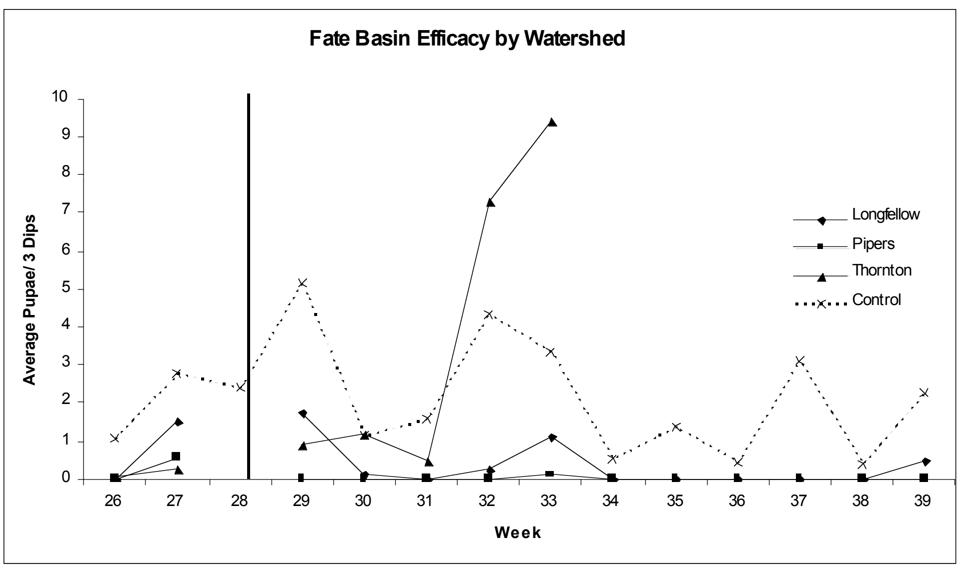

Figure 11. Average pupae per three dips for the fate basins through Week 39 (9/24/07-9/28/07). Data are presented by watershed (Longfellow: N=8; Pipers: N=9; Thornton: N=13). No basins were monitored during treatment week (Week 28). All points to the right of the vertical line are from the 50 untreated basins monitored as part of the citywide efficacy assessment.

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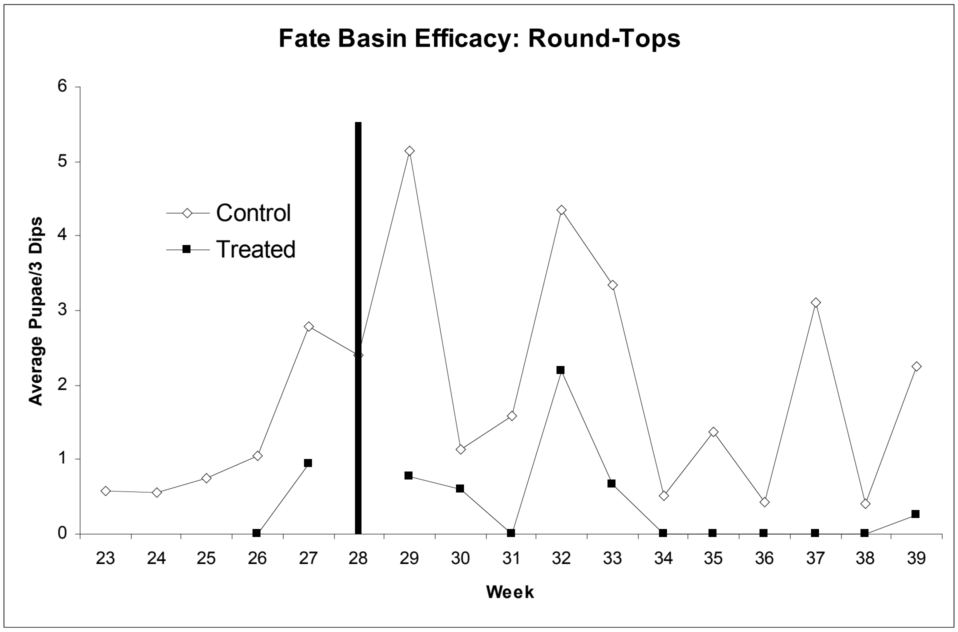

Figure 12. Average number of mosquito pupae per three dips for round top fate basins (N=17), compared to controls. No basins were monitored during treatment week (Week 28). All points to the right of the vertical line are those that occurred post-treatment. Control data are from the 50 untreated basins monitored as part of the citywide efficacy assessment.

top basins (Fig. 9, p. 28). This hypothesis is supported by the larvicide fate data presented in the next section of this report. Spore counts 1-week post treatment were low relative to those observed in the catch basins monitored in the Longfellow and Pipers Creek watersheds. In addition, a statistical comparison of water quality parameters within round top and grated basins monitored citywide indicated that only water temperature differed between the two basin types (Table 10, p. 36), and that difference was small (average round top basins = 19.83 C; grated = 19.08 C).

Table 8. Statistical analysis comparing average pupae counts for the fate basins within the Thornton Creek watershed to the 10 grated top control basins within the citywide efficacy assessment using one-sided Mann-Whitney test (alpha=0.05). The analysis compared pupae counts between control basins and those treated with VectoLex® CG at 1, 3, and 5 weeks post treatment. Thornton Creek basins were only monitored for 5 weeks because of a lack of efficacy at Week 5. Differences were considered statistically significant if $P \leq 0.05$*.

Treatment Watershed	Week	Week Post Treatment	Statistical Significance
Thornton	29	1	0.0170*
	31	3	0.0130*
	33	5	0.0935

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Table 9. Statistical analysis comparing average pupae counts for the fate basins within the Longfellow and Pipers Creek watersheds to the 50 round top control basins within the citywide efficacy assessment using one-sided Mann-Whitney test (alpha=0.05). The analysis compared pupae counts between control basins and those treated with VectoLex® CG at 1, 3, 5, and 7 weeks post treatment. Differences were considered statistically significant if P≤0.05*.

Treatment Watershed	Week	Week Post Treatment	Statistical Significance
Pipers	29	1	0.0015*
	31	3	0.0320*
	33	5	0.0065*
	35	7	0.0300*
Longfellow	29	1	0.0225*
	31	3	0.0400*
	33	5	0.0300*
	35	7	0.0565

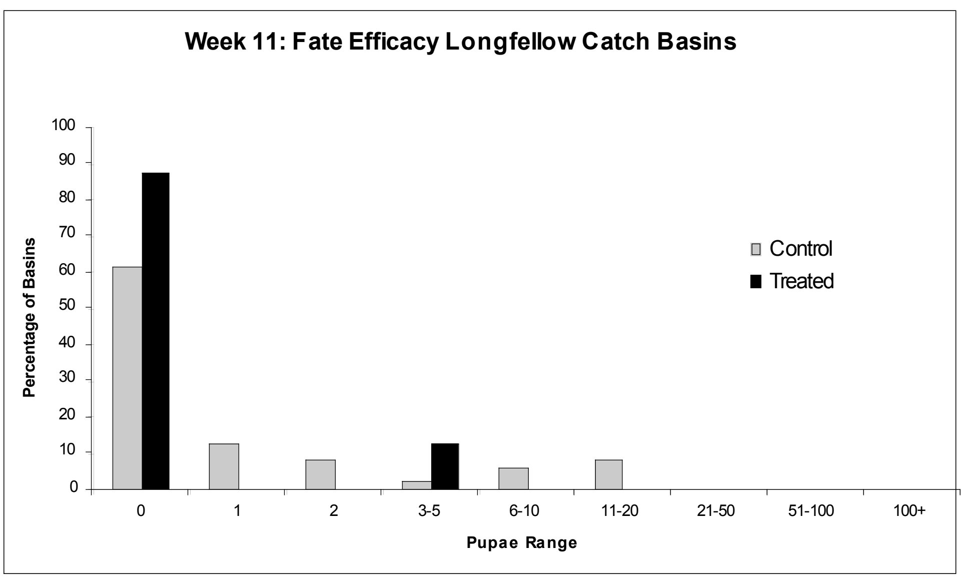

Figure 13. Percentage of basins with specified ranges of mosquito pupae for fate basins (N=8) within the Longfellow Creek watershed. Data were collected 11 weeks post treatment (Week 39).

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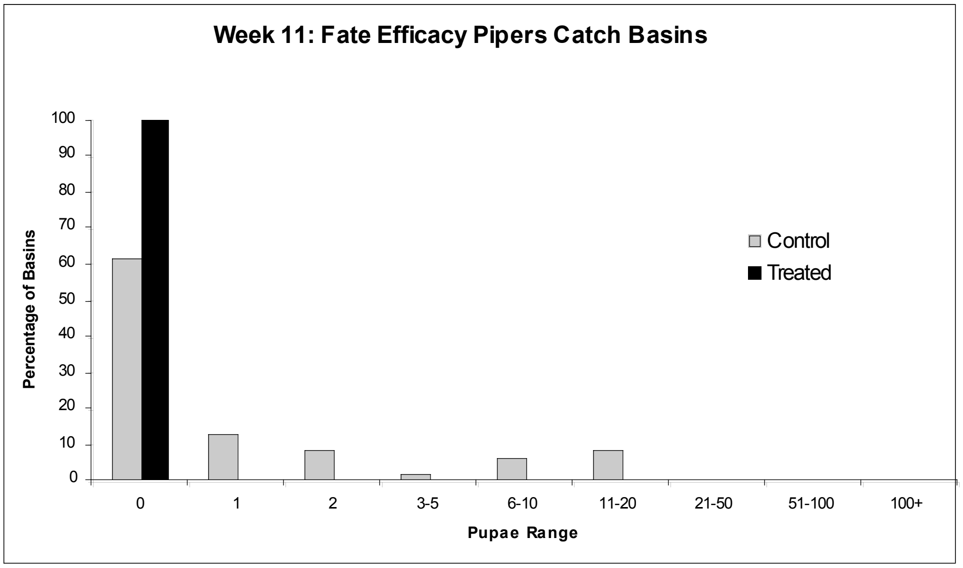

Figure 14. Percentage of basins with specified ranges of mosquito pupae for fate basins (N=9) within the Pipers Creek watershed. Data were collected 11 weeks post treatment (Week 39).

Fate of the Larvicide within Treated Basins — Bacterial cultures from the water samples collected from the catch basins within each of the three watersheds before and after treatment indicate that larvicide was present in concentrations significantly above background in the basins within the Longfellow and Pipers Creek watersheds only 1 week after treatment (Figs. 15 and 16). Average concentrations were ca. 300,000 and 140,000 spores of *Bacillus thuringiensis* (BT) per ml with essentially no *Bacillus sphaericus* (BS) detected in the samples. Very little if any BT was detected pre-treatment in basins within the two watersheds (Figs. 15 and 16). BT was detected in catch basins within the Thornton Creek watershed before and after treatment, but at much lower

Table 10. Results of a two-way ANOVA (P values, alpha = 0.05) comparing water quality parameters between basin type (Round top vs. Grated) and treatments (Control *vs* BS-treated). Also reported are the results for a Levene's Test of Equality of Error Variance. Water temperature was greater in round top basins (mean = 19.83 C, n = 100, Week 2 basins) than grated basins (mean = 19.08 C, n=20, basins selected in Week 2) from Weeks 2 – 39. Differences between treated and control basins were not significant.

	pH	D.O. (mg/L)	Conductivity (us)	Temperature (C)	Water Depth	Sediment Depth
Basin Type	0.697	0.988	0.976	0.001*	0.338	0.201
Treatment	0.784	0.427	0.510	0.274	0.865	0.795
Error Variance	0.175	0.804	0.878	0.273	0.222	0.577

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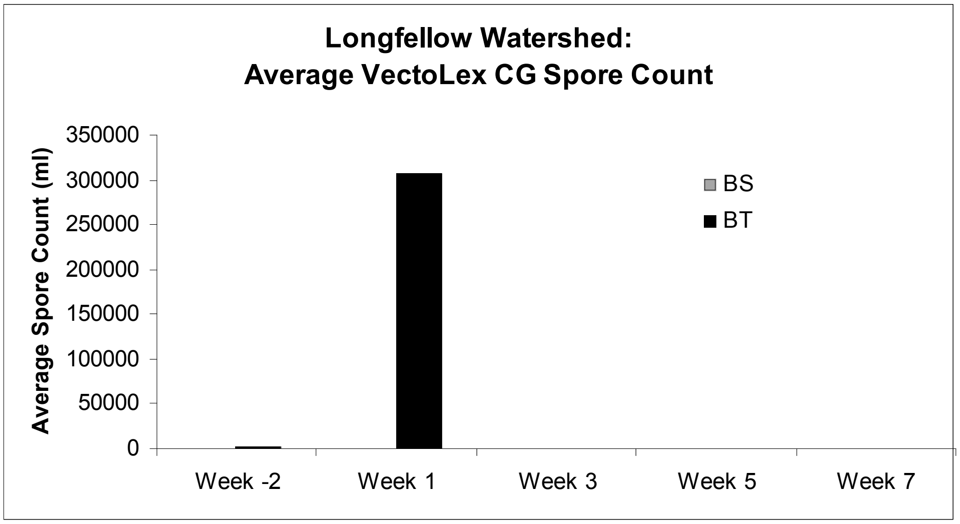

Figure 15. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) in catch basins (N=8) within the Longfellow Creek watershed before and after treatment with VectoLex® CG (Week 0).

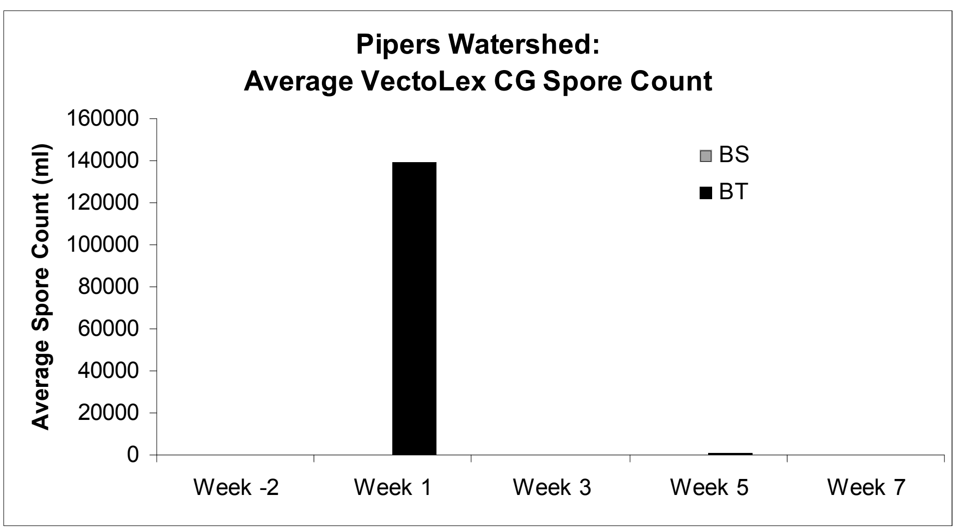

Figure 16. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) in catch basins (N=9) within the Pipers Creek watershed before and after treatment with VectoLex® CG (Week 0).

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concentrations (2,000-4,000 spores/ml; Fig. 17). Again, very little BS was detected. Laboratory analyses confirmed that the BT detected is the same strain as that within BTI larvicides (Mosquito Dunks®). Results suggest that the concentrations of BS were severely reduced by precipitation events shortly after application and that the extended efficacy observed in the basins monitored in the Longfellow and Pipers Creek watersheds was largely the result of the initial inoculation and not the continued presence of the larvicides within the water column of the basins. However, the average concentration of BTI within the catch basins 1 week after treatment with VectoLex® WSP in 2006 was only ca. 5,500 spores per ml (Grue et al. 2006), two orders of magnitude lower than BTI concentrations within the basins in the Longfellow and Pipers Creek watersheds. Corresponding concentrations of BS in 2006 were ca. 7,000 spores per ml and concentrations of both larvicides decreased with time, but were still detectable 5 weeks post treatment (Grue et al. 2006). Concentrations in 2006 were associated with very good efficacy for a period of at least 5 weeks (Grue et al. 2006).

The impact of frequent flushing of the basins during precipitation events may have contributed to the observed efficacy. The relatively low remaining concentrations of the larvicides may have been adequate to control larval development between precipitation events. Alternatively, concentrations of the bacteria may have been greater within the upper portions of the sediment not adequately sampled by our integrated water column sampler, although this sampling technique was effective in documenting elevated concentrations of the two bacteria during efficacy trials in 2006. Water temperatures within the treated basins in 2007 were lower than those in the basins treated with BS in 2006 (Fig. 18), and the water in the basins in 2007 appeared clearer with less organic

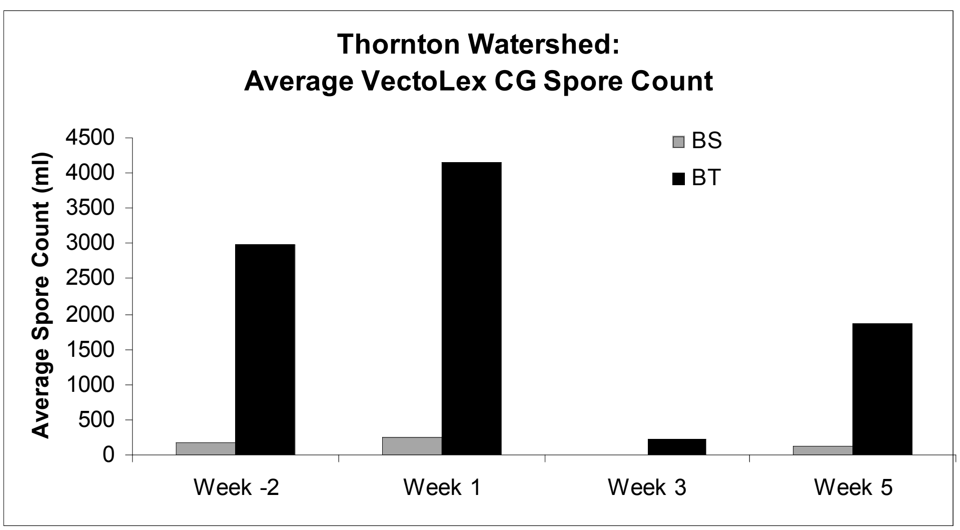

Figure 17. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) in catch basins (N=13) within the Thornton Creek watershed before and after treatment with VectoLex® CG (Week 0). Basins were only monitored through Week 5 due to a lack of efficacy at that time.

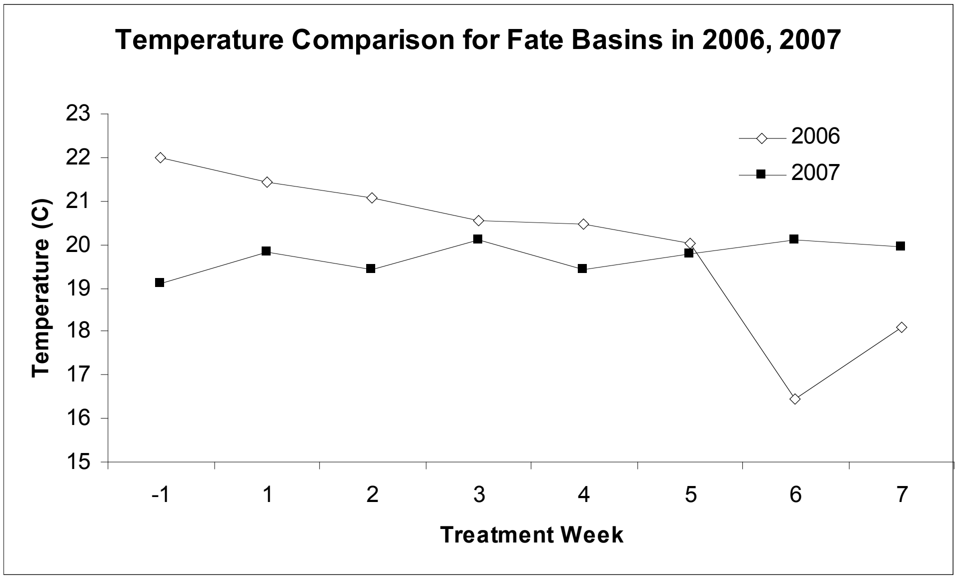

Figure 18. Comparison of temperature (C) within BS "Fate" basins in 2006 (N = 15) and 2007 (N = 30). In 2006, basins were only located within the Northwest sector, while in 2007 basins were located in the Northwest, Northeast, and Southwest sectors.

matter. BS is believed to be "…particularly effective in controlling mosquito larvae in waters high in organic matter such as catch basins…" (Valent BioSciences Corporation, 2008, www.valentbiosciences.com/agricultural_products/agricultural_products_9.asp). Concentrations of BS (VectoLex® WSP) were also lower than expected in toxicity tests with juvenile salmonids in which the water temperature was 12± 2 C and the water was dechlorinated city water with no organic matter other than that associated with the test fish, whereas BTI concentrations (Mosquito Dunks® and Bits®) were several orders of magnitude greater (Tables 11 and 12; M. R. Sternberg, unpublished data). Should this be the case, BS may not be the best choice for controlling mosquito larvae within catch basins potentially subject to frequent flushing from precipitation events. Differences in efficacy and fate of BS within treated catch basins in 2006 (no precipitation) and 2007 (frequent precipitation) support this hypothesis.

BS and BT were negligible in water samples collected from the catch basins in the Longfellow and Pipers Creek watersheds before treatment (Figs. 15 and 16, p. 37), but were present in samples from basins in the Thornton creek watershed 2 weeks before the City's treatment program (Fig. 17). The average concentration of BT pre-treatment (ca. 3,000 spores/ml) was similar to that measured 1-week post treatment (ca., 4,000 spores/ml). These results suggest that the basins may have been treated before the scheduled start of the City's treatment program, or larvicide entered the basins from other sources. Contractor records indicate that the subject basins were not treated early. These data match the concentrations of BT detected in the baseline flows from the outflow pipes feeding Thornton Creek 1 week before the scheduled start of the treatment program.

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Table 11. Average *Bacillus sphaericus* (BS) and *Bacillus thuringiensis* (BT) colony counts at hour 0 and 96 h in water treated with VectoLex® WSP (one pouch) or Mosquito Dunks® and Bits® (2 dunks + 1 tbsp bits) comparable to that used in 2006 efficacy and fate catch basins. Water was treated 24 h before the start of the toxicity test (T0). Juvenile Coho Larvicide Exposure Test 1 (WACFWRU 2007; M.R. Sternberg, unpublished data).

Product	Time (h)	BS (colony forming spores/ml)	BT (colony forming spores/ml)
VectoLex®WSP	0	61	180
VectoLex®WSP	96	34	2,300
Mosquito Dunks®, Bits®	0	<10	91,200
Mosquito Dunks®, Bits®	96	<10	280,000

Table 12. Average *Bacillus sphaericus* (BS) and *Bacillus thuringiensis* (BT) colony counts at hour 0 and 96 h in water treated with combinations of VectoLex® WSP (one pouch), Mosquito Dunks® and Bits® (2 dunks + 1 tbsp bits), and Aquaprene® XL briquettes (one briquette). Water was treated with the bacterial larvicides 24 h before the start of the toxicity test (T0); 7 days prior for Aquaprene. Juvenile Coho Larvicide Exposure Test 2 (WACFWRU 2007; M.R. Sternberg, unpublished data).

Product	Time (h)	BS (colony forming spores/ml)	BT (colony forming spores/ml)
VectoLex®WSP, Mosquito Dunks®, Bits®, Aquaprene®	0	1,440	257,000
VectoLex®WSP, Mosquito Dunks®, Bits®, Aquaprene®	96	16,667	507,000
VectoLex®WSP, Aquaprene®	0	160	407,000
VectoLex®WSP, Aquaprene®	96	75	3,450
Mosquito Dunks®, Bits®, Aquaprene®	0	150	145,000
Mosquito Dunks®, Bits®, Aquaprene®	96	<10	96,500
VectoLex®WSP, Mosquito Dunks®, Bits®	0	100	110,000
VectoLex®WSP, Mosquito Dunks®, Bits®	96	100	500,000

Concentrations of Larvicide within Outflows and Creeks — We monitored concentrations of BS and BT in the outflows from the three watersheds into their respective creeks during normal (baseline) flows and during precipitation events. Concentrations of BT in the outflows into Longfellow Creek during baseline flows 1-week post treatment were comparable to those within the basins (ca., 130,000 spores/ml, Fig. 19). Neither BS nor BT was detected in any of the other baseline sampling of the outflows into Longfellow Creek (Fig. 19). Elevated concentrations of the two bacteria were only detected in the outflows during the precipitation event that occurred in Week 5 post treatment when rainfall for the week was 1.01 inches, the most during the study period (Fig 20). The maximum concentration of BT was ca. 40,000 spores per ml and for BS, ca. 2,500 spores/ml (Fig. 20). Comparable graphs with greater resolution on the Y-axis (spore-counts/ml) to better represent low concentrations of BS and BTI are provided in Appendix 3.

Maximum concentrations of the two bacteria (only BT was detected) in the baseline outflows into Pipers Creek 1-week post treatment (ca., 9,000 spores/ml, Fig. 21) were a fraction of those within the basins at that time (ca., 140,000 spores/ml, Fig. 16, p. 37). Little BT was detected in any of the other baseline sampling of the outflows into Pipers Creek (Fig. 21). Elevated concentrations of BT were detected in the outflows during precipitation events that occurred 1 week prior to treatment and during subsequent precipitation events during Weeks 1 and 3 post-treatment (Fig. 22, p. 43). BS concentrations were very low when detected (Fig. 22). The elevated concentration of BT in outflow water before the start of the City's treatment program suggests that there were other sources of BT within the watershed that entered Pipers Creek.

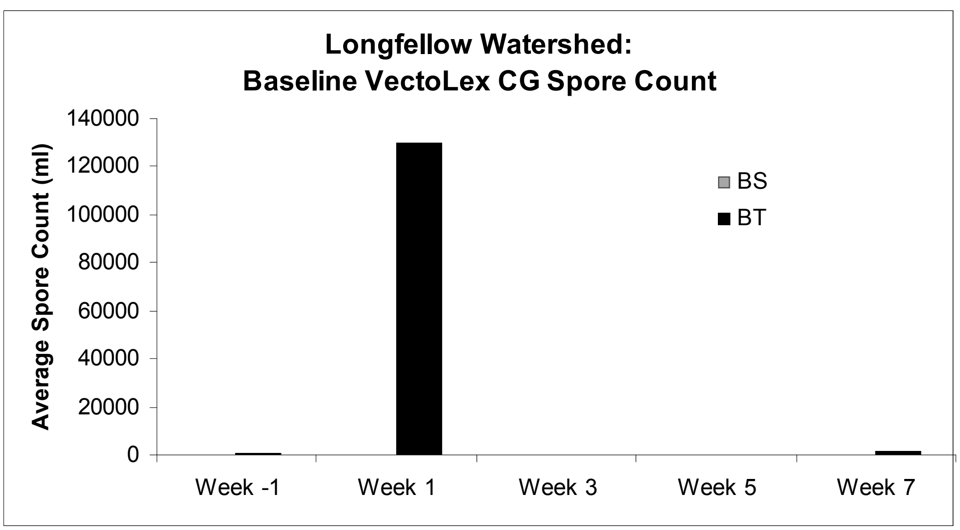

Figure 19. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) in outflow pipes (N=3) within the Longfellow Creek watershed measured during baseline flows before and after treatment of basins within the watershed with VectoLex® CG (Week 0).

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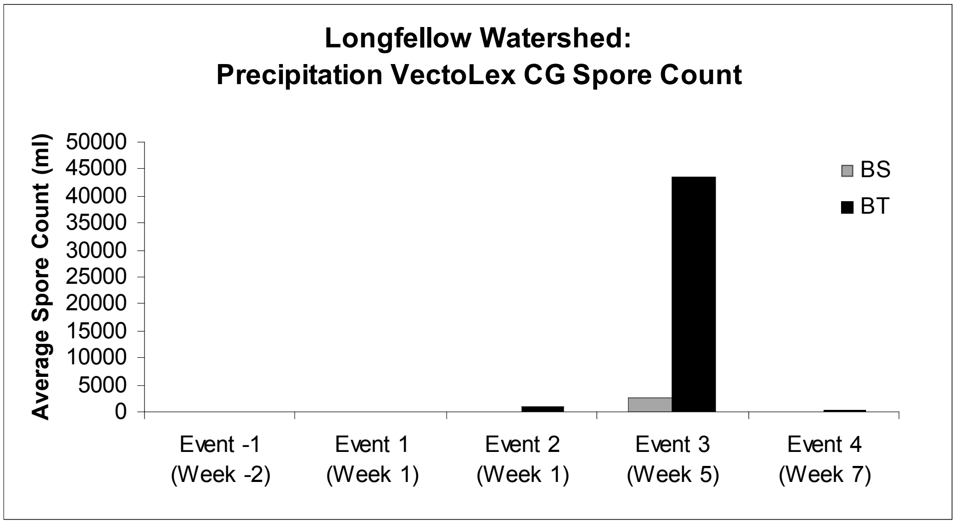

Figure 20. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) in outflow pipes (N=5) within the Longfellow Creek watershed measured during precipitation events before and after treatment of basins within the watershed with VectoLex® CG (Week 0). Rainfall during Event 3 was 1.01 inches, Events 2 and 4 = 0.30, 0.31 inches, and Events -1 and 1 = 0.12, 0.05 inches.

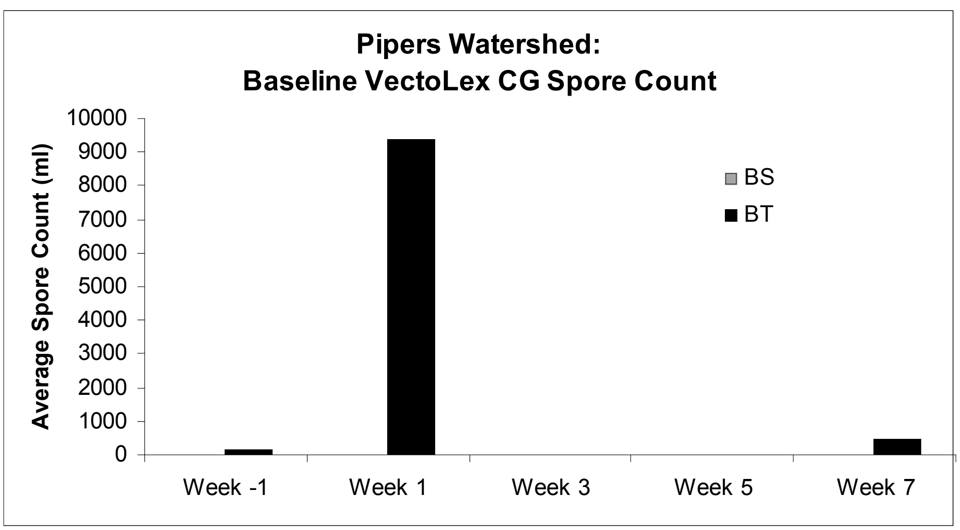

Figure 21. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) in outflow pipes (N=1; one sample lost by the laboratory) within the Pipers Creek watershed measured during baseline flows before and after treatment of basins within the watershed with VectoLex® CG (Week 0).

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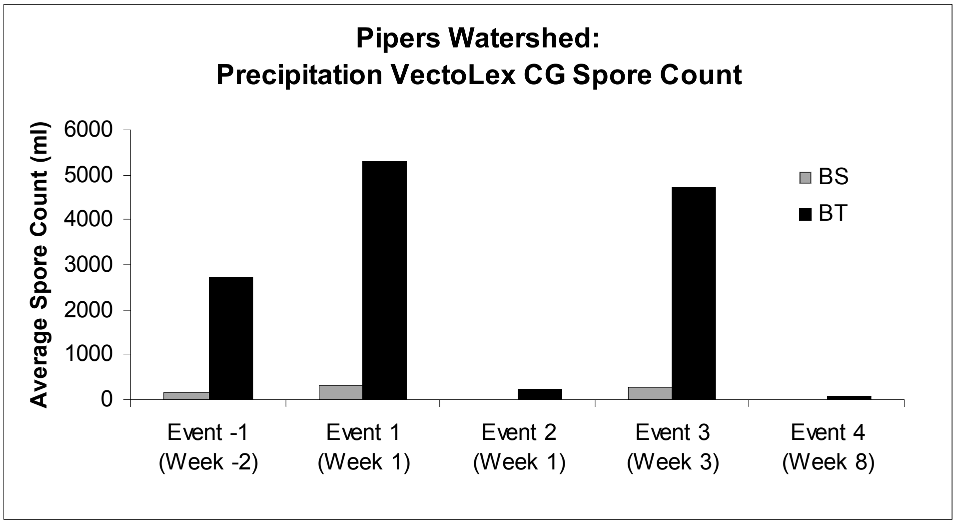

Figure 22. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) in outflow pipes (N=2) within the Pipers Creek watershed measured during precipitation events before and after treatment of basins within the watershed with VectoLex® CG (Week 0). Rainfall during Events -1-4 were 0.26, 0.05, 0.31, 0.05, and 0.74 inches.

Within the outflows into Thornton Creek, elevated concentrations of BT were detected within base flows 1 week prior to the start of the City's treatment program (ca., 1,500 spores per ml) and again during Week 5 post treatment (ca., 800 spores per ml). Little BT was detected 1-week post treatment (Fig. 23). Concentrations of BT within the outflows during precipitation events were elevated 1-week pre-treatment (ca., 4,500 spores per ml) and during two precipitation events in the week after treatment (maximum = ca., 8,000 spores per ml; Fig. 24). The later corresponded to the greatest rainfall (0.31 inches) during the times we monitored the outflows at Thornton Creek. Very little BS was detected (Fig. 24). Results suggest that basins were treated prior to the scheduled start of the City's treatment program and/or other sources of BT existed within the watershed. Contractor records, however, indicate that the subject basins were not treated early.

Results of the analyses for BS and BT within the creeks themselves during base flows and precipitation events are shown in Tables 13 and 14 (pp. 45-46). Neither BS nor BT was detected in the majority of the water samples collected during base flows before or after treatment of the catch basins within the watersheds. The maximum baseline concentration of either larvicide was 350 spores of BT per ml in a water sample collected from Thornton Creek 5 weeks post treatment (Table 13, p. 45). Both larvicides were detected in the creeks during precipitation events prior to the start of the City's control program, but concentrations were low (≤110 spores per ml). Following treatment, concentrations of the larvicides were frequently greater than pre-treatment levels with concentrations reaching 7,800 spores of BT per ml in a water sample collected in Pipers Creek 1 week post treatment (Table 14, p. 46).

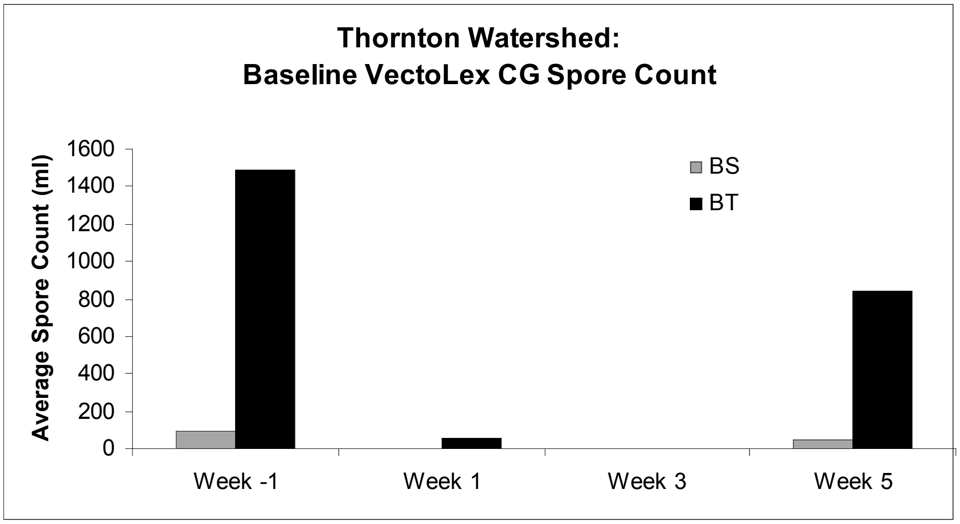

Figure 23. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) in outflow pipes (N=5) within the Thornton Creek watershed measured during baseline flows before and after treatment of basins within the watershed with VectoLex® CG (Week 0).

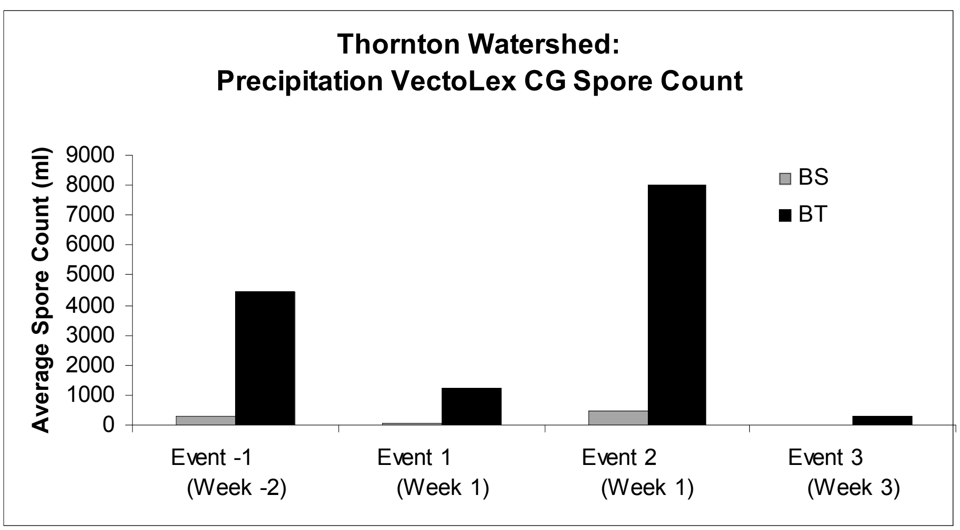

Figure 24. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) in outflow pipes (N=5) within the Thornton Creek watershed measured during precipitation events before and after treatment of basins within the watershed with VectoLex® CG (Week 0). Rainfall during Event -1-3 was 0.12, 0.05, 0.31, and 0.05 inches.

Table 13. Presence of BS and BT (spores/ml) within water samples collected from Longfellow, Pipers, and Thornton creeks during base flows (outside of precipitation events) before and after treatment of catch basins with VectoLex® CG. Baseline times were chosen randomly within each of the designated weeks. — = samples lost by IEH.

Watershed	Week	Week Post Treatment	*Bacillus* Type	Bacterial Count (spores/ml)
Longfellow	27	-1	BS	0
Longfellow	27	-1	BT	10
Longfellow	29	1	BS	0
Longfellow	29	1	BT	0
Longfellow	31	3	BS	0
Longfellow	31	3	BT	0
Longfellow	33	5	BS	0
Longfellow	33	5	BT	30
Longfellow	35	7	BS	0
Longfellow	35	7	BT	10
Pipers	27	-1	BS	—
Pipers	27	-1	BT	—
Pipers	29	1	BS	0
Pipers	29	1	BT	30
Pipers	31	3	BS	0
Pipers	31	3	BT	0
Pipers	33	5	BS	0
Pipers	33	5	BT	20
Pipers	35	7	BS	0
Pipers	35	7	BT	0
Thornton	27	-1	BS	0
Thornton	27	-1	BT	40
Thornton	29	1	BS	0
Thornton	29	1	BT	20
Thornton	31	3	BS	0
Thornton	31	3	BT	0
Thornton	33	5	BS	20
Thornton	33	5	BT	350

Potential for Non-target Effects within Surface Waters — We reviewed the existing literature on the non-target toxicity of BS and BTI. Unfortunately, data in which concentrations of the larvicides are expressed as spores 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 recently conducted toxicity tests with several larvicides, singly and in combination, and juvenile coho salmon (*Oncorhynchus kisutch*; ca. 18 g). 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 (Tables 11 and 12, p. 40).

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Table 14. Presence of BS and BT (spores/ml) within water samples collected from Longfellow, Pipers, and Thornton creeks during precipitation events before and after treatment of catch basins with VectoLex® CG.

Watershed	Week	Week Post Treatment	*Bacillus* Type	Bacterial Count (spores/ml)
Longfellow	26	-2	BS	10
Longfellow	26	-2	BT	110
Longfellow	29	1	BS	0
Longfellow	29	1	BT	10
Longfellow	29	1	BS	0
Longfellow	29	1	BT	10
Longfellow	33	5	BS	0
Longfellow	33	5	BT	50
Longfellow	35	7	BS	0
Longfellow	35	7	BT	10
Pipers	26	-2	BS	10
Pipers	26	-2	BT	90
Pipers	29	1	BS	480
Pipers	29	1	BT	7,800
Pipers	29	1	BS	0
Pipers	29	1	BT	10
Pipers	31	3	BS	10
Pipers	31	3	BT	100
Pipers	36	8	BS	20
Pipers	36	8	BT	490
Thornton	26	-2	BS	10
Thornton	26	-2	BT	110
Thornton	29	1	BS	0
Thornton	29	1	BT	80
Thornton	29	1	BS	0
Thornton	29	1	BT	30
Thornton	31	3	BS	20
Thornton	31	3	BT	390

Conclusions

With the exception of catch basins within the Thornton Creek watershed, treatment of the basins with VectoLex® CG reduced the number of pupae to the point where, in most cases, no pupae were detected. The lack of efficacy in the Thornton Creek watershed appeared to be primarily associated with grated basins as average pupae counts for round top basins remained below the control average. Even by 11 weeks post treatment, pupae were not detected within more than 85% of the basins within the Longfellow Creek watershed and 100% within the Pipers Creek watershed. Reasons for the reduced efficacy within the Thornton Creek watershed are not known. Run-off associated with precipitation events may have a greater flushing effect within grated catch basins. Similarly, efficacy in the grated basins monitored as part of the citywide assessment was also short-lived in comparison to round top basins.

Bacterial cultures from the water samples collected from the catch basins within each of the three watersheds before and after treatment indicate that larvicide was present in concentrations significantly above background in the basins within the Longfellow and Pipers Creek watersheds only 1 week after treatment. Very little, if any, BT was detected pre-treatment in basins within the two watersheds. BT was detected in catch basins within the Thornton Creek watershed before and after treatment, but at much lower concentrations. Very little BS was detected overall. Results suggest that the concentrations of BS were severely reduced by precipitation events shortly after application and that the extended efficacy observed in the basins monitored in the Longfellow and Pipers Creek watersheds was largely the result of frequent flushing of the basins during precipitation events.

BS and BT were negligible in water samples collected from the catch basins in the Longfellow and Pipers Creek watersheds before treatment, but were present in samples from basins in the Thornton creek watershed 2 weeks before the City's treatment program. Results suggest BT entered the basins from other sources. These data match the concentrations of BT detected in the baseline flows from the outflow pipes feeding Thornton Creek 1 week before the scheduled start of the treatment program.

Concentrations of BT in the outflows into Longfellow Creek during base flows 1-week post treatment were comparable to those within the basins. Neither BS nor BT was detected in any of the other baseline sampling of the outflows into Longfellow Creek. Elevated concentrations of the two bacteria were only detected in the outflows during the precipitation event in which rainfall for the week was 1.01 inches, the most during the study period. In contrast, maximum concentrations of the two bacteria in the base outflows into Pipers Creek 1-week post treatment were a fraction of those within the basins at that time. Little BT was detected in any of the other baseline sampling of the outflows into Pipers Creek. Elevated concentrations of the BT were detected in the outflows during precipitation events that occurred 1 week prior to treatment and during subsequent precipitation events. The elevated concentration of BT in outflow water before the start of the City's treatment program suggests that there were other sources of BT within the watershed that entered Pipers Creek. Within the outflows into Thornton Creek, elevated concentrations of BT were detected within base flows 1 week prior to the start of the City's treatment program and again during Week 5 post treatment. Little BT was detected 1-week post treatment. Concentrations of BT within the outflows during precipitation events were elevated 1-week pre-treatment and during two precipitation events in the week after treatment. Very little BS was detected. Similar to Pipers Creek, these results suggest other sources of BT existed within the watershed.

Neither BS nor BT was detected in the majority of the water samples collected during base flows before or after treatment of the catch basins within the watersheds. Both larvicides were detected in the creeks during precipitation events prior to the start of the City's control program, however concentrations were low. Following treatment, concentrations of the larvicides were frequently greater than pre-treatment levels. Unfortunately, data on non-target toxicity in which concentrations of the larvicides expressed as spores per ml are lacking, making a direct comparison of the results of existing studies of non-target effects to the concentrations we detected within the outflows and creeks difficult. However, results of recent toxicity tests with several larvicides, singly and in combination, and juvenile coho suggest that the concentrations detected in each of the creeks pose little hazard to juvenile salmonids.

Research Needs

Efficacy within the grated basins within the Thornton Creek watershed was short-lived compared to round top basins within the other two watersheds and within the City as a whole. Although reasons for the reduced efficacy are not known, the differences observed are likely due to differences in the effects of precipitation on the levels and/or persistence of the larvicide within the basins. As suggested previously in this report, the City may want to test the efficacy of the extended release formulation of Aquaprene® (XL, active ingredient = methoprene) as an alternative to bacterial larvicides as the briquettes are less likely to be flushed from the basins, efficacy is regained rapidly after flushing, and recent toxicity tests with the product and juvenile coho salmon indicate little potential for hazard to salmonids within surface waters that may receive product from treated catch basins following precipitation events.

Frequent precipitation events likely contributed to the observed efficacy of BS (VectoLex® CG) in the round top basins citywide. BS concentrations in the basins treated in 2007 were lower than those reported in 2006 (basins treated with VectoLex® WSP) when precipitation was not a factor. Water temperatures were lower in 2007 and there appeared to be less organic matter as a result of frequent flushing; conditions that may not be the most favorable for BS. The relative contribution of BTI in the VectoLex® formulations used in 2006 (WSP) and 2007 (CG) to the observed efficacy is also not clear. The relationship between the effects of precipitation (water temperature and organic matter) need to be investigated either through discussions with the manufacturers and other municipalities, or research specifically designed to identify the effects of these variables, in order to answer the following operational questions: Is BS the best choice for controlling mosquito larvae within catch basins potentially subject to frequent flushing from precipitation events? Would products containing BTI or methoprene as the active ingredient be a better choice? In the long term, what is the best combination of larvicides to prevent the development of resistance?

The elevated concentrations of BT within catch basins and outflows before the start of the City's treatment program suggests that there are other sources of BT within the watersheds we monitored. The City may wish to consider having some of the samples further analyzed to identify the specific strain of BT within the samples, although it is likely BTI, as the larvicide is available to the general public through retail stores.

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Additionally, it would be helpful to know the extent to which these "other" inputs are occurring within the basins, outflows and creeks, in order to more effectively address questions of non-target toxicity.

The toxicity tests we conducted with the most frequently used larvicides (BTI, BS and methoprene) and juvenile salomonids (young coho salmon, ca. 18 g), as well as studies by others, suggest little potential for direct toxicity. However, comparable data for younger salmonids and sensitive invertebrates using a common metric for quantifying exposure of bacterial larvicides are lacking. In addition, our field studies in 2006 and laboratory studies in 2007 indicate that methoprene is effective at extremely low concentrations (<0.2 ppb). Should methoprene be considered as an alternative for controlling mosquito larvae in grated basins, comparisons of methoprene concentrations in outflows to those associated with toxicity to sensitive invertebrate taxa would also be warranted. These data would enhance our ability to evaluate the potential for non-target effects associated with inputs of these larvicides to surface waters following treatment of catch basins.

References

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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.

Appendix 1. Frequency distributions of the numbers of mosquito pupae within catch basins treated with VectoLex® CG and control (untreated) basins within the City of Seattle in 2007.

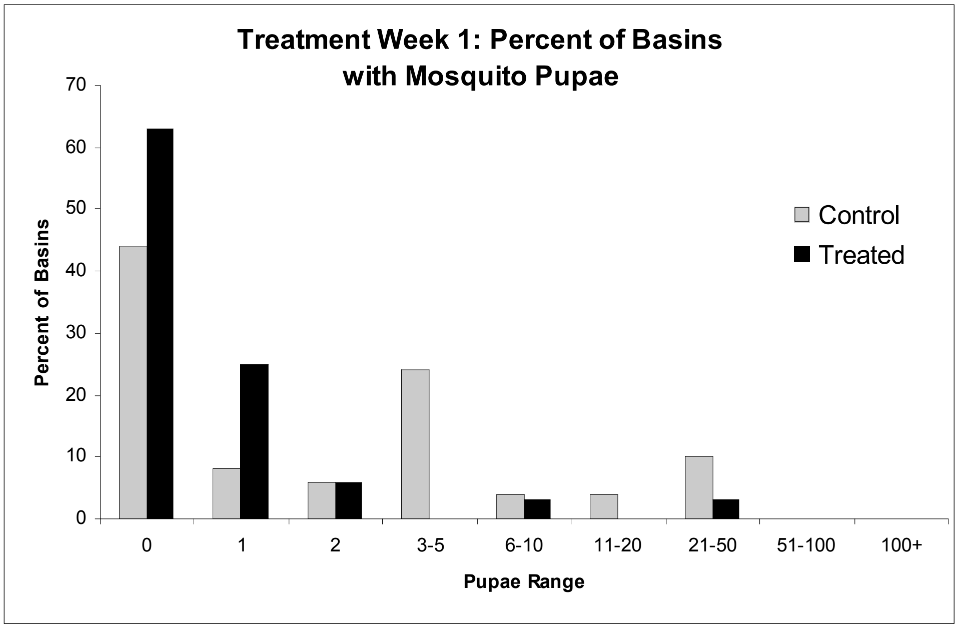

Figure A1-1. Percentage of basins with specified ranges of mosquito pupae within round top basins treated during Week 1 (N=50) and control basins (N=50). Data collected 1 week post-treatment.

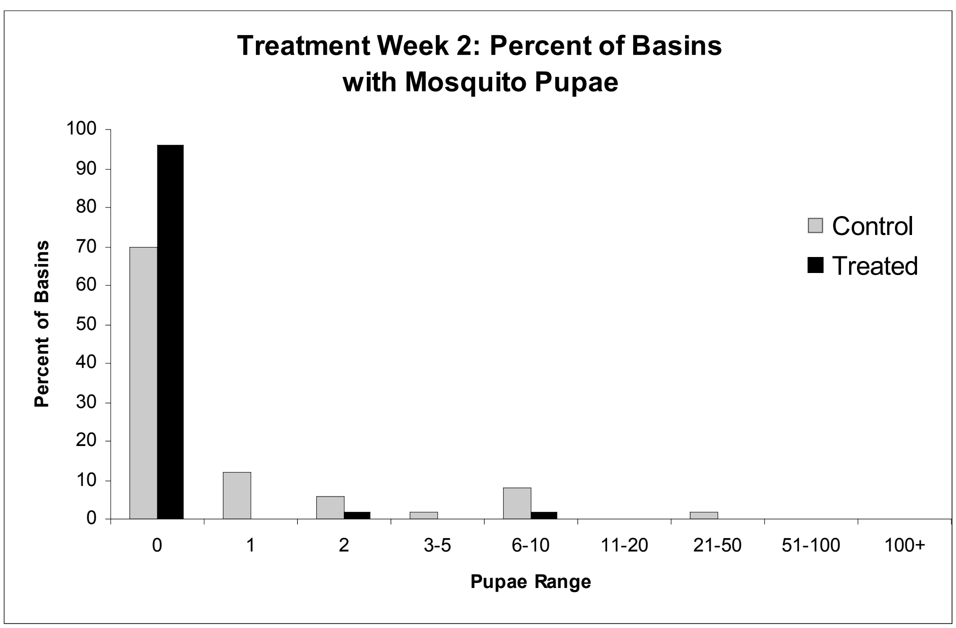

Figure A1-2. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the second week of treatment. Data collected 1 week post-treatment.

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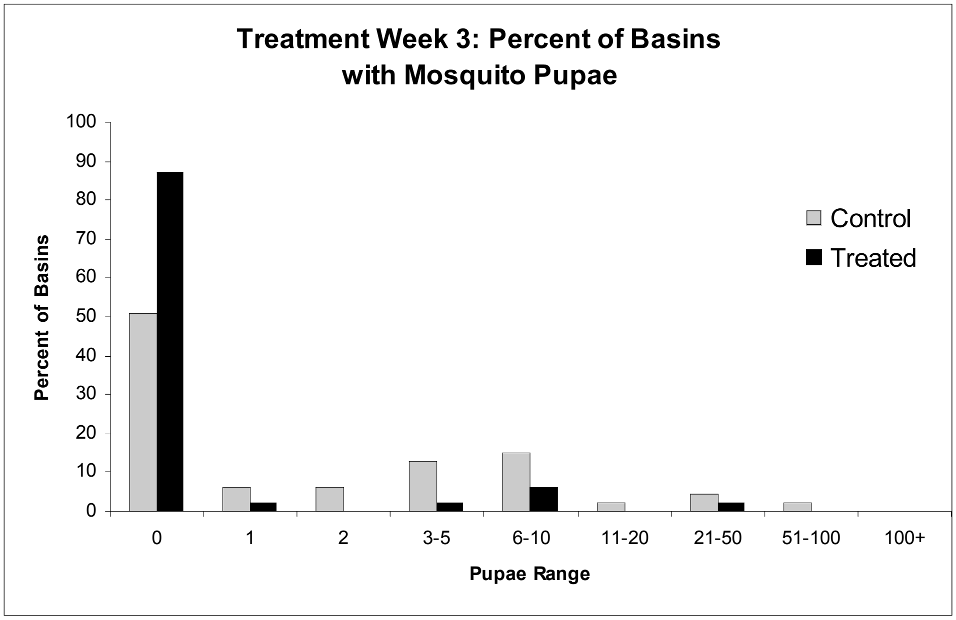

Figure A1-3. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the third week of treatment. Data collected 1 week post-treatment.

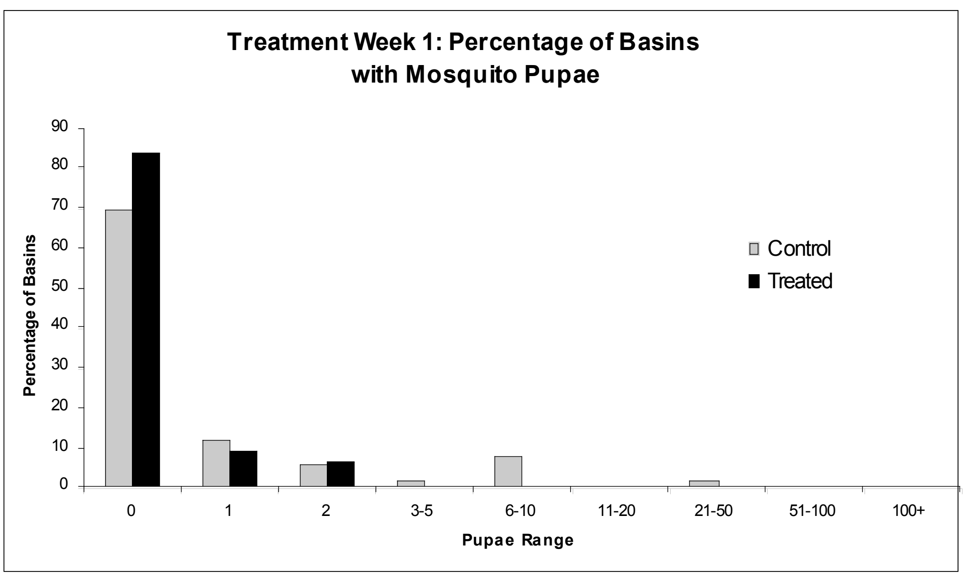

Figure A1-4. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the first week of treatment. Data collected 3 weeks post-treatment.

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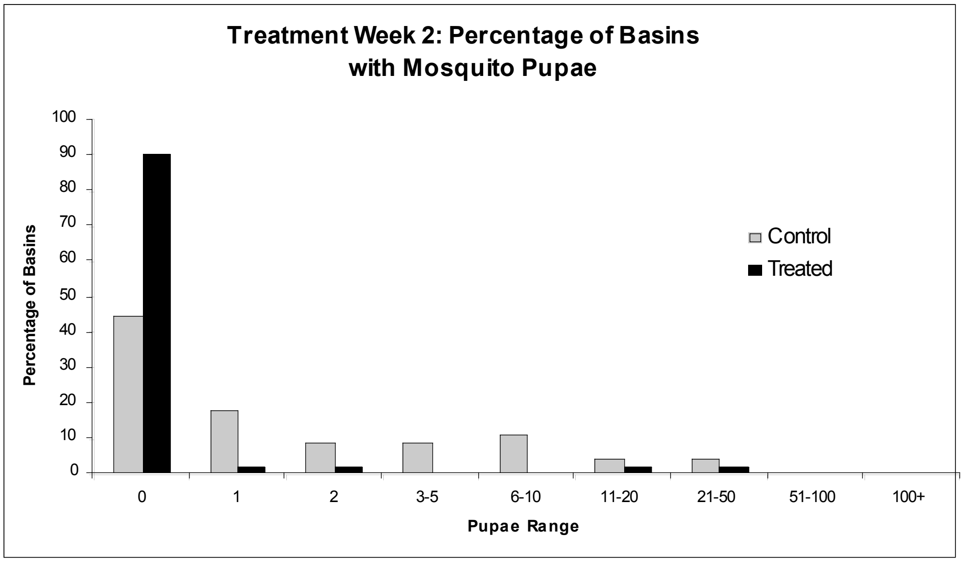

Figure A1-5. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the second week of treatment. Data collected 3 weeks post-treatment.

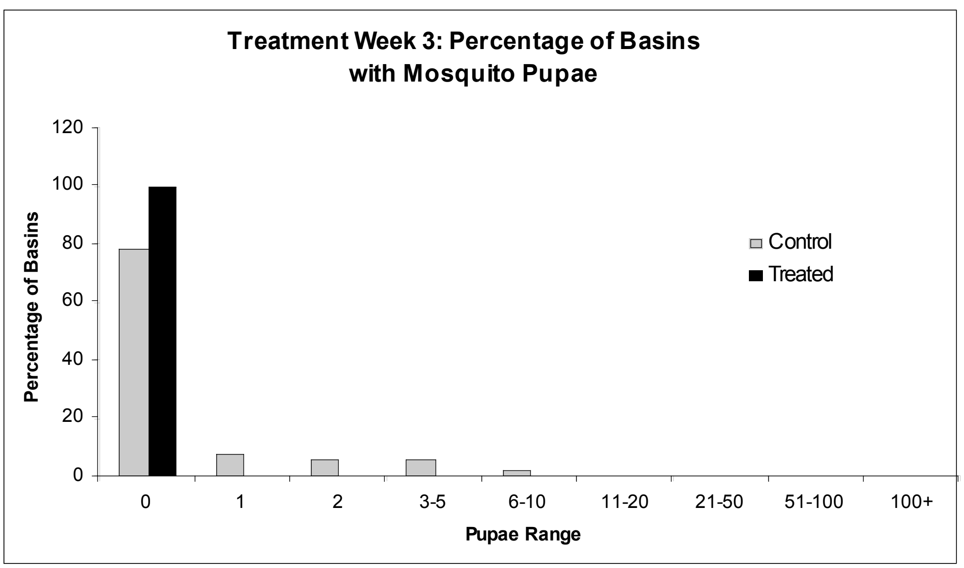

Figure A1-6. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the third week of treatment. Data collected 3 weeks post-treatment.

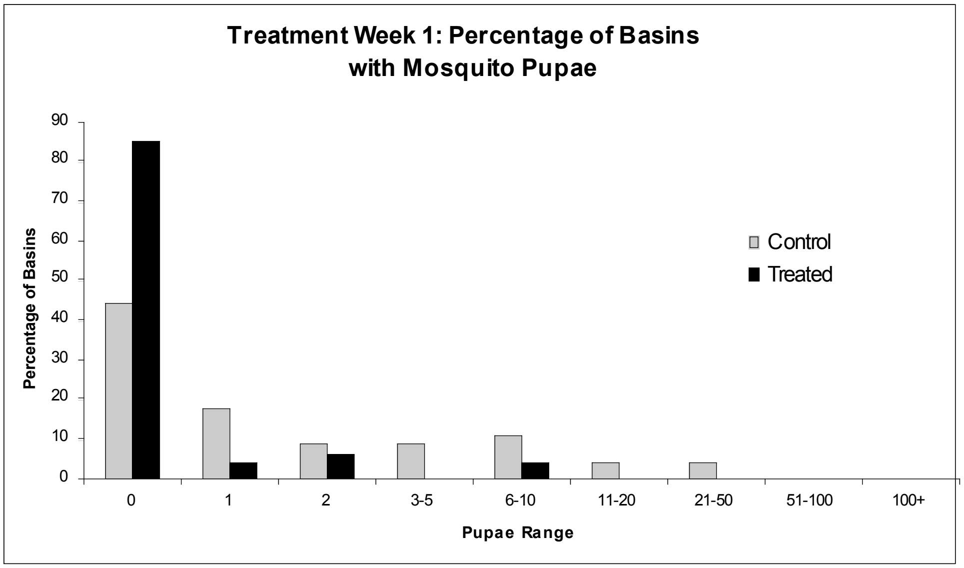

Figure A1-7. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the first week of treatment. Data collected 5 weeks post-treatment.

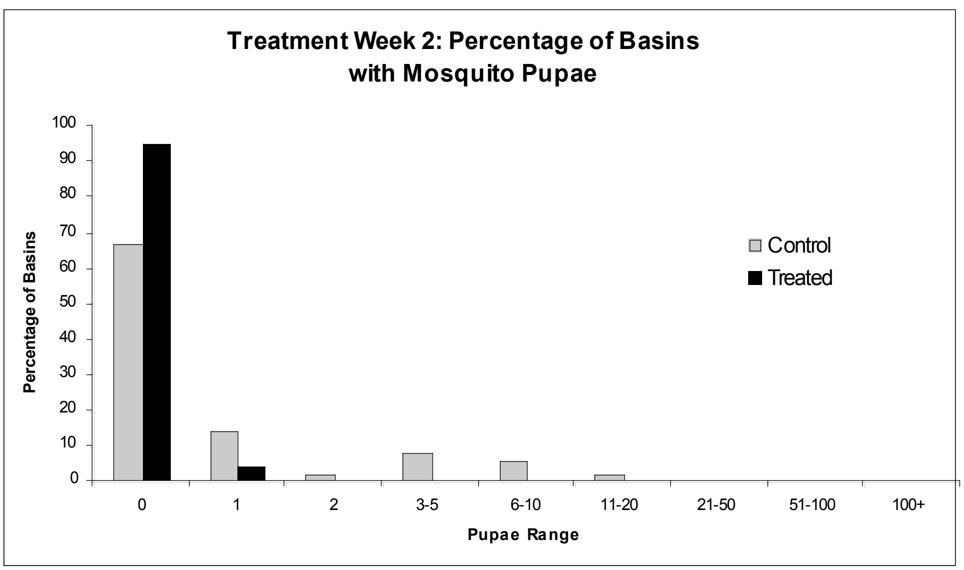

Figure A1-8. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the second week of treatment. Data collected 5 weeks post-treatment.

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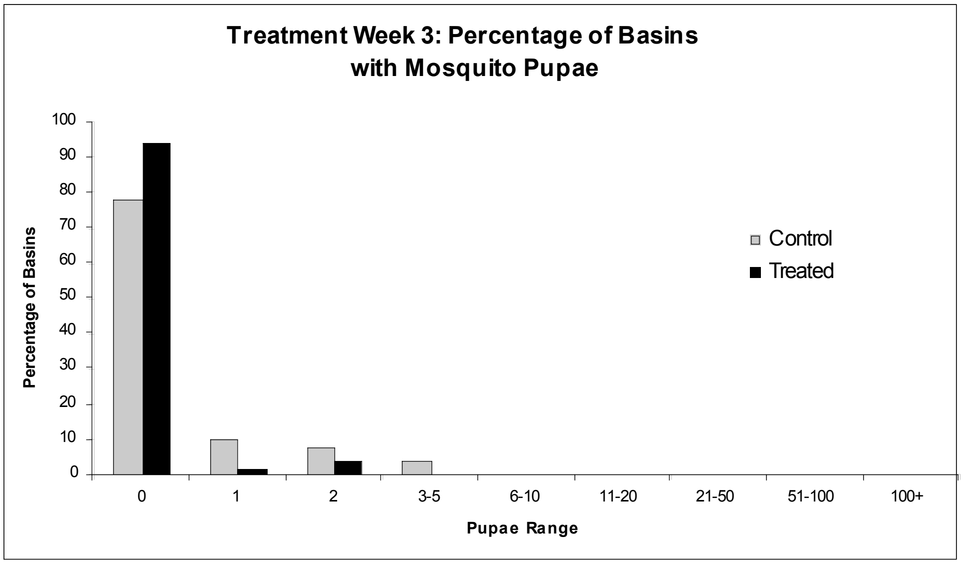

Figure A1-9. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the third week of treatment. Data collected 5 weeks post-treatment.

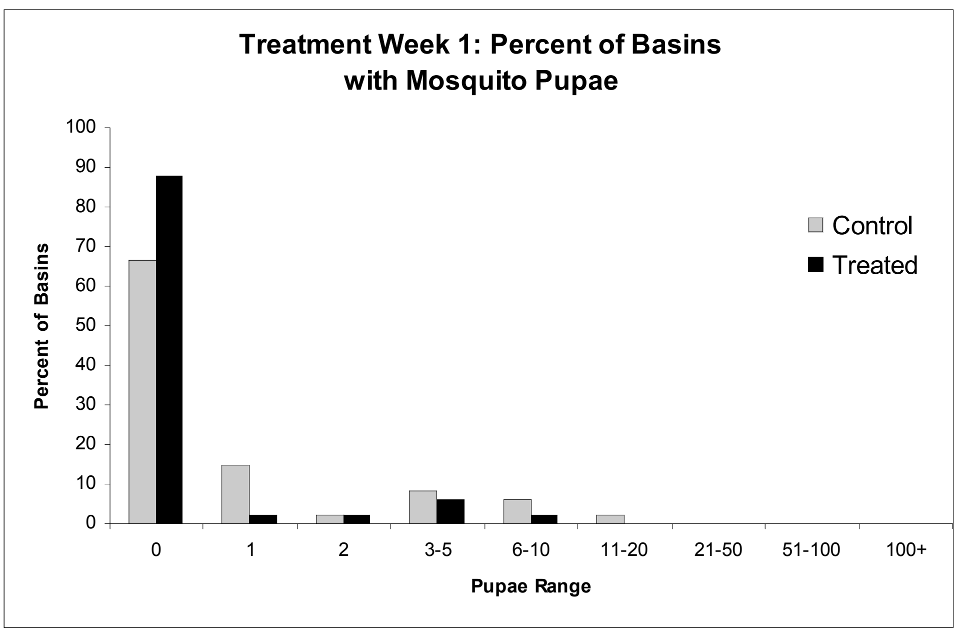

Figure A1-10. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the first week of treatment. Data collected 7 weeks post-treatment.

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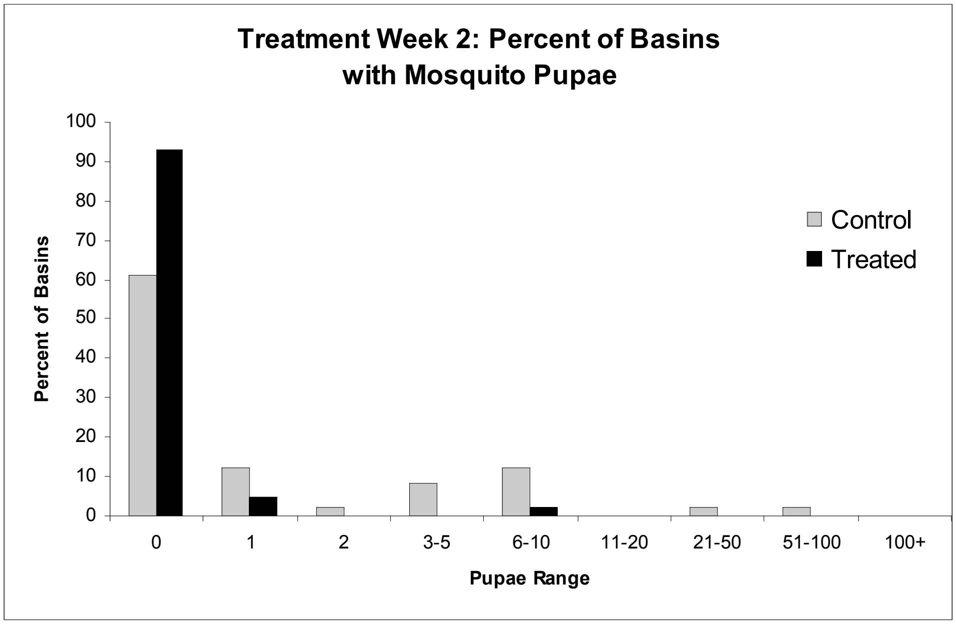

Figure A1-11. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the second week of treatment. Data collected 7 weeks post-treatment.

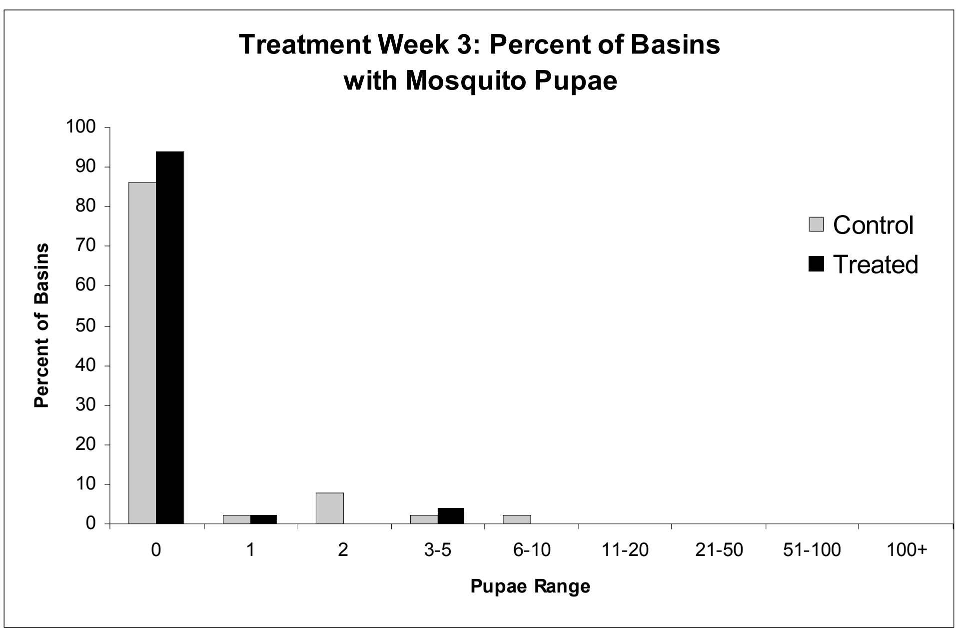

Figure A1-12. Percentage of basins with specified ranges of mosquito pupae for round top basins selected during the third week of treatment. Data collected 7 weeks post-treatment.

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Appendix 2. Locations of catch basins within the watersheds (sub-basins) of Longfellow (F23-F30), Pipers (F14-F22), and Thornton (F01-F13) creeks in which the efficacy and fate of VectoLex® CG was monitored in 2007.

Basin Number	Cross Street	Corner Direction	Basin Type
F01	NE 92nd and 35th NE	Southwest (on 92nd)	Grated
F02	NE 96th and 35th NE	Southwest (on 96th)	Grated
F03	NE 125th and 23rd NE	Southwest (on 125th)	Grated
F04	Roosevelt and 8th NE	Northwest	Round top
F05	NE 95th and Roosevelt	Southwest (on Roosevelt)	Grated
F06	N 107th and Meridian N	Southeast (on 107th)	Grated
F07	N 117th and 1st NE	Southeast (on 117th)	Grated
F08	NE 91st and 1st NE	Northeast (on 91st)	Grated
F09	NE 98th and 5th NE	Southeast (on 98th)	Grated
F10	NE 95th and 5th NE	Southeast (on 5th)	Grated
F11	NE 102nd and 5th NE	Northeast (on 102nd)	Grated
F12	NE 100th and 1st NE	Northwest (on 1st)	Grated
F13	NE 97th and 5th NE	Southeast	Round top
F14	NW 90th and 4th NW	Northeast	Round top
F15	NW 90th and 2nd NW	Northeast	Round top
F16	N 95th and 1st NW	Northwest (on 1st)	Grated
F17	NW 90th and 1st NW	Southeast	Round top
F18	NW 97th and 6th NW	Northeast	Round top
F19	NW 97th and 3rd NW	Southwest	Round top
F20	NW 87th and 3rd NW	Southwest	Round top
F21	NW 92nd and 4th NW	Northeast	Round top
F22	NW Woodbine and 14th NW	Southeast	Round top
F23	SW Hudson and Delridge SW	Southeast	Round top
F24	SW Myrtle and 31st SW	Southwest	Round top
F25	SW Thistle and 20th SW	Northwest (on 20th)	Grated
F26	SW Thistle and 22nd SW	Southeast	Round top
F27	SW Thistle and 24th SW	Southeast	Round top
F28	SW Barton and 30st SW	Northwest	Round top
F29	SW Roxbury and 20th SW	Southeast	Round top
F30	SW Barton and 31st SW	Northwest	Round top

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Appendix 3. Selected frequency distributions of the numbers of mosquito pupae within catch basins treated with VectoLex® CG and control (untreated) basins within the City of Seattle in 2007 in which the resolution on the Y-axis (spore counts/ml) has been increased to better represent low values of BS and BTI.

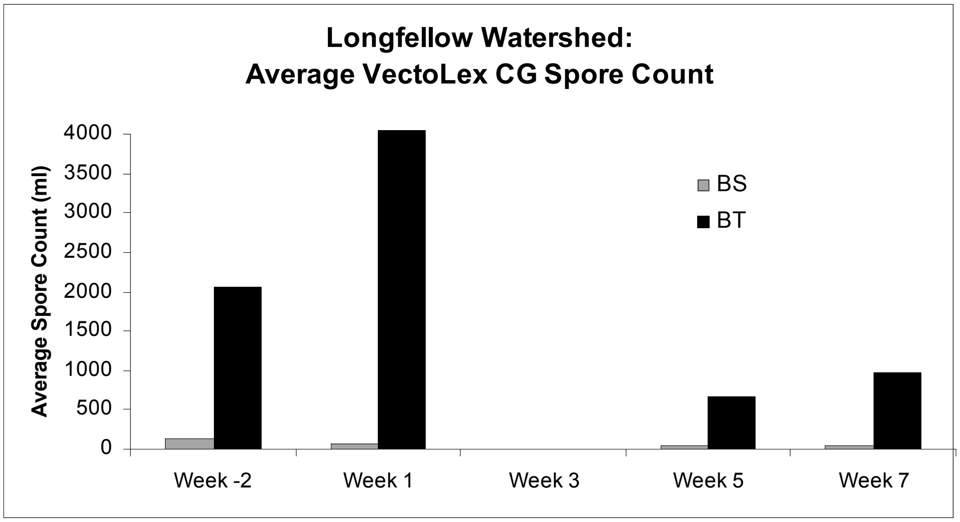

Figure 3A-1. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) within the Longfellow Watershed catch basins measured in spores/ml (N = 8). Y-axis values are re-scaled to include smaller values. Week 1 BT counts exceed the scale on this graph; the actual value for BT within week 1 is 306,250 spores per ml.

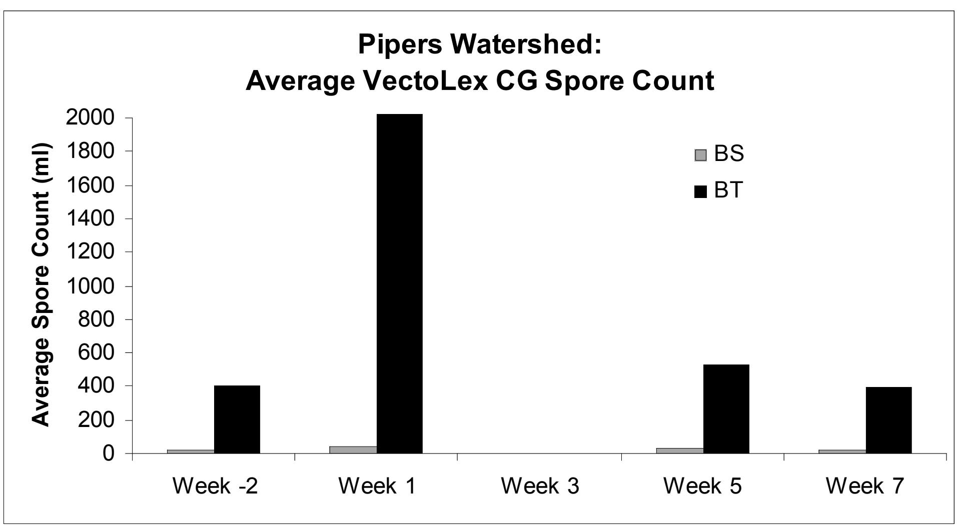

Figure A3-2. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) within the Pipers Watershed catch basins measured in spores/ml (N = 9). Y-axis values are re-scaled to include smaller values. Week 1 BT counts exceed the scale on this graph; the actual value for BT within week 1 is 138,789 spores per ml.

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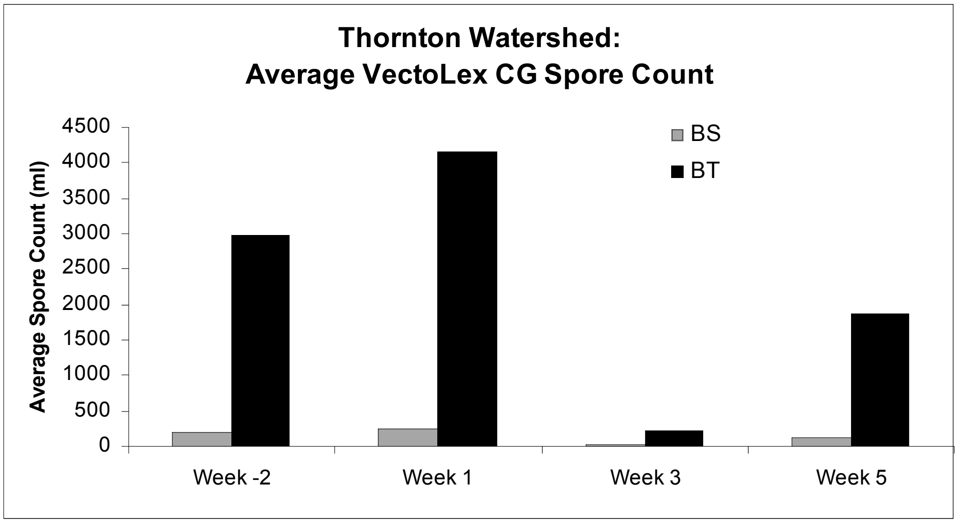

Figure A3-3. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) within the Thornton Watershed catch basins measured in spores/ml (N = 13).

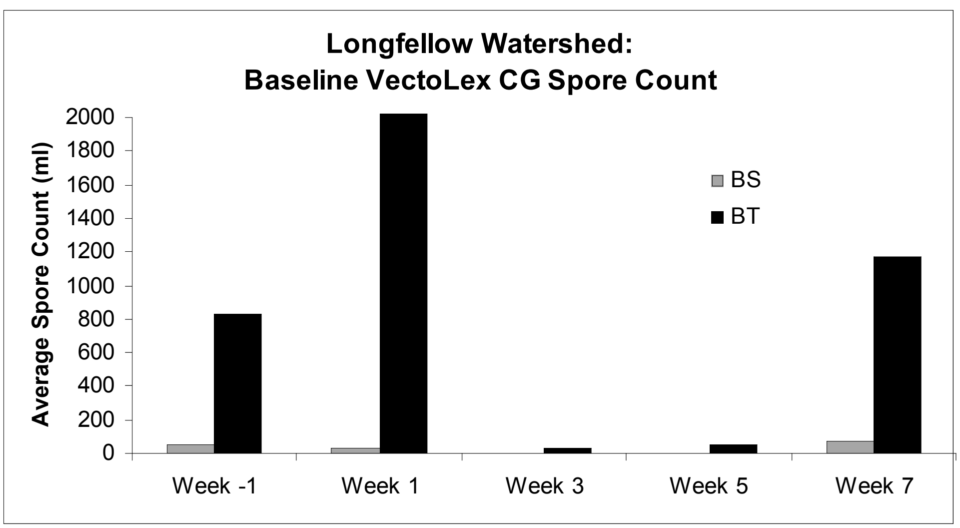

Figure A3-4. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) within the Longfellow Watershed outflow pipes measured for baseline analysis in spores/ml (N = 3). Y-axis values are re-scaled to include smaller values. Week 1 BT counts exceed the scale on this graph; the actual value for BT within week 1 is 130,023 spores per ml.

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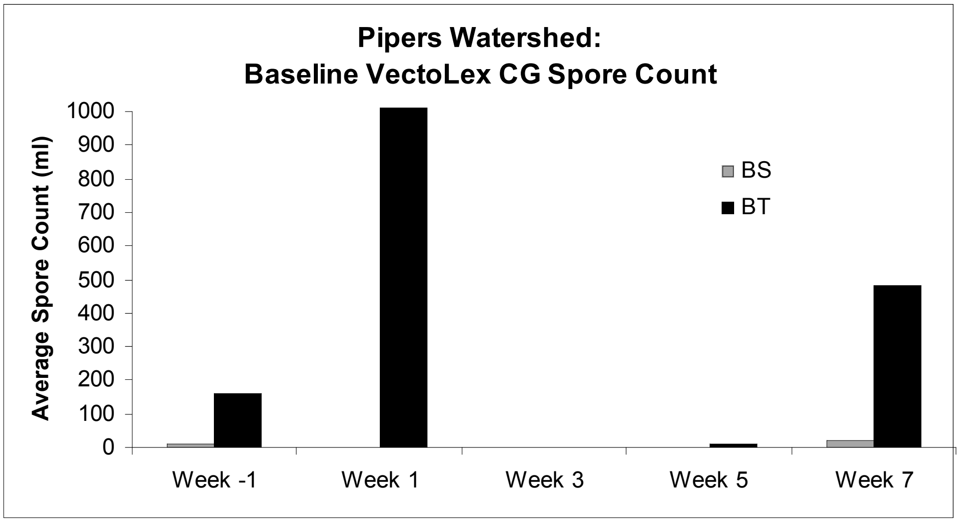

Figure A3-5. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) within the Pipers Watershed outflow pipes measured for baseline analysis in spores/ml (N = 1). Y-axis values are re-scaled to include smaller values. Week 1 BT counts exceed the scale on this graph; the actual value for BT within week 1 is 9,400 spores per ml.

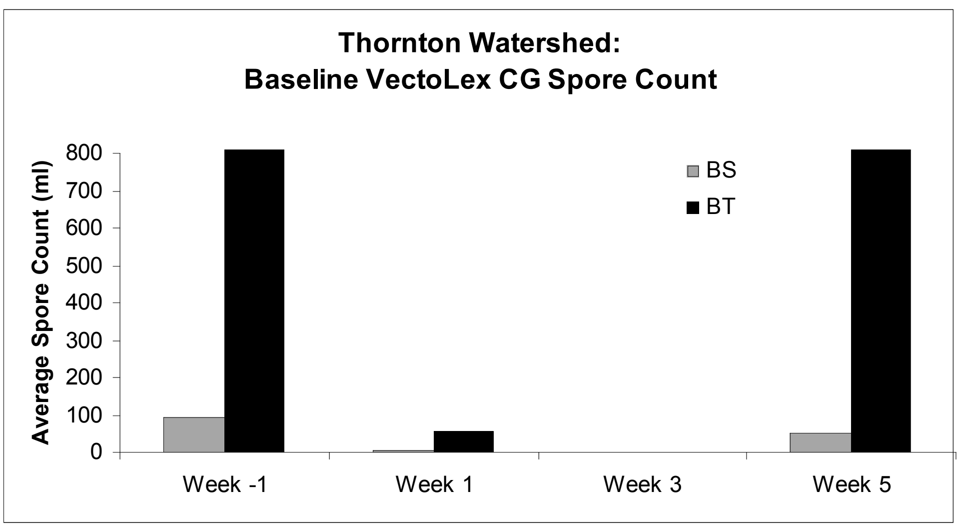

Figure A3-6. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) within the Thornton Watershed outflow pipes measured for baseline analysis in spores/ml (N = 5). Y-axis values are re-scaled to include smaller values. Week -1 and Week 5 BT counts exceed the scale on this graph; the actual value for BT within Week -1 is 1, 485 spores per ml and the actual value for BT within Week 5 is 844 spores per ml.

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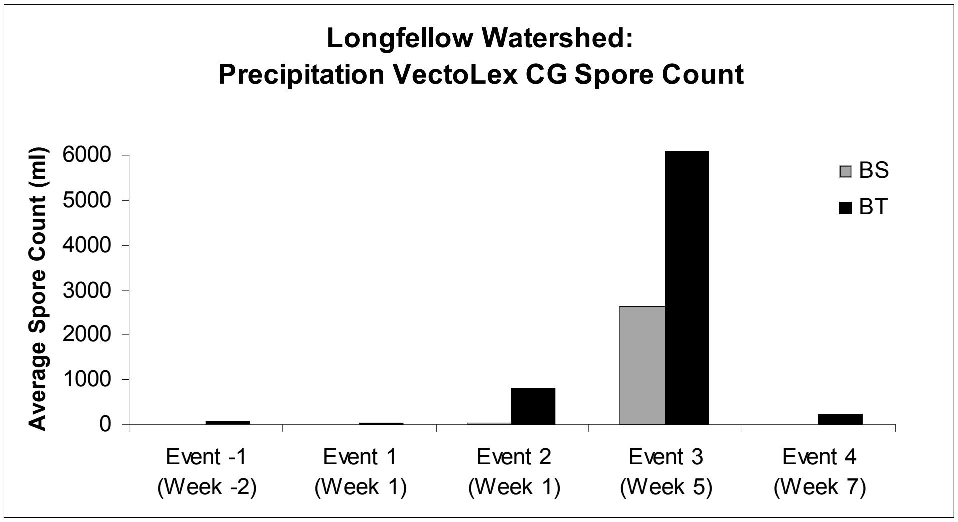

Figure A3-7. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) within the Longfellow Watershed outflow pipes measured during a precipitation event in spores/ml (N = 3). Y-axis values are re-scaled to include smaller values. Event 3 (Week 5) BT counts exceed the scale on this graph; the actual value for BT within Event 3 is 43,597 spores per ml.

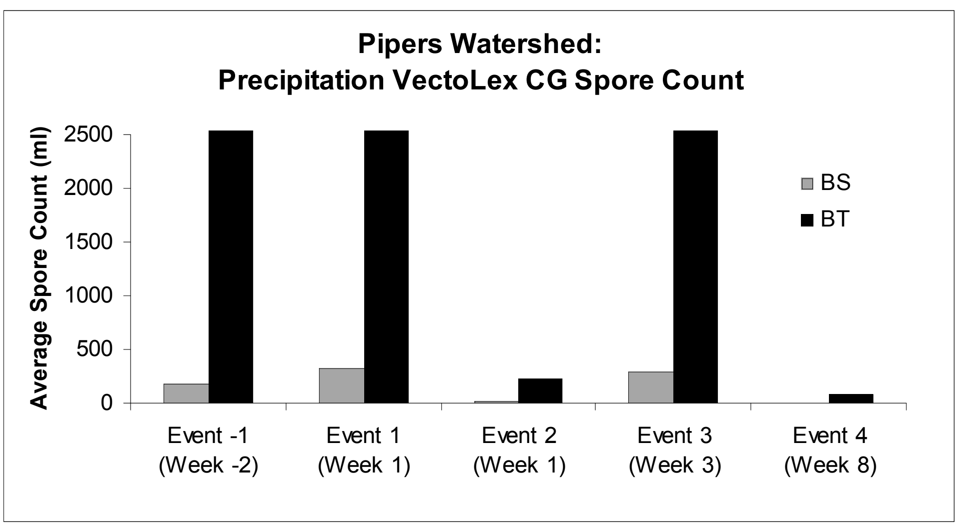

Figure 3A-8. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) within the Pipers Watershed outflow pipes measured during a precipitation event in spores/ml (N = 2). Y-axis values are re-scaled to include smaller values. Event -1 (Week -2), Event 1 (Week 1), and Event 3 (Week 3) BT counts exceed the scale on this graph; the actual value for BT within Event -1 is 2,745 spores per ml, within Event 1 is 5,300 spores per ml, and within event 3 is 4,695 spores per ml.

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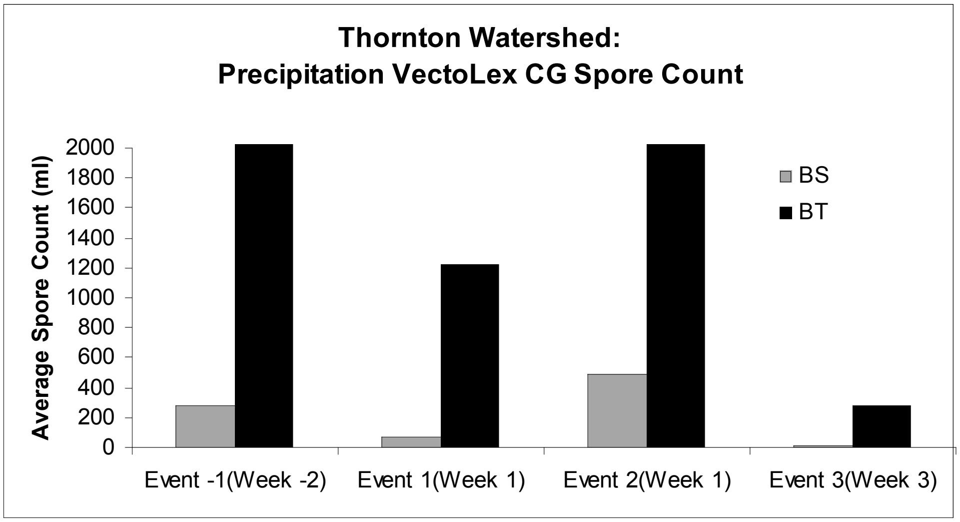

Figure 3A-9. Counts of *B. sphaericus* (BS) and *B. thuringiensis* (BT) within the Thornton Watershed outflow pipes measured during a precipitation event in spores/ml (N = 5). Y-axis values are re-scaled to include smaller values. Event -1 (Week -2) and Event 2 (Week 1) BT counts exceed the scale on this graph; the actual value for BT within Event -1 is 4,432 spores per ml and within Event 2 is 7,984 spores per ml.

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