Boise Creek Bacterial Source Tracking Study:

2011 Summary of Findings

March 2012

King County

Department of Natural Resources and Parks
Water and Land Resources Division
Science Section
King Street Center, KSC-NR-0600
201 South Jackson Street, Suite 600
Seattle, WA 98104

Alternate Formats Available

206-296-7380 TTY Relay: 711

Boise Creek Bacterial Source Tracking Study:

2011 Summary of Findings

Submitted by

Raymond Timm
King County Water and Land Resources Division
Department of Natural Resources and Parks

King County
Department of
Natural Resources and Parks
Water and Land Resources Division

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Acknowledgements

This document was dramatically improved because of helpful and timely reviews by a number of people in the Science Section of the King County Water and Land Resources Division. In particular, Sally Abella, Deb Bouchard, Kate O'Laughlin and Dave White provided insight that improved both content and readability. Tom Ventur patiently formatted several iterations of the document. Also, many thanks go to personnel from the King County Environmental Laboratory for their willingness to help guide this effort from conception through reporting. Special thanks go to Lab Project Manager Colin Elliott, the Microbiology Group, especially Eric Thompson, Joe Clark, Debbie Turner and the Field Science Unit, in particular Ben Budka and Stephanie Hess.

INTRODUCTION AND BACKGROUND

This study focused on the water quality impairments in Boise Creek because of its history of bacterial contamination. This report describes the study and findings from the first year of investigation into the sources of microbial contamination, as well as continuing work toward solving the problem. The goals of this project were to investigate and identify sources of bacterial contamination along Boise Creek and its tributaries. The primary objective was to discriminate among the potential sources of bacteria and group them according to human or agricultural origin to more effectively inform cleanup strategies.

In the 2011 adopted budget, the King County Council allocated the first round of limited funds for studying rural sources of water quality impairment in areas of the County that are outside of the King County Wastewater Treatment Division service area. Water quality impairments in rural King County include such things as high temperatures or nutrient concentrations, deviations from natural pH, and bacterial contamination.

The federal Clean Water Act requires that a total maximum daily load (TMDL) be developed for each of the water bodies on the state's 303(d) list of polluted waters. The TMDL identifies pollution problems in the watershed, and then specifies how much pollution needs to be reduced or eliminated to achieve clean water. The Puyallup River Watershed has many listings on the federal Clean Water Act 303(d) list as impaired for fecal coliform bacteria. Boise Creek, which is a tributary in the Puyallup River drainage has exhibited bacterial contamination in exceedance of water quality standards regularly since the problem was first formally identified in 1996. Since then, a number of stations in Boise Creek have been monitored along the creek and its tributaries by Ecology, King County and the City of Enumclaw.

In the Puyallup River fecal coliform (FC) TMDL, Boise Creek was the largest FC bacteria loading source of any tributary in the study area. It also required the largest FC reduction of any dry season source within the Puyallup River Basin. The Boise Creek watershed was identified as the number one priority for improvement for this TMDL (Mathieu and James, 2011). The completed water quality improvement report and implementation plan for the Puyallup River system can be located at: http://www.ecy.wa.gov/biblio/1110040.html .

High bacterial concentrations occur in Boise Creek during summer low flow and winter high discharge conditions. Fecal coliform (FC) load reduction targets are substantially higher during low flow conditions and have been defined for more locations than during winter months. But, during both seasons, FC reduction targets are high, ranging from 57% to 92% in summer, to 61% to 67% in winter in order to achieve Washington State water quality guidelines of 200 colony forming units (cfu) per 100mL (Mathieu and James, 2011). The high summer FC loadings suggest there is a potential for non-storm driven sources such as leaking sewer pipes, septic systems, or cross-connections that could be causing increased bacterial loadings. High numbers during the wet season likely implicate runoff from the landscape as a contributor as well. As some potential sources are human in origin, increased exposure via contact with water from Boise Creek to bacterial and viral pathogens could be a significant public health concern.

Fecal coliform bacteria are the basis for the State water quality standard. However, because they are found in the intestinal tracts of all warm blooded animals, they are not very helpful in discriminating among sources. This study employed a number of different approaches being developed jointly by the King County Science Section and the Environmental Lab in the effort to determine sources of microbial contamination. The approach uses multiple organisms that have varying degrees of specificity to both human and ungulate hosts

STUDY AREA

Headwaters of Boise Creek begin in the Cascade Mountains east of the City of Enumclaw, Washington and drains more than 18 square miles (nearly 12,000 acres). More than 65% of the drainage area lies within the Forest Production District, with the remainder of the drainage dominated by agricultural land cover (13%), and the City of Enumclaw (11%). In addition, slightly more than 3% of the land area is comprised of farms that are enrolled in farmland preservation programs of the County which means that they will maintain their agricultural character into the foreseeable future (King County, 2012). In addition, there are many small tributaries and ditches that drain from agricultural land within the drainage (Figure 1).

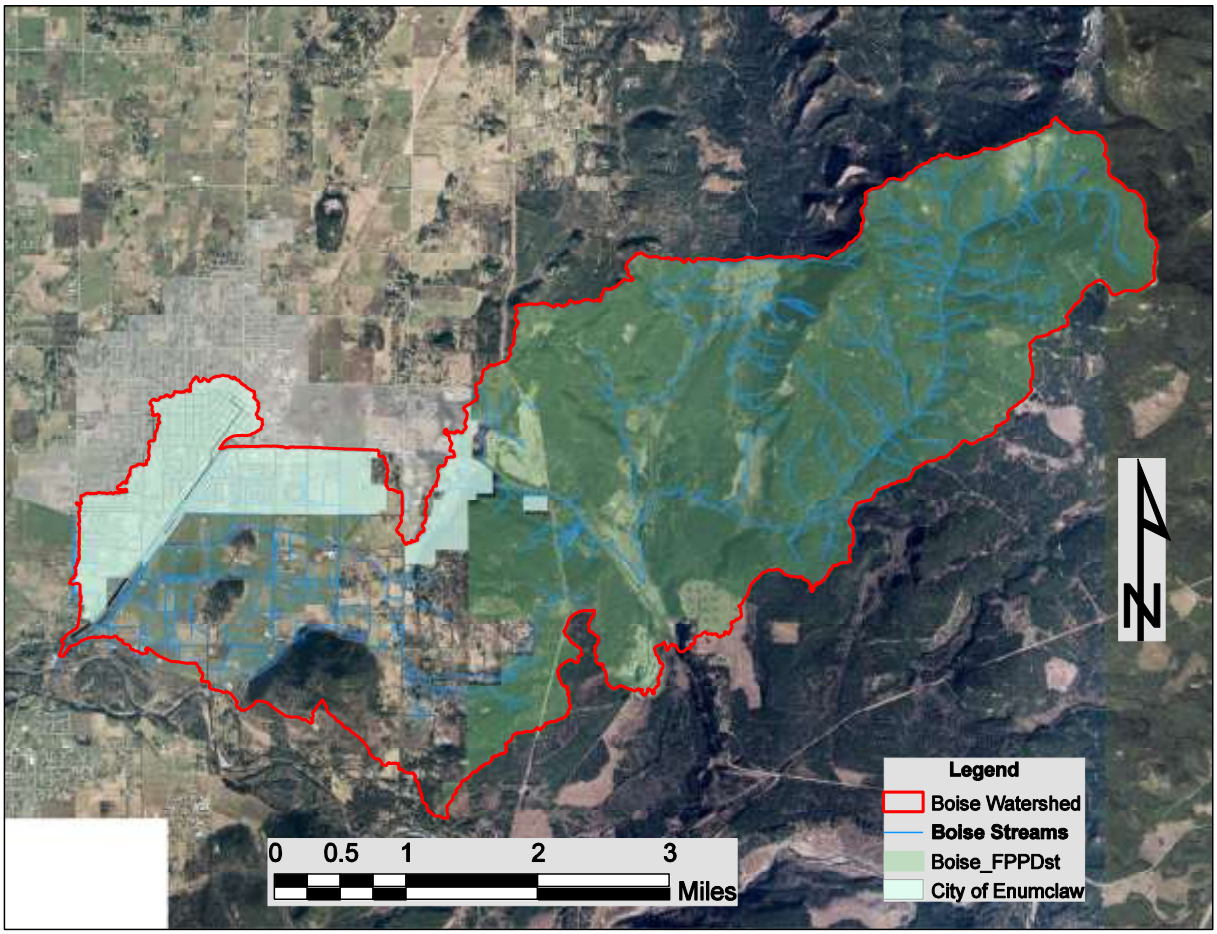

Figure 1. Overview of Boise Creek drainage. The eastern two thirds of the drainage is in forestry production, the northwestern portion is within the City of Enumclaw, and the remainder is rural agricultural – primarily dairy.

This microbial source tracking study incorporated twelve locations within the lower one third of the drainage, focusing on the agricultural and urban portions of the drainage (Figure 2). Sampling locations were distributed on both branches of tributary junctions in order to isolate sources of bacterial contamination geographically. Sampling sites were located within public rights of way and on public property, beginning near the mouth of the stream and proceeding upstream along branches of the creek. The sampling locations were split evenly between the City of Enumclaw (sites 3, 4, 5, 7, 11, and 12) and unincorporated King County (sites 1, 2, 6, 8, 9, and 10). The Enumclaw sites were all tributary locations, while the County sites were along the mainstem, except for site # 10 which was on a small roadside ditch at its confluence with the creek (Figure 2). Additionally, the bacterial analyses aimed to discriminate between human and ruminant sources of bacteria. This information should inform cleanup efforts of bacterial contamination in the creek drainage, ensuring that resources are spent effectively.

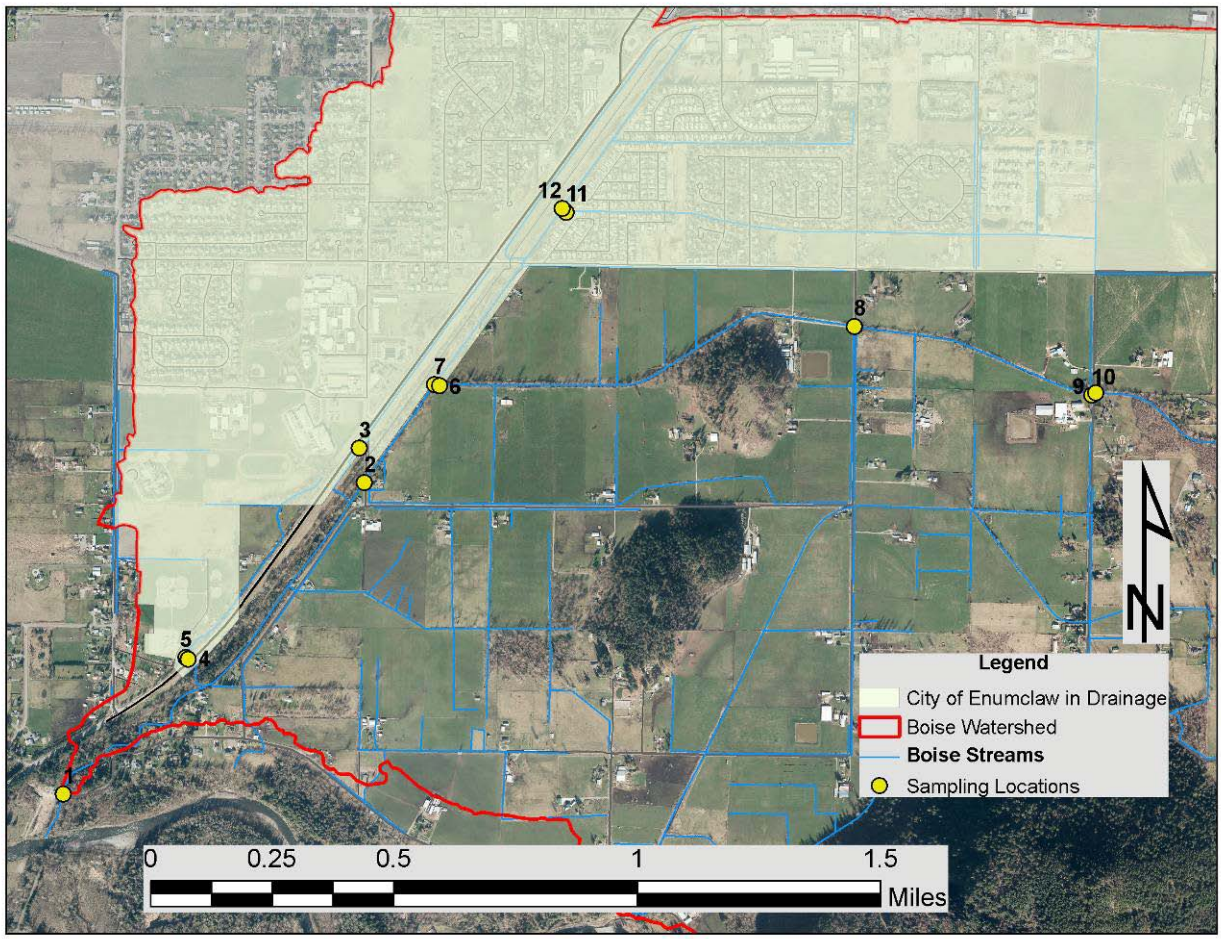

Figure 2. Twelve Boise Creek Microbial Source Tracking Sampling Locations. Sites 4, 5, 7, 11, and 12 are within the city of Enumclaw, WA. Sites 1,2,3,8,9, and 10 are in agricultural lands of unincorporated King County.

STUDY DESIGN

The design focused on sampling for total Fecal Coliform, and *Escherichia coli* (*E. coli*), *Rhodococcus coprophilus*, sorbitol – fermenting *Bifidobacteria*, and *Bacteroides thetaiotaomicron* to look for differences in bacterial community composition and concentrations related to land use or waste system failures. The Microbiology Unit of the King County Environmental Laboratory adopted a "toolkit" approach for microbial source tracking using these indicator species following Plummer and Long (2009). This project was a continuation of the field testing of these methods to provide information on the usefulness of these indicators in identifying the presence of grazing animal or human-source waste in surface waters (pers. comm. Eric Thomson, 2012).

All organisms were cultured on live media except for *Bacteroides,* which was analyzed using quantitative polymerase chain reaction (qPCR) molecular methods to isolate the s16 rRNA sequence specific to that organism. Quantitative PCR presents definitive results for positive samples but the units are in terms of numbers of individuals because the qPCR method quantifies the number of specific DNA chains found in respective samples. Data quality, specificity, and sensitivity are still under investigation (USEPA, 2011; King County, 2011).

Four sampling events were scheduled so that two occurred during the typical summer low flow period (July to September) and two during wet weather months. Sampling occurred during dry weather to capture bacterial pollution levels during base flow conditions, and wet season events targeted the creek during a rising hydrograph when turbidity should be higher than normal, indicating runoff from the surrounding landscape is reaching the stream. An antecedent dry period of at least 24 hours with no more than 0.02 inches of rain was incorporated to ensure that creek flows were close to base flow when sampling began. However, laboratory preparation of culture media required several days of advance notice. So, weather predictions were actually used more to guide the sampling calendar than to respond to current weather and hydrologic conditions.

Planning specific sampling events was informed, to the degree practicable by readily available local precipitation gauges and stream discharge information. The King County hydrologic conditions website reports data from a telemetered rain gauge located within the Boise Creek drainage located at:

http://green.kingcounty.gov/WLR/Waterres/hydrology/GaugeMetaData.aspx?G_ID=155

There is also a continuous flow gauge maintained by the County on Boise Creek at 276th Ave SE. The data from this stream gauge can be obtained at:

http://green.kingcounty.gov/WLR/Waterres/hydrology/GaugeMetaData.aspx?G_ID=1073

In addition, the US Geological Survey (USGS) operates a flow gauge near the confluence with the White River. Data from this gauge can be obtained at:

http://waterdata.usgs.gov/wa/nwis/uv?site_no=12099600

FIELD SAMPLING

Sampling locations (Figure 2) were spatially arranged at tributary junctions in order to isolate potential sources geographically. Sample collection was scheduled during the morning to allow ensure samples were delivered to the lab within the prescribed holding times (King County, 2011). This scheduling allowed for all samples to be processed at KCEL within the 6 hour holding time for *Bifidobacteria* samples. Each location (Figure 2 and Table 1) was investigated prior to sampling to ensure safety and facilitate collection so that holding time criteria were met.

Grab samples for bacteria were collected at each sampling location for every designated sampling run. If site access from the streambank was limited, or if stream discharge made it unsafe to wade the stream, a sampling pole with a one 1iter (L) bottle holder was used to collect the grab sample. A digital photograph was taken at each sampling location during the initial site visit, focusing on the wetted portion of the stream where the sample was collected. Field observations were recorded by sampling personnel related to stream flow conditions or other factors observed that could impact data quality.

Collection for *Bifidobacter* analysis required sample transfer from the collection bottle to a sterile bottle containing sodium sulfite preservative. In addition, *Bifidobacter* had other specific sampling and culturing considerations due to its anaerobic nature. For the collection of *Rhodococcus*, samples were collected directly into a 250 ml sterile, glass bottle. Containers were not pre-rinsed with sample prior to collection. All filled sample containers were stored immediately in ice-filled coolers for transport to the lab (King County, 2011).

Table 1. Sampling Station Location and Coordinates*. Sites are located in road rights of way or on public property in the City of Enumclaw.

LIMS Locator	Creek/tributary name	site street/intersection/address	X	Y	Lat	Long
Bse_1Mud MtnRd	Boise Creek near the mouth	Mud Mountain Road bridge	66500.6229	1345664.0215	47 10.572	-122 1.115
Bse_4_Bse CrPkCulvert	Tributary to Boise Creek	Confluence of Bse_5_BseCrPkDitch and small wetland, in culvert just upstream of Hwy 410	67966.8066	1347018.7901	47 11.129	-122 0.332
Bse_5_Bse CrPkDitch	Tributary to Boise Creek	Trib near Boise Creek Park upstream of confluence with Bse_4_BsCrPkCulvert	67983.2171	1346990.3365	47 11.192	-122 0.353
Bse_3_410 Ditch	Tributary to Boise Creek	Ditch downstream of 252 Ave SE and SR 410 intersection	70254.2840	1348871.4497	47 10.813	-122 0.792
Bse_2_252 AveSE	Boise Creek at 252nd Ave	Main stem downstream of 252 Ave SE	69878.1451	1348923.6213	47 10.81	-122 0.785
Bse_6Main stem	Boise Creek	Boise Creek near Foothills Trail upstream confluence with Bse_7TribDitch	70931.9530	1349738.4103	47 11.305	-122 0.14
Bse_7TribD itch	Tributary to Boise Creek	Tributary that runs parallel to Foothills Trail upstream of confluence with Bse_6Mainstem	70941.7962	1349684.6290	47 11.307	-122 0.152
Bse_8_268 AveSE	Boise Creek at 268th Ave	Boise Creek upstream of 268th Ave SE crossing	71573.1687	1354236.8509	47 11.426	-121 59.06
Bse_9_276 AveSE	Boise Creek at 276 Ave	Boise Creek upstream of 276th Ave SE	70854.0331	1356849.4959	47 11.309	-121 58.42
Bse_10_27 6Ditch	Tributary to Boise Creek	276th Ave SE bridge (lateral ditch DS of bridge)	70828.8103	1356812.2576	47 11.305	-121 58.43
Bse_11Trail erPkDitch	Tributary to Boise Creek	Trib draining trailer park upstream of Warner Ave.	72811.2199	1351115.1855	47 11.607	-121 59.81
Bse_12Enu mclawDitch	Tributary to Boise Creek	above Trib junction upstream of Warner Ave.	72849.6499	-1351071.2654	47 11.623	-121 59.82

*NAD 1983 HARN State Plane Washington North FIPS 4601 Feet; and degree decimal minutes.

RESULTS

Four sampling events occurred during 2011; April 13, June 1, July 13, and October 15. All twelve locations (Figure 2) were sampled during each event. Laboratory analysis was performed consistent with an extensive quality control process (King County, 2011), and then posted into the King County laboratory information management system (LIMS) database.

In general, numbers were lowest during the April 13 sampling event and increased as summer progressed and water levels approached summer base flow conditions. During that event, fecal coliform bacteria exceeded state standards at station 11 (Figures 2, 3, Table 2). During the three subsequent sampling events (June 1, July 13, and October 5), every sampling location was in exceedance of state water quality guidelines for both parameters except station 9, which was slightly below state standards during the July 13 sampling event (Table 2). It is notable that most of the water quality exceedances were during the summer and fall sampling events and that mean exceedance values as well as the variability around those means increased continuously (Figure 4).

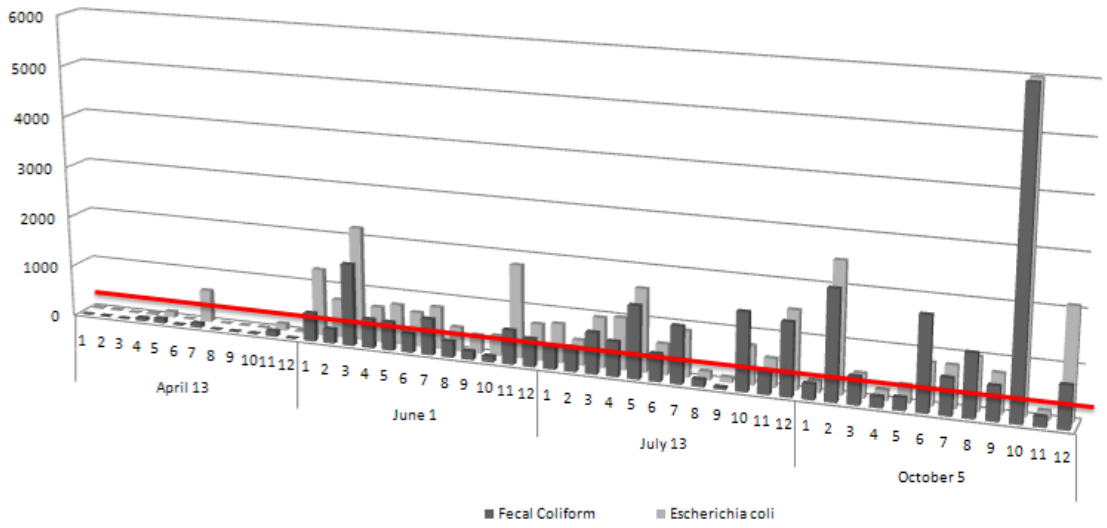

Figure 3. Fecal coliform and *E. coli* levels during four sampling events at twelve locations during 2011.
See Figure 2 (above) for locations of each sampling location. Values on the Y axis indicate colony forming units / 100mL of sample. The broad red line indicates the level below which contamination is within State of Washington water quality standards.

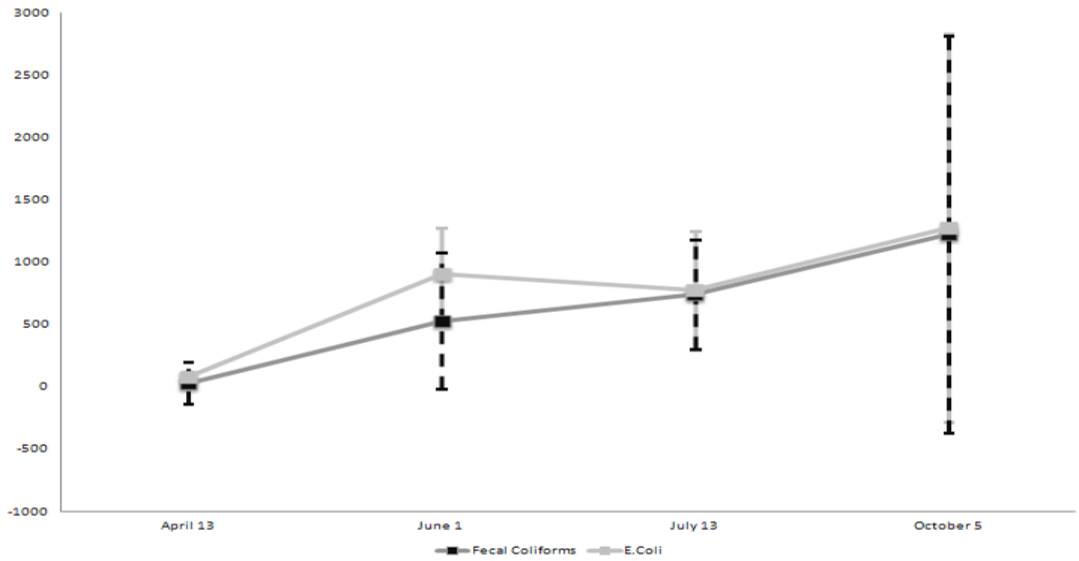

Figure 4. Monthly mean colony forming units (cfu) for fecal coliforms and E. coli bacteria in the Boise Creek drainage.
Means were generated from all 12 sampling locations (see Figure 2). Error bars represent +/- 1 standard deviation. The Y axis units are cfu.

Table 2. Microbial* source tracking results from twelve sampling locations in Boise Creek across four dates during 2011.

	14-Apr					17-Jun			13-Jul					5-Oct			
Site	**Rhodo ‡**	**Bifido§**	**Fecals**	**E.Coli**	**Bacteroides**	**Fecals**	**E.Coli**	**Bacteroides**	**Rhodo**	**Fecals**	**E.Coli**	**Bacteroides**	**Bifido**	**Fecals**	**E.Coli**	**Bacteroides**	**Bifido**
1	<5	30	5	21	205	550	1300	140	<5	440	750	841	Pos	300	230	257	<1
2	R+3	90	58	35	679	680	560	5005	5	670	1000	7820	<1	210	200	758	<1
3	R+3	Pos	90	110	2049	550	770	987	10	1400	1600	24170	<1	240	340	917	<1
4	<5	<1	4	7	475	1600	2200	1086	<5	800	2200	2888	<1	530	460	1266	<1
5	<5	<1	7	21	182	280	750	457	5	960	750	1208	<1	2100	2500	825	<1
6	<5	<1	10	8	304	370	660	208	<5	530	590	785	<1	1800	800	278	<1
7	<5	<1	84	630	1495	700	810	5608	<5	1100	890	4730	Pos	700	800	617	<1
8	<5	<1	3	4	167	310	460	260	<5	140	160	338	<1	1200	1000	230	<1
9	<5	<1	1400	750	8750	160	350	201	<5	40	95	173	<1	650	750	278	<1
10	<5	<1	9	8	1440	110	380	1413	<5	1500	750	21834	<1	6000	6000	44391	<1
11	<5	<1	110	130	1870	660	1800	766	15	450	550	949	<1	190	160	238	100
12	<5	<1	4	7	589	540	700	2827	<5	1400	1500	8750	Pos	810	2100	7165	<1

*Parameters sampled include *Rhodococcus spp.*, sorbitol-fermenting *Bifidobacteria spp*., fecal coliform bacteria, *Escherichia coli*, and *Bacteroides spp*.. *Bacteroides* was assessed using qPCR methods and quantified according to the number of times DNA was positively identified. All others were cultured and enumerated as colony forming units (cfu).

‡ R+3indicates that *Rhodococcus* was detected but could not be identified beyond a suite of three speices. The detection limit for *Rhodococcus spp*. is 5.

§ The detection limit for *Bifidobacteria spp*. is 1. "Pos" results indicate the presence of *Bifido* in numbers greater than 1, but still too small to enumerate.

When plotted on a log scale (Figure 5) to assess temporal patterns in the data, the only obvious result was that overall bacterial counts were lower, but more variable across species in April than during the other three sampling periods. There was no geographic consistency across time among positive results for *Bifidobacteria* species. Recall that *Bifidobacteria spp*. were included in this investigation because they are only found in the gut of humans and are a clear indicator of a close sewage source. By contrast, *Rhodococcus spp*. are strong indicators of pasture animals. Again, there was no consistent geographic pattern to *Rhodococcus spp*. except for station 4 had two positive results for that organism. That was somewhat of a surprise because station 4 is not associated with a pasture (see Figures 1 and 2).

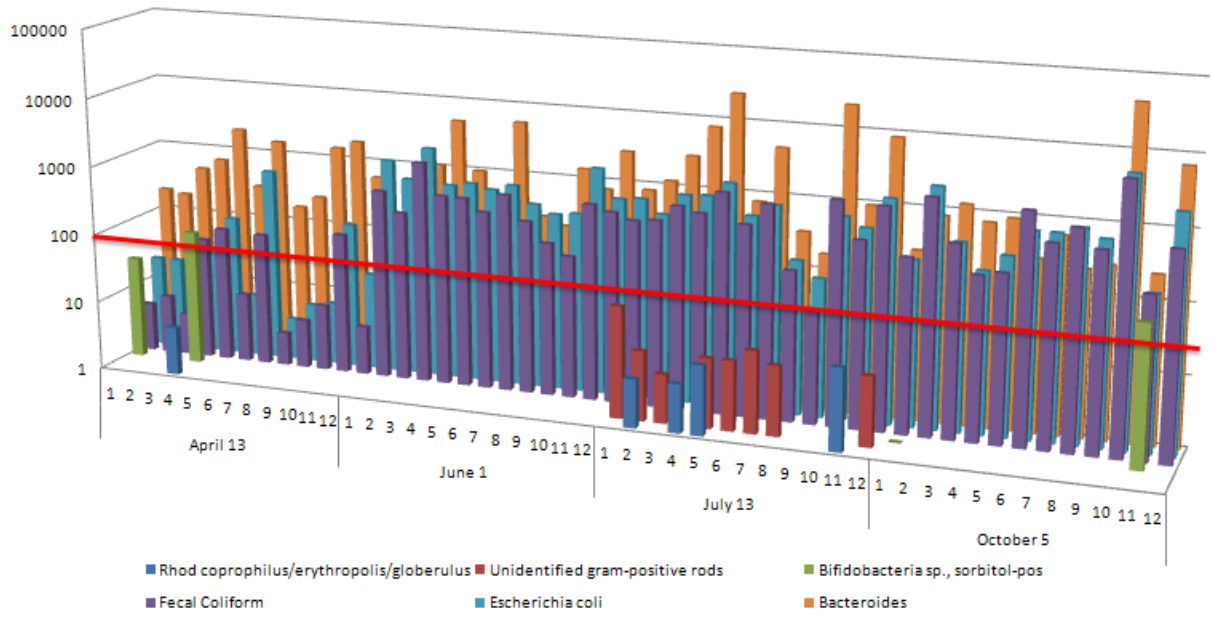

Figure 5. Complete 2011 biological results from Boise Creek microbial source tracking investigation.
Values plotted on a log scale to visually compare across all sampling events.
Horizontal red line indicates State water quality value for fecal coliform and *E. coli* bacteria.

DISCUSSION

The organisms targeted in this study were chosen because of their relative specificity for their host organisms. The species of *Bacteroides* and *Bifidobacteria* in this study are highly specific to humans (Long et al., 2003). *Rhodococcus coprophilus* is associated with pastures and grazing animals (Mara and Oragui, 1981). Fecal coliform bacteria are the basis of the State water quality standard and often highly correlated with *E. coli* concentrations. However, *E. coli* as a subset of FC, are found in the gut of numerous warm blooded animals and very commonly occurring in the environment. In general, *E. coli* is considered to be more specifically correlated with human health risk than FC. Therefore, it is commonly measured in studies of bacterial contamination in

aquatic ecosystems even though it is not regulated by the state standard. However, because *E. coli* is often the most commonly occurring fecal coliform bacterium in the environment, state standards can be appropriated for evaluating measurements (Sargeant et al., 2011).

Values were reported in units of colony forming units (cfu) per 100mL for all organisms except *Bacteroides spp.* which was reported as total number of cells. The State of Washington water quality standards for bacteria are limited to fecal coliform bacteria (WAC 173-201A-200(2)(b)). Fecal coliform geometric mean concentrations cannot exceed 100 cfu / 100mL with no more than 10 per cent of samples exceeding 200 cfu / 100mL under state guidelines. The other organisms evaluated in this study are part of a larger pilot study undertaken by King County of microbial source tracking methods for which no water quality standards exist (pers. comm. Eric Thomspson, 2012).

Results from this study indicate highest levels of fecal coliform and *E. coli*. during the summer through fall. *Bacteroides* qPCR for s16 rRNA, which is a human indicator, was also high across all sampling stations for all time periods. Given the differences among human densities in the drainage, this was something of a surprise. Expectations prior to sampling were that there would be a clear line of demarcation between human sources from the urbanized portions of the drainage, and ruminant sources from the dairy-dominated agricultural portions of the drainage. However, it appears that failing septic systems may play a bigger role in the rural portions of the drainage than previously thought.

Bifidobacter and *Rhodococcus* were rarely found in the samples. But these results may be inconsistent with the presence of fecal material from their host organisms. Difficulty in culturing *Rhodococcus* or unknown sampling challenges may lead to false negative results. In addition, the fact that *Rhodococcus* is not a normal constituent of the intestinal flora of ruminants, but is instead coincident with pastures may make it inappropriate as a definitive indicator of livestock in some circumstances, particularly where dairy cattle are fed primarily hay or silage. Similar challenges exist for sensitive indicator organisms like *Bifidobacteria* with the added difficulty of very specific sampling and culturing methods requirements.

Because there are currently no reliable and well-tested rapid assessments that can clearly discriminate among microbial sources, investigations into bacterial pollution often rely on multiple lines of evidence to pinpoint sources (Long et al., 2006). In this instance, it is clear that nearly everywhere we sampled in Boise Creek, there were high bacterial levels, and given the concentrations and geographic distribution of positive *Bacteroides* results, human contamination appears to be a substantial problem throughout the drainage. However, in the agriculturally dominated portions of the watershed, human density is low relative to domestic animals and yet spatial distributions of *Bacteroides* results are fairly consistent irrespective of land use.

Caution is urged when interpreting the results from the first year of this study. The absence of *Rhodococcus* does not mean that ruminant sources are not important contributors to the bacterial pollution in the drainage. Similarly, the absence of *Bifidobacter* does not indicate that human waste is not present. Negative results should not be viewed as an absence of fecal material from their respective host organisms.

NEXT STEPS

One of the major objectives of this study is the identification of microbial sources so that remediation steps can be taken to clean up the waterway. We understand more about the spatial and temporal distribution of contamination than before the 2011 sampling. But, many questions remain about specific locations of sources and causes of contamination in Boise Creek. We will incorporate the knowledge gained during this first year of study to inform future efforts. During 2012, approximately fifteen additional sampling sites will be added to the existing twelve sites in the 2011 study. The additional sampling locations will be placed at tributary junctions with the continued goal of isolating sources by stream branch. Two residential developments that are known to be on septic systems will be spatially isolated with this increased effort. In addition, some of the industrial forestry land drains the upper-most locations that will be sampled above the residential development to provide background information on microbial levels (Figure 6).

In addition to changing the geographic extent of our investigation, knowledge gained during the first year of the study will guide which organisms will be sampled for during 2012. As highlighted earlier, highest levels of bacterial contamination were recorded during the summer and fall sampling events of 2011. That information will be used to focus efforts during a similar period of 2012. Moreover, some of the organisms included in 2011 yielded little in the way of positive results during 2011 sampling. So for 2012, the plan is to focus sampling into a week of low flow conditions and sample twice each day. Our focus will be on known ruminant specific *Bacteroides* organisms for which molecular methods have been developed (Reischer et al., 2006). In this way, molecular methods for both human- and ruminant-specific species of *Bacteroides* will be applied to discriminate between host organism sources. We will continue sampling organisms collected during 2011 to see if comparisons yield additional information.

Ultimately, the information derived from this study will be used in public outreach and education programs to help guide best management practices implementation for agricultural operations, and to target failing human sewerage systems. King County, the City of Enumclaw, the Washington Department of Ecology, and the King Conservation District all have a direct stake in the outcomes from this work. The definitive knowledge generated by this effort can guide microbial pollution cleanup efforts, not only in Boise Creek, but in other areas of King County, Puget Sound, and beyond.

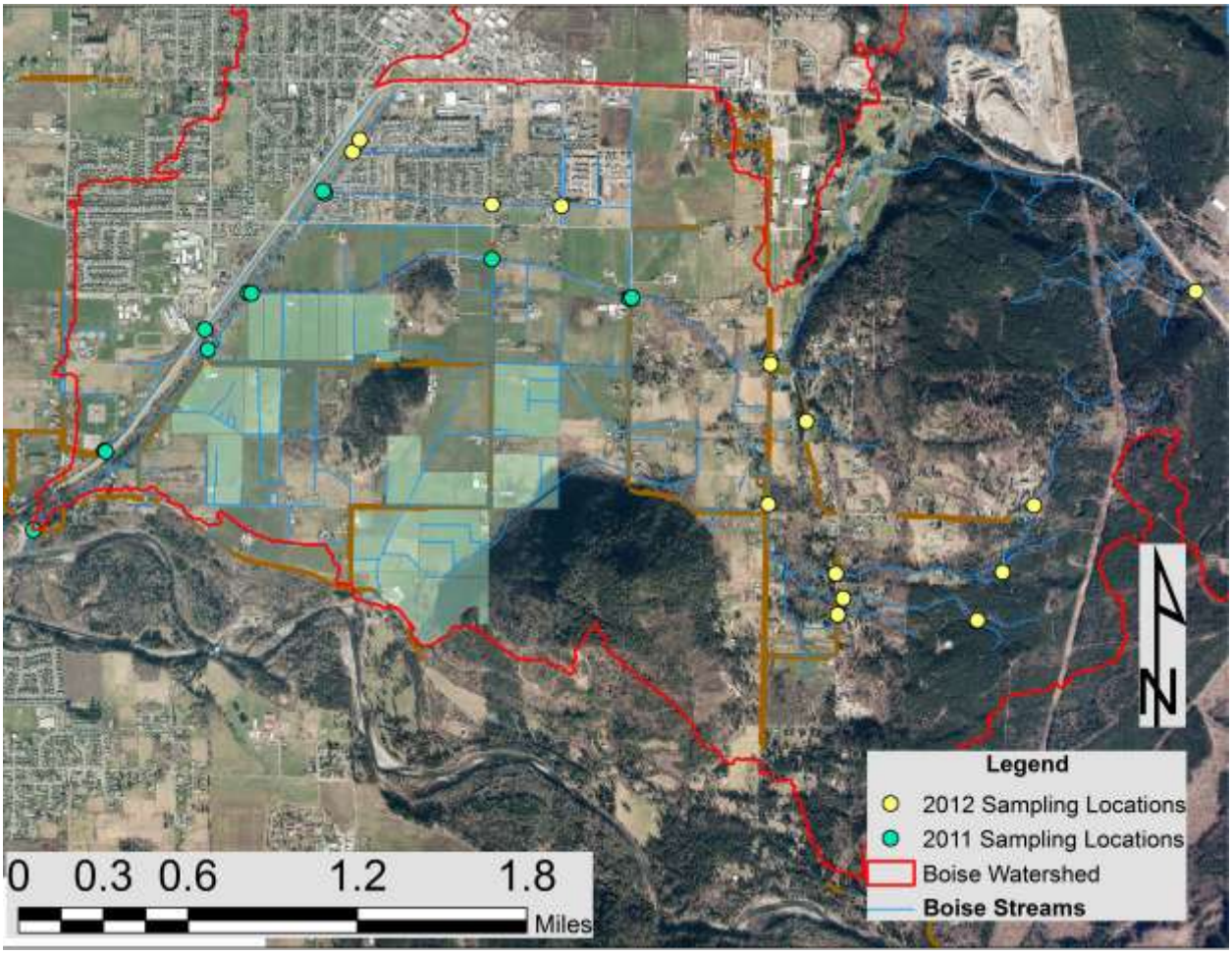

Figure 6. 2012 updated sampling stations. Bright yellow dots represent added sites. Green dots represent locations sampled in 2011 that will be repeated during 2012.

REFERENCES

American Public Health Association. 1998. Method 9213 D. Natural Bathing Beaches. Clesceri, L.S., A.D. Eaton, and A.E. Greenberg (ed). *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. American Public Health Association, Washington, DC.

American Public Health Association**.** 1998. Method 9222 D. Fecal Coliform Membrane Filter Procedure. Clesceri, L.S., A.D. Eaton, and A.E. Greenberg (ed). *Standard Methods for the Examination of Water and Wastewater* 20th Edition. American Public Health Association, Washington, DC.

Converse, Reagan R., A. Denene Blackwood, Marek Kirs, John F. Griffith and Rachel T. Noble. 2009. Water Research 43. 4828 – 4837.

Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., and Franson, M.H. (editors). 2005. Standard Methods for the examination of water and wastewater. New York: American Public Health Organization. Twenty-first edition. Long, Sharon. Personal communication April 2010. Internal UW method titled: "Enumeration, Isolation and Identification of Sorbitol-fermenting Bifidobacter" University of Wisconsin Laboratory of Hygiene.

King County, 2011. Boise Creek Bacterial Source Tracking Study: Sampling and Analysis Plan. Prepared by Raymond Timm, King County Science Section. Seattle, WA. 19 pp.

King County, 2012. Farmland Preservation Program.

Long, S.C. Personal communication April 2010. Internal UW method titled: "Filtration/Resuspension Method for Enumeration of *Rhodococcus coprophilus*" University of Wisconsin Laboratory of Hygiene.

Long, S.C., E. Shafer, C. Arango, and D. Siraco. 2003. Evaluation of three source tracking indicator organisms for watershed management. Journal of Water Supply, Research and Technology. 52(8): 565-575.

Long, S.C., J.D. Plummer, T. Tauscher, and M. Aull. 2006. Using a weight-of-evidence approach for management of watersheds. Water Science and Technology. 54(3): 71-76.

Mara, D.D., and J.I. Oragui. 1981. Occurrence of *Rhodococcus coprophilus* and Associated Actinomycetes in Feces, Sewage, and Freshwater. Applied and Environmental Microbiology. 42(6): 1037-1042.

Mathieu, N., and C. James. 2011. Draft Puyallup River Watershed Fecal Coliform Total Maximum Daily Load Water Quality Improvement Report and Implementation Plan. Washington State Department of Ecology Olympia, Washington 98504-7710

Plummer, J.D., and S.C. Long. 2009. Identifying sources of surface water pollution: A toolbox approach. Journal of the American Water Works Association. 101(9): 75-88.

Reischer, G.H., D.C. Kasper, R. Steinborn, R.L. Mach, and A.H. Farnleitner. 2006. Quantitative PCR method for sensitive detection of ruminant fecal pollution in freshwater and evaluation of this method in alpine karstic regions. Applied Environmental Microbiology. 72(8): 5610-5614.

Sargeant, D., W.R. Kammin, and S. Collyard. 2011. Review and Critique of Current Microbial Source Tracking (MST) Techniques. Publication No. 11-03-038. Environment Assessment Program. Washington State Department of Ecology. Olympia, WA. 66pp.

Stoeckel and Harwood. 2007. Performance, design, and analysis in microbial source tracking studies. Applied and Environmental Microbiology p. 2405-2415.

Thomson, E. 2012. Personal communication. Laboratory approaches to microbial source tracking. King County Environmental Laboratory, Microbiology Unit.

USEPA, 2011. Using Microbial Source Tracking to Support TMDL Development and Implementation. U.S. Environmental Protection Agency, Region 10 Watersheds Unit. Prepared by Tetra Tech and Herrera Environmental Consultants. 64pp.

WAC 173-201A: Water Quality Standards for surface waters in the State of Washington. Washington State Department of Ecology, Olympia, WA. www.ecy.wa.gov/laws-rules/ecywac.html