Boise Creek Bacterial Source Tracking Study:

2012 Summary of Findings

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King County

Department of Natural Resources and Parks
Water and Land Resources Division
Science Section
King Street Center, KSC-NR-0600
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Submitted by

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

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INTRODUCTION AND BACKGROUND

This microbial source tracking (MST) study focuses on water quality impairments in Boise Creek related to a history of bacterial contamination. This report describes the study and findings from the second year of investigation into sources of microbial contamination, as well as continuing work toward solving the problem. The goals of this project are to investigate and identify sources of bacterial contamination along Boise Creek and its tributaries. The primary objective is to discriminate among the potential sources of bacteria and group them according to human or agricultural origin to more effectively inform cleanup strategies.

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 watersheds, and then specifies pollution reduction quantities necessary to achieve clean water. The Puyallup River Watershed has multiple listings on the federal Clean Water Act 303(d) list as impaired because of high fecal coliform bacteria concentrations. Boise Creek, which is tributary to the Puyallup River 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 locations in Boise Creek have been monitored along the creek and its tributaries by the Washington State Department of Ecology, King County and the City of Enumclaw.

In the Puyallup River fecal coliform (FC) TMDL area, Boise Creek is the largest FC bacteria loading source of any tributary in the study area. It also requires the largest FC reduction of any dry season source within the Puyallup River Basin. The Boise Creek watershed has been identified as the number one priority for improvement within the TMDL study area (Mathieu and James, 2011). The completed water quality improvement report and implementation plan for the Puyallup River system can be accessed 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. Depending on location in the watershed, reduction targets range from 57% to 92% in summer, to 61% to 67% in winter in order to achieve Washington State water quality guidelines of 100 colony forming units (cfu) per 100mL (Mathieu and James, 2011). The high summer FC loadings suggest non-storm driven sources such as leaking sewer pipes, septic systems, or cross-connections that could be implicated in bacterial contamination. High numbers during the wet season likely suggest 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 of Washington 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. Because this effort is aimed at finding sources, we employ 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 ruminant hosts (Table 1). Information generated in this study will be used to guide cleanup efforts.

Table 1. Parameters sampled during 2012, methods used to quantify response, and whether the indicator organism was human or ruminant.

Parameter	Laboratory Method	Units	Host Organism	Sampled in 2011
Total Fecal Coliform	Live culture	CFU/ 100mL	Not specific	Yes
Escherichia coli	Live culture	CFU/ 100mL	Not specific	Yes
Bacteroides thetaiotaomicron	qPCR*	Cells/ 100mL	Human	Yes
Sorbitol-fermenting *Bifidobacteria spp*.	Live culture	CFU/ 100mL	Human	Yes
Rhodococcus coprophilus	Live culture	CFU/ 100mL	General pasture animals	Yes
Rhodococcus coprophilus	qPCR	Present/ Not Found	General pasture animals	No
Bacteroidales spp.	qPCR	Present/ Not Found	Ruminants	No

*qPCR indicates a real time polymerase chain reaction method was used to detect target organism.

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).

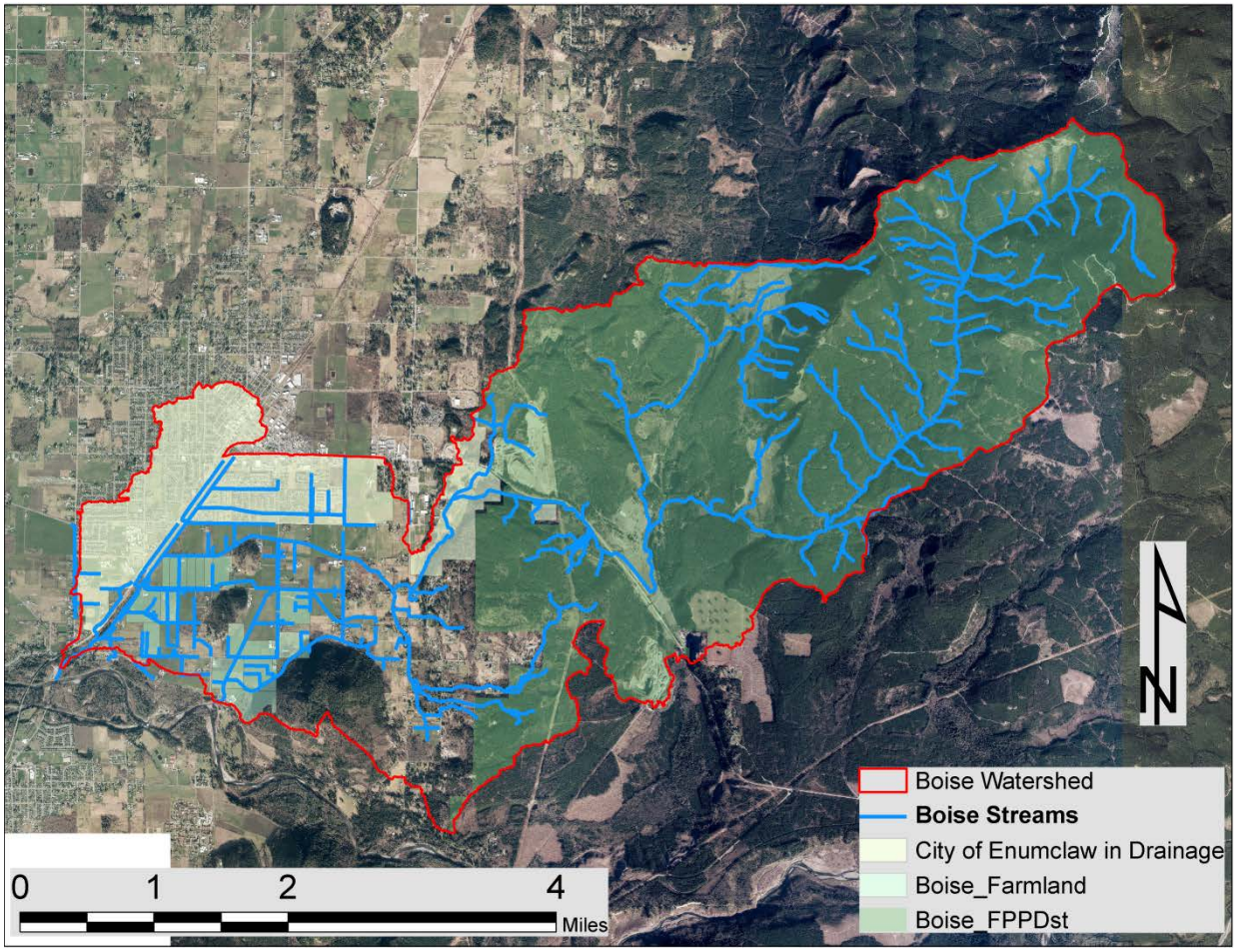

Figure 1. Overview of Boise Creek drainage. The eastern two thirds of the drainage is in forestry production, the northwestern portion is within the City of Enumclaw, and the remainder is rural agricultural – primarily dairy.

This microbial source tracking study incorporated twenty 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 detect differences in bacterial concentrations by stream segment. 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. Twelve of the sampling locations were repeated from the 2011 study and 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 near its confluence with the creek. Eight sites were added during 2012, all of which were located upstream of sites previously studied (Figure 2).

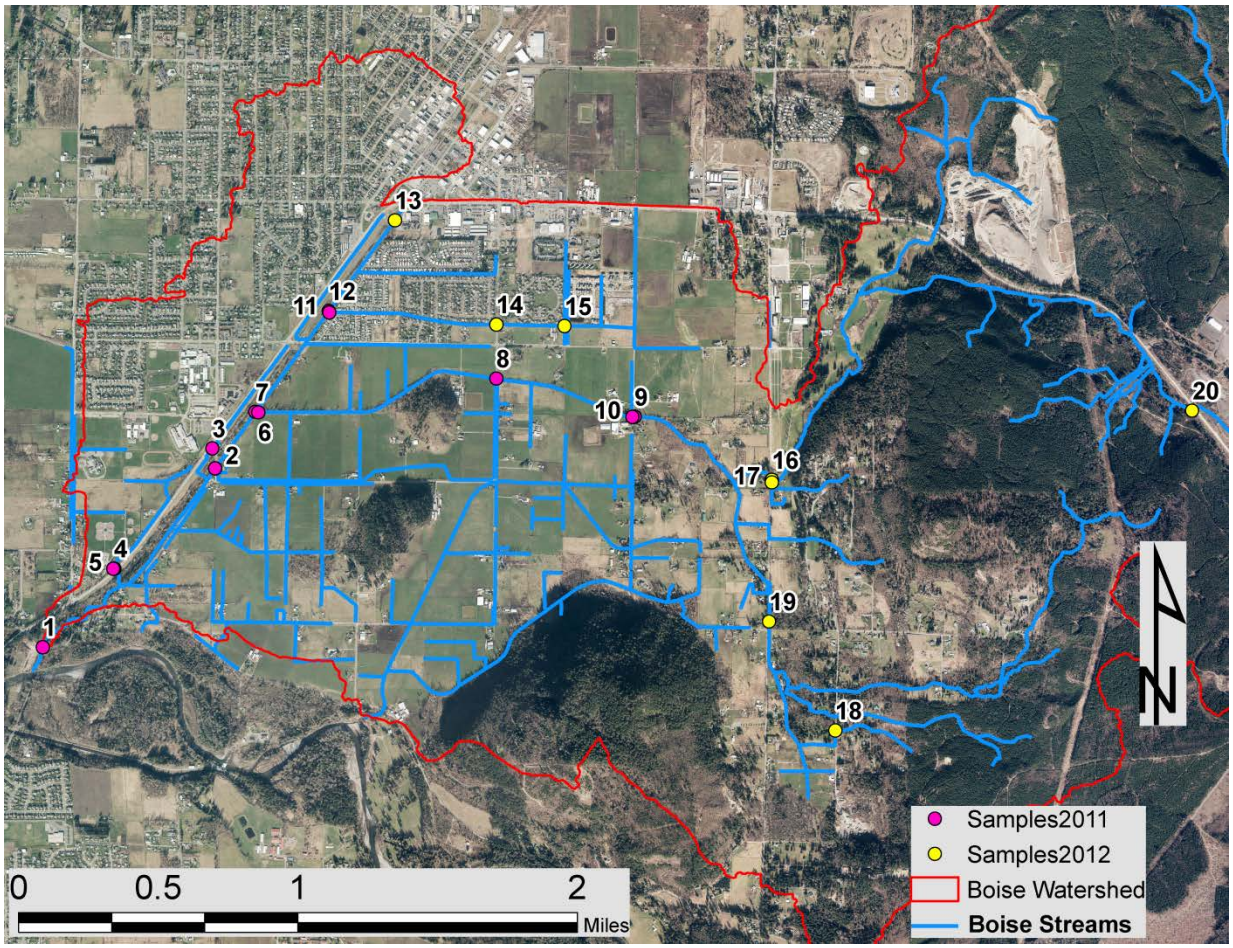

Figure 2. Twenty Boise Creek Microbial Source Tracking Sampling Locations. Sites 4, 5, 7, 11, 12, 13, 14, and 15 are within the city of Enumclaw, WA. Sites 1,2,3,8,9, 10, 16, 17, 18, 19, and 20 are in rural lands of unincorporated King County. Pink colored dots represent sites sampled in 2011 and 2012. Yellow dots represent sites added during 2012.

Laboratory Approach

The design focused on sampling for total fecal coliform (FC), *Escherichia coli* (*E. coli*), sorbitol – fermenting *Bifidobacter*, *Bacteroides thetaiotaomicron*, *Rhodococcus coprophilus*, and ruminant *Bacteroidales spp*. to look for differences in bacterial community composition and concentrations related to agricultural land use or human waste system failures (Table 1). 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). The toolkit approach employs organisms with varying degrees of specificity in a "weight of evidence" framework. In this way, likely sources of contamination can be inferred and used to guide more targeted sampling and confirmation (Table 2). Refinement of the toolkit is ongoing to improve the usefulness of these indicators for identifying the source hosts (i.e., human or ruminant) of bacterial contamination in surface waters (pers. comm. Eric Thomson, 2012).

All organisms were quantified by counting live colonies cultured on media except for *Bacteroides thetaiotaomicron, Rhodococcus coprophilus* and ruminant *Bacteroidales spp*., which were analyzed using a real time polymerase chain reaction technique (qPCR; Table 1). These molecular based methods target the genes that code for the 16S Ribosomal RNA unit (16S rRNA). Real time PCR methods provide definitive results for each target species or groups of organisms that share a genetic component. Real time PCR can be used to determine whether the target organism is present (or not found) in a sample or in some instances, to estimate bacterial cell numbers in a sample. Quantitative numbers are given as cells / ml. Data quality, specificity, and sensitivity are still under investigation (USEPA, 2011; King County, 2011).

Table 2. Biological indicators and important values included in the King County "toolkit" approach to microbial source tracking.

Organism/ Group	Threshold concentration	Indications
Fecal coliform bacteria	<= 100 CFU/100mL	State Water Quality requirement
Escherichia coli	> 100 CFU/100mL	Bacterial contamination
Bifidobacteria spp.	Presence	Close and recent human source
Bacteroides thetaiotaomicron	> 300 cells/100mL	Highly likely human source
Bacteroidales spp.	Presence	Ruminant source
Rhodococcus coprophilus	Presence	Pasture animal source

Field Sampling

Sampling locations (Figure 2) were arranged at tributary junctions in order to isolate potential sources geographically. Sample collection was scheduled during the morning and again in the early afternoon so that samples could be delivered to the King County Environmental Laboratory (KCEL) within the prescribed 6 hour holding time for *Bifidobacteria* samples (King County, 2011). Sampling occurred during dry weather to measure bacterial levels during base flow conditions. Minimal precipitation occurred in the fifteen days prior to the sampling event. Only two days during that period (July 26, and 27) received any rainfall, 0.01 and 0.05 inches respectively. Sampling under these flow and precipitation conditions biases the sampling toward human sources because it is assumed that bacteria entering the creek come through groundwater contributions that could result from broken or improperly connected plumbing systems. Discharge conditions for Boise Creek and local precipitation records were downloaded from 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

Each location (Figure 2) 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 each 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 spp.* analysis required sample transfer from the collection bottle to a sterile bottle containing sodium sulfite preservative. *Rhodococcus coprophilus* 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).

RESULTS

Twice-daily sampling occurred in the morning and afternoon of August 6, 7, and 8, 2012 during the typical summer low flow period. All twenty locations (Figure 2) were sampled during each event except for site 5 which was dry during all sampling events. 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, fecal coliform bacteria (FC) concentrations were variable across the study area. However, eleven locations were consistently out of compliance with State of Washington water quality standards (Figure 3a, 3b). For both morning (Figure 3a) and afternoon (Figure 3b) sampling time periods, more than half the sites exhibited FC exceedances. Additionally, the spatial characteristics of bacterial concentrations revealed compelling information. In locations where *Bacteroides* levels consistently indicated human sources of contamination during 2012, they were very tightly coupled with urban landscapes in the watershed (Figure 3c, 3d). Sampling locations 2 and 7 were the only exceptions to this observation. Station 19 was also outside of the urban area, but drains a mixed landscape of large lot rural development and hobby farm agriculture (Figure 4).

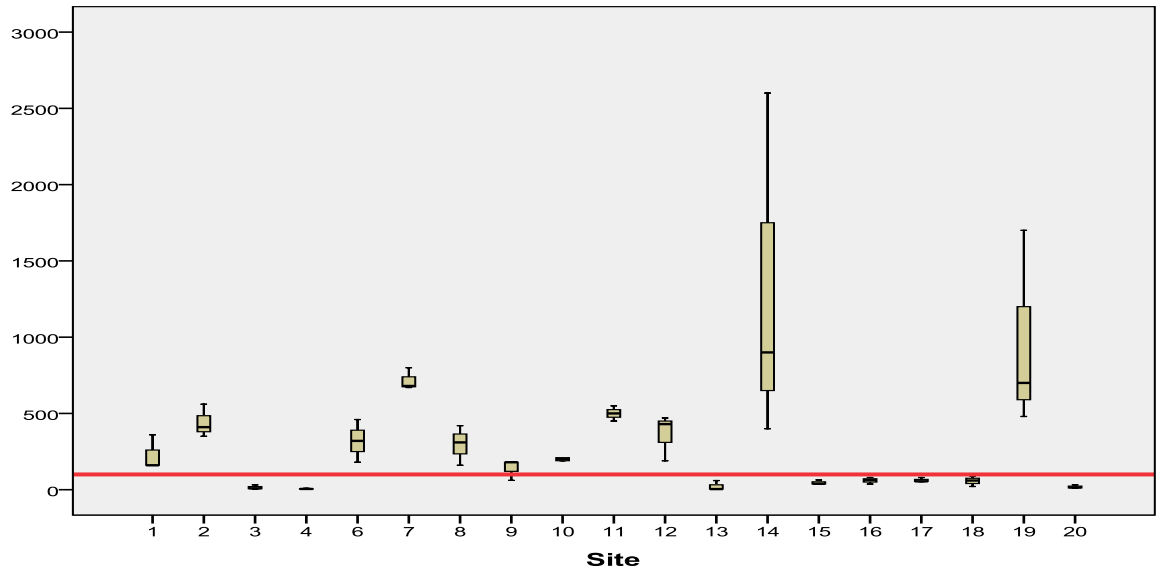

Figure 3a. Box and whisker plot of fecal coliform concentrations (CFU/ 100mL) during morning sampling events August 6, 7, and 8, 2012. The red line indicates the State of Washington water quality standard. Above the red line, fecal coliform concentrations are out of compliance with State water quality requirements. See Figure 2 (above) for locations of each sampling location. Values on the Y axis indicate colony forming units / 100mL of sample.

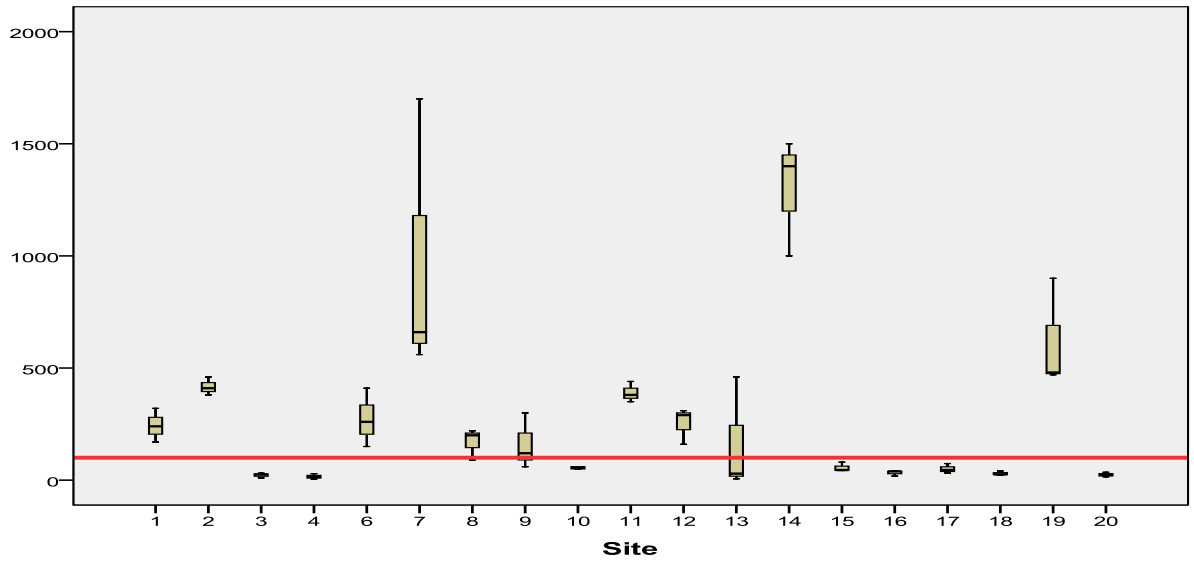

Figure 3b. Fecal coliform concentrations (CFU/ 100mL) from afternoon sampling events. The red line indicates the State Water Quality standard. Values on the Y-axis indicate colony forming units (CFU) / 100mL.

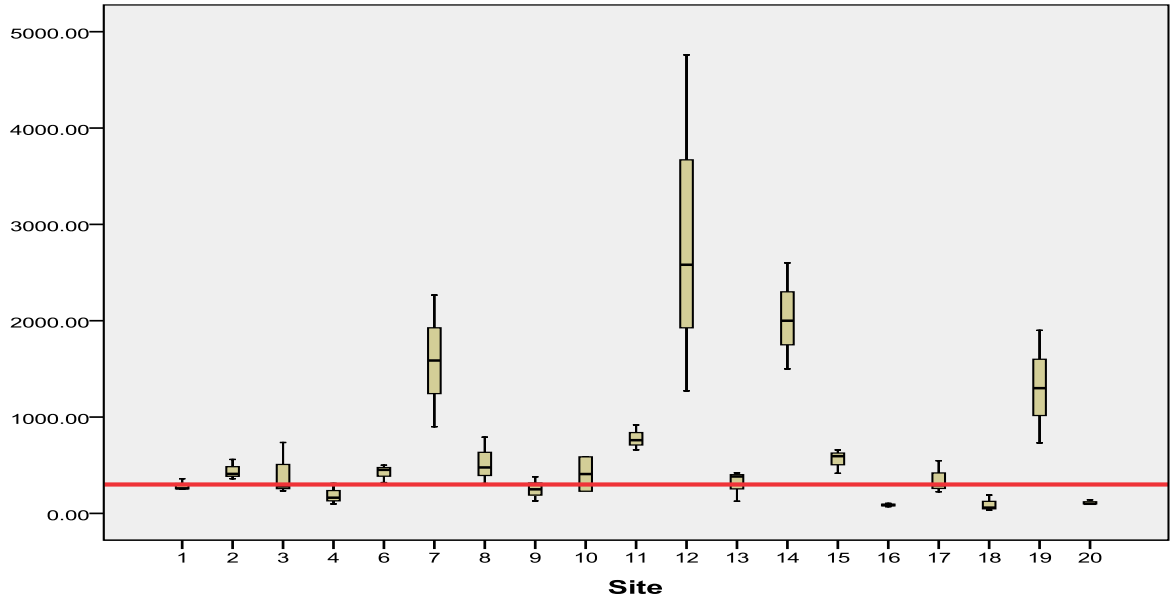

Figure 3c. *Bacteroides thetaiotaomicron* levels from morning sampling events on August, 6, 7, and 8, 2012. The red line indicates the level above which there is high confidence of a human source. Sampling location is on the X-axis. See Figure 2 for location of each sampling location.

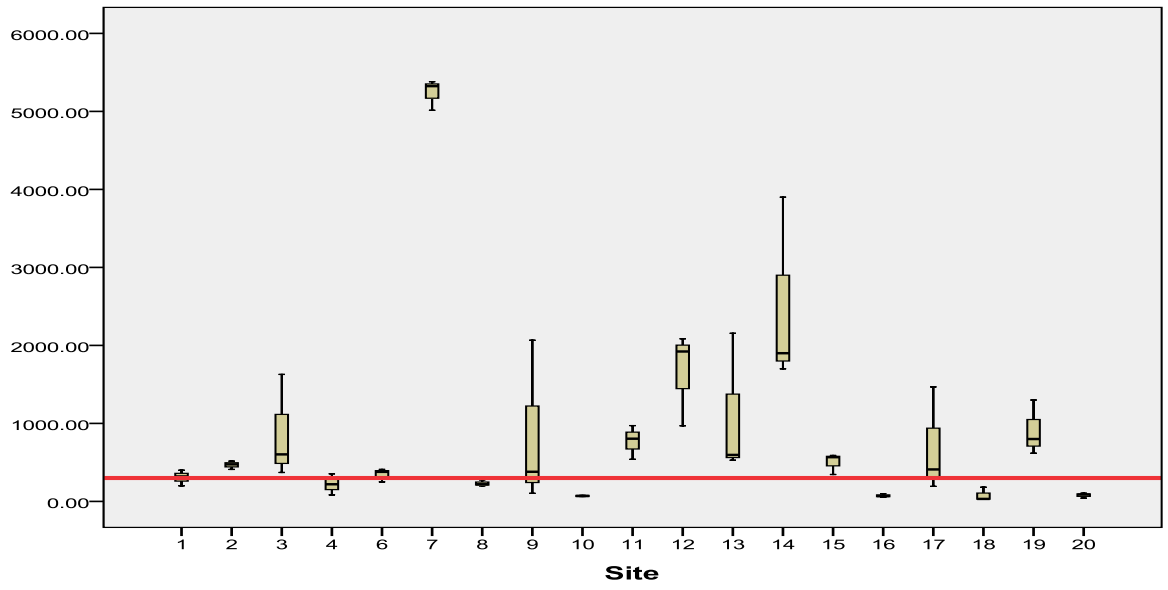

Figure 3d. Afternoon sampling results for *Bacteroides thetaiotaomicron*. Site number is on the X-axis. Cells per 100mL is represented on the Y-axis. The red line represents the value above which there is high confidence of a human source.

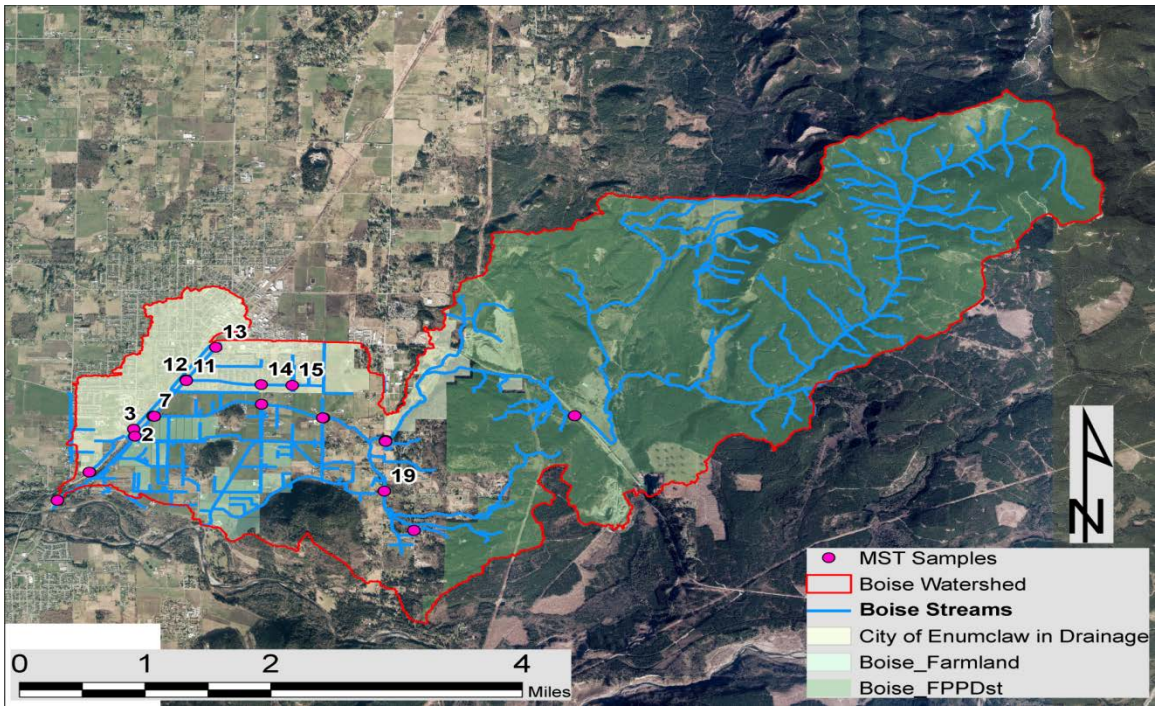

Figure 4. Sampling locations where fecal coliforms and E. coli bacteria in the Boise Creek drainage exceeded state water quality guidelines for the three days of sampling during 2012. Numbered sites indicate chronic exceedance locations.

Similarly, the organisms indicating ruminant sources of bacterial contamination (i.e., Ruminant *Bacteroidales*, *R. coprophilus*) were variable in time in space across the watershed. However, of the eleven sites that tested positive for ruminant *Bacteroidales*, seven of them were positive at each sampling event (i.e., each day, morning and afternoon). All seven of those locations occurred in the agriculturally dominated portions of the watershed (Figure 5). The morning and afternoon sampling scheme only yielded differing results on six occasions. On August 6, there was a positive result in the afternoon only at site 12. On August 7, sites 2 and 14 yielded positive results only in the morning. On August 8, sites 15 and 16 yielded positive results in the morning only while site 14 was positive in the afternoon only. The two sites that yielded positive results for *Rhodococcus coprophilus*, by contrast, were only associated with ruminant *Bacteroidales* at site 14. In addition, *R. coprophilus* was only detected during morning sampling at sites 14 and 18 (Figure 6).

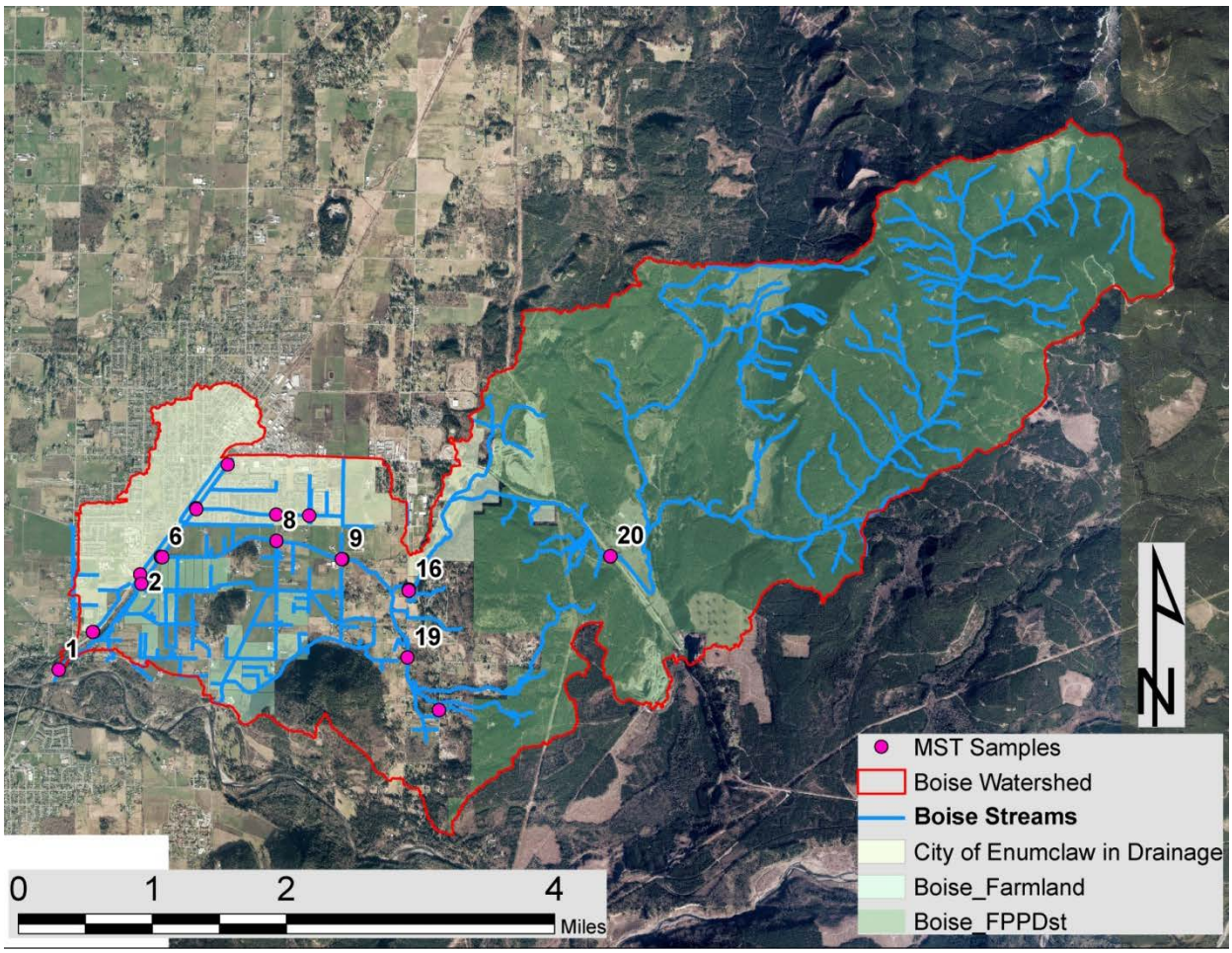

Figure 5. Sites where ruminant *Bacteroidales* were found on three consecutive days of sampling during August, 2012. These sites all occur in the agricultural areas of the watershed except for site 20 which lies near the bottom extent of the industrial forrestry lands. Numbered sites indicate locations of chronic contamination.

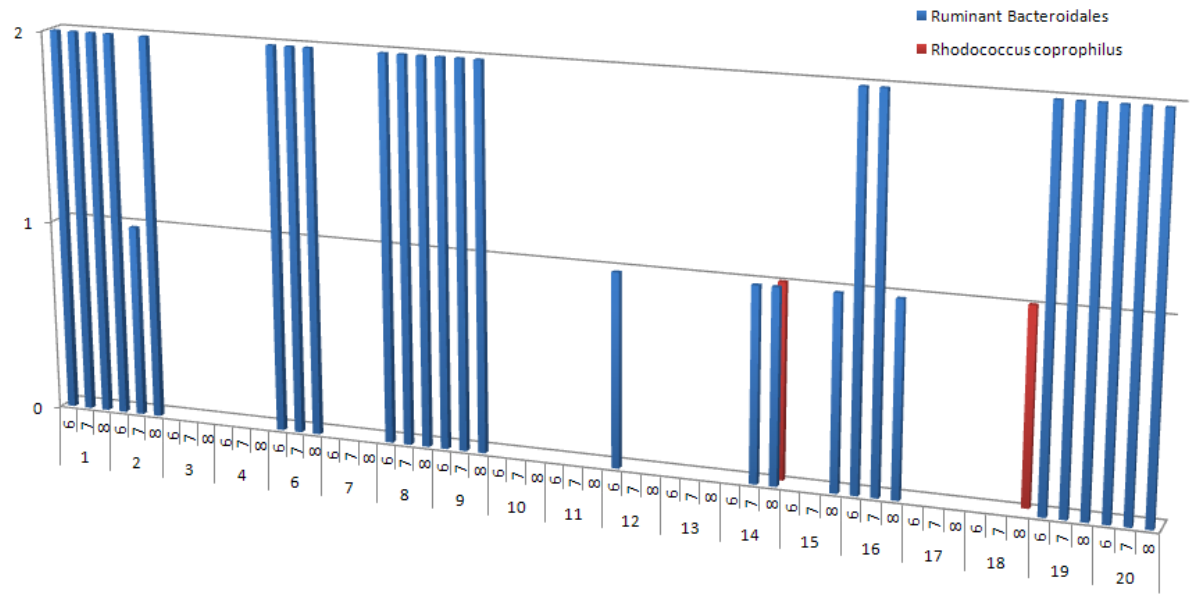

Figure 6. Ruminant *Bacteroidales* and *Rhodococcus coprophilus* positive results during 2012 sampling. At the bottom of the X axis is the sampling location (1-20) and just above that is the day of sampling (August 6,7,8). Values for positive results evaluate to 1 and "Not Found" = 0. Positive results were summed by day to indicate if indicator organisms were found in both morning and afternoon sampling events.

DISCUSSION

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). Values were reported in units of colony forming units (cfu) per 100mL for FC bacteria, *E. coli*, and *Bifidobacteria spp*. *Bacteroides spp.*, ruminant *Bacteroidales*, and *Rhodococcus coprophilus* were all reported as total number of cells. *Bacteroidales spp.* represents a family of intestinal flora specific to ruminants, and *Rhodococcus coprophilus* are associated with pastures and grazing animals (Mara and Oragui, 1981). Results from *Bacteroidales* and *Rhodococcus* were also reported as total number of cells.

The organisms targeted in this study were chosen because of their relative specificity for their host organisms. Fecal coliform bacteria are the basis of the State water quality standard and often highly correlated with *E. coli* concentrations. In this study, FC and *E. coli* were reasonably well

correlated for morning and afternoon samples (Spearman Rank Order Correlation *P=0.691* in the morning; *P=0.787* in the afternoon). 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). The species of *Bacteroides thetaiotaomicron* and *Bifidobacteria* in this study are highly specific to humans (Long et al., 2003) and have been selected to decrease uncertainty around contributing source organisms. Because the units are different among FC, *E. coli* and *Bacteroides*, correlations are most useful to see if variables behave similarly through time, rather than as a measure of statistical dependence. In this study, Spearman Rank Order Correlations between FC and Bacteroides indicated relatively high agreement during morning (*P=0.735*) and afternoon (*P=0.631*) sampling events, providing further support for its use in microbial source tracking investigations.

In a spatial sense, results from this study indicate highest consistent levels of FC, *E. coli.* , and *Bacteroides thetaiotaomicron* are associated with the urban areas of the drainage. By contrast, highest concentrations of ruminant *Bacteroidales* are associated with the agricultural portions of the drainage (Figures 4 and 5). However, it appears that failing septic systems may play a bigger role in the rural portions of the drainage than previously thought because *Bacteroides thetaiotaomicron* was found throughout the sampling area during all sampling events. Given the concentrations and broad geographic distribution of positive *Bacteroides* results, human contamination appears to be a contribution throughout the drainage. Surprisingly, in the agriculturally dominated portions of the watershed, human density is low relative to domestic animals and yet *Bacteroides* results indicate human sources are likely contributing to overall levels of bacterial contamination.

In terms of temporal findings during 2012, statistical comparisons using a Shapiro-Wilk test of the concentrations of bacteria between morning and afternoon sampling efforts indicate that there is no significant difference between time periods for *Bacteroides* (*P=0.900*), FC (*P=0.699*), or E. coli (*P=0.620*). In other words, when morning and afternoon concentrations were compared, there were no significant differences between sampling periods. This could suggest that there wasn't enough time between samples to show the diurnal variation we expected. It could also indicate that sources were spatially distant and that the creek was thoroughly mixed where we sampled relative to presumed upstream bacterial source locations. Alternatively, it's possible that introductions are fairly constant temporally throughout the system and do not vary on a daily basis.

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. Since *Rhodococcus* is not a normal constituent of the intestinal flora of ruminants, but can be associated with many different animals found in pastures, it may be 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 *Bifidobacter* 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). Caution is urged when interpreting the results until statistical analyses yield more definitive results. The absence of ruminant bacteria in samples collected during low flow summer time conditions does not mean that ruminant sources are not important contributors to the overall bacterial pollution in the drainage. The sampling approach during 2012 was purposefully biased toward finding human sources because low flow conditions occur when ground water is the predominant source of water in the creek. In addition, there was nearly zero precipitation in the weeks preceding the study. So, overland flow through livestock areas was presumed to be minimal. The fact that ruminant bacteria were found during low flow conditions could indicate that sewage lagoons or other livestock waste management facilities are interacting with shallow ground water in the drainage.

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 years of study to inform future efforts. During 2012, eight additional sampling sites were added to the existing twelve sites in the 2011 study. The additional sampling locations were 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 were spatially isolated with this increased effort and provided justification for more intensive investigation. In addition, assumed baseline conditions were sampled at the downstream extent of the industrial forestry land (Site 20, Figure 1).

In addition to changing the geographic extent of our investigation, knowledge gained during the first year of the study was used to guide which organisms were sampled during 2012. Ruminant *Bacteroidales* was added, as was a molecular method for *Rhodococcus coprophilus*. Moreover, some of the organisms included in 2011 yielded little in the way of positive results during 2011 and again during 2012 sampling. Specifically, *Rhodococcus* will be dropped from future sampling because it has only been found twice. Additionally, sorbitol-fermenting *Bifidobacteria spp.* will be used in a more targeted approach in future investigations because of its highly sensitive nature. It is a strong indicator of close, recent human sources. But, it has not provided much helpful information in the broad microbial screening approach that has been used up until now. During 2012, sampling occurred during a week of summer low flow conditions and sample twice each day. Molecular methods for both human- and ruminant-specific species of *Bacteroidales* were applied with some success to discriminate between host organism sources.

During 2013, sampling effort will be refined further based on findings from 2012 and 2011. For example, rather than sample twice each day for three days in a row, the same effort will be distributed over one summer low flow event and one event later in the fall as ground water starts to recharge and stream discharge starts to increase from the fall rains. We will drop *Rhodococcus coprophilus, Bifidobacteria spp.,* and *E. coli* from those efforts. The cost savings achieved by not sampling for those organisms during each event will allow for more targeted sampling of stream segments where there are suspected bacterial introductions. During these targeted investigations,

we will sample for Bifidobacteria spp., Bacteroides, Bacteroidales, FC, and possibly some organic chemicals that are highly associated with humans such as caffeine, cholesterol, Coprostanol, and triclosan.

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.

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