Intensive Beach E.coli and Bacteriodales Monitoring at Matthews Beach in Seattle, Washington

Introduction

Nearly 3,000 beaches in the US are tested at least weekly for fecal indicator bacteria (FIB) (Dorfman & Stoner 2007). These indicators are used to estimate the presence of sewage pollution and potential pathogenic bacteria in water that may impact human health risk from swimming in contaminated waters (Prüss 1998). Elevated counts of FIB occur when sewage is present in the waters, however high bacteria counts do not always indicate the presence of human sewage pollution because many other mammals and birds can contribute this type of bacteria to the water (King County 2005; Boehm 2009). Water quality criteria and swimming beach bacteria studies tend to focus on the type of FIB and the relative counts of these various indicators to trigger management actions.

A need to develop appropriate sampling designs to account for variation associated with spatial and temporal factors, such as hourly, daily and seasonal variation was identified by EPA (EPA 2003) as well as an acknowledgement that FIB densities exhibit high variability at multiple time and length scales, with the greatest temporal variability in FIB densities attributed to rain events. Much of the statistical focus has been on evaluating the association between the type of indicator and bacteria level and relative risk of swimming associated pollution related infectious disease (Cabelli 1989) not on sampling methodology designed to address FIB spatial and temporal variability. Even a review study states that gastro-intestinal symptoms are the most common health problem related to the count of indicator bacteria in recreational waters, inferring that the count is a static or minimally a relatively consistent representative number (Prüss 1998). Results from the King County swimming beach monitoring program (king County 2005) do not support an inference of a static or minimally constant FIB counts.

The use of indicator microorganisms for assessing water quality of exposure is one of the major sources of bias and which FIB indicator to use is typically driven by local regulations. This inconsistency in FIB adds to uncertainty in estimating risk because of the different linkages of the various indicators to epidemiological results. This limited precision of methods for quantifying indicator organisms adds substantial measurement error (Prüss 1998). In addition, there are several sources of temporal and spatial variability in both sampling and analyzing FIB that add uncertainty to estimating water contact risk. FIB concentrations in surface water are variable over time scales from minutes to years owing to variation in local water mixing, sunlight induced inactivation, failing or constant or episodic failure of sanitary infrastructure, seasonal cycles of rainfall and run-off and multiple often unknown local environmental conditions (EPA 2010).

The scientific and regulatory literature is quite sparse on how to design a bacterial sampling program that is representative of the known spatial and temporal variability of FIB in the aquatic environment (Boehm 2009) or how this uncertainty effects the efficacy of using FIB in managing swimming beaches. Variability in temporal and spatial FIB counts is substantial, and difficult to relate to individual bathers or exposure risk (Prüss 1998). The use of multi-week or seasonal means for bacterial criteria compliance, rather than more frequent temporal measurements further increases the inaccuracy of estimating the bacteria exposure and diminishes the applicability of FIB results as timely information for beach use decisions. Spatial variability has also been documented over scales of 10 m and more (Boehm et al. 2002; Whitman & Nevers 2004) and has been hypothesized at the beach they studied to be driven by local wind and wave patterns and stream or stormwater inputs (Nevers and Whitman 2005). Spatial and temporal variability at similar short local scales has been documented in Thornton Creek adjacent to Matthews Beach on Lake Washington (Frodge 2013). Also, FIB concentrations vary more quickly than monitoring results can be obtained using USEPA-approved or other culture-based methods, typically 24 hrs. (Boehm et al. 2009).

The spatial and temporal variability evident in FIB data sets as well as the delay in obtaining analytical results decrease the applicability of a single sample standard (USEPA 1986) for routine water quality notification purposes. Rapid methods, unproven and still in development, may help address this issue, particularly in identifying beaches impacted by episodic or ephemeral bacteria inputs. These methods are less applicable to many management situations due to equipment costs, analytical complexity, and other logistical issues (Boehm et al. 2009).

A proposed alternative approach to estimating swimming beach and water contact risk is development of simple statistical models. Nevers and Whitman (2005) propose that these models do not necessarily require an understanding of processes and mechanisms controlling bacteria indicator fate and transport for daily water quality assessment and public notification of water quality. Such “now-casting” models relate environmental factors such as rainfall, wind and waves to water quality at a specific beach in real time and allow early warnings to be issued by managers (Nevers & Whitman 2005; Francy et al. 2006). However useful locally these site-specific models are, they are intensively local data driven and because they are intentionally site-specific have reduced utility for general application.

Another approach to identifying human fecal pollution exposure is the use of human-specific bacteria identification and quantification. The order *Bacteroidales* are anaerobic, gram negative, non-endospore-forming bacillus bacteria that are commonly found in human fecal material. *Bacteroidales* comprise a significant component of human fecal material and therefore are an abundant target for human fecal contamination. Their presence in environmental waters can be an indication of recent human pollution due to their short survival time and inability to reproduce in the environment. While the use of these human-specific bacteria reduces the problem of non-human bacteria confounding the identification of sources of bacteria, Bacteriodales are still subject to the temporal and spatial variability of less source specific FIB. These human-specific bacteria may be more susceptible to the spatial and temporal variability, as the in-situ quantification of these bacteria is less known than the traditional FIB.

The lack of understanding of the temporal and spatial variability of the FIB samples used to make beach closure decisions remains a major source of error in using FIB as an indicator of water contact public health risk. This study developed an initial estimate of spatial and temporal *E.coli* and Bacteriodales FIB variability at Matthews Beach, (Seattle, King County WA) and synoptically in adjacent Thornton Creek (Figure 1). King County, Washington began weekly monitoring of approximately 30 selected public swimming beaches during the summer starting in 1996, with beaches monitored to estimate levels of FIB as an indicator of exposure risk. The intent of this program was not to quantify the FIB counts at a high level of accuracy in the swimming waters, but to provide weekly information on relative swimming related exposure risk.

The initial sampling design of single weekly grabs allocated sampling capacity geographically over more frequent or replicated sampling in order to cover the greatest number of local public beaches. This design resulted in less sampling capacity available for replication or increased temporal sampling at specific beaches and resulted in a lack of statistical power in quantifying estimates. This approach potentially de-emphasized both spatial variability and short-term temporal variability within the swimming beach by some unknown amount. There are obvious weaknesses in a FIB monitoring program designed with no means to estimate spatial or temporal variability or confidence around a sample. In order to address some of the spatial viability, the monitoring protocol recently increased sampling from a single sample collected weekly to three samples collected one day a week.

This study was conducted to develop an estimate of spatial and temporal variability for *E.coli* and Bacteriodales counts at a popular swimming beach and was not scheduled as a response to a known or suspected pollution event. A better understanding of the variability in the FIB sampling could help inform sampling design and closure protocols. Although local swimming beach models call for site specific data, and it is likely there are local differences between individual beaches, the estimates for spatial and temporal variability from this sampling will be used as an initial estimate of variability at all of the beaches in the King County monitoring program until better estimates are available.

Methods and Materials

Matthews Beach Park is located along the northwest shore of Lake Washington in Seattle, Washington. Thornton Creek enters Lake approximately 520 ft south of the designated swimming area in the park. Thornton Creek drains a 7,402-acre (11 sq. mi.) watershed into Lake Washington. The watershed is 96% developed with only 3% forest (Kerwin 2000). Seattle, where the beach is located on the northwest side of Lake Washington, typically has wet winters and dry summers. Western Washington has a maritime climate with average precipitation of 99 cm falling primarily in late fall to early spring. Average monthly precipitation during the June - early September swimming season is less than ~1.5 inches, and days with precipitation between 9 – 5 days (U.S. Climate Data 2019).

Figure 1. Matthews Beach is in northeast Seattle on Lake Washington. FIB samples were collected at ~3 ft. depth evenly spaced across the designated swimming area. The stream sampling (SB434) site is ~520 ft south and 350 ft upstream from the beach sampling sites and is the same location where citizen volunteer samples (TCA) were collected. A long-term homeless camp site was ~1400 ft upstream of the stream sampling location and was occupied until removed and cleaned in late July 2018, just three weeks prior to sampling.

Spatial and temporal sampling design

Multiple FIB samples were collected to estimate temporal and spatial variability and generate standard errors (SE) around these the estimates. Swimming beach samples were collected hourly (11 AM – 6 PM) on multiple days at three evenly spaced locations (north, middle, south) in the Matthews Beach swimming area and in adjacent Thornton Creek (Figure 1). The 11 AM to 6 PM sampling is based on the time of day that the swimming area is officially open and staffed with lifeguards.

FIB samples were collected at the three beach sites and creek sites hourly from 11 AM to 6PM on Sunday August 19, Sunday August 26, Tuesday August 28, and Monday Labor Day) September 3, 2018. A single set of samples were also collected at these four locations on Tuesday August 21, Wednesday August 22, Wednesday August 29, and Thursday August 30, 2018. Grab samples were collected at three locations from near the middle, far right, and far left laterally along the shoreline, ~60 ft apart at thigh depth (~3 ft), and at a site (SB434) near the mouth of Thornton Creek (Figure 1). Sampling in these shallow locations in the designated swimming area is where maximum bather exposure was assumed to occur, as it is frequently occupied by younger swimmers and transited by anyone going into deeper water. The Thornton Creek site was in the lowest flowing section of the creek, just above the slack backwater from Lake Washington closest to the swimming area (~520 ft).

Bacteria sampling

Water samples for E.coli and Bacteriodales were collected in the swimming area and adjacent creek site (Table 1). To avoid contamination of the samples personnel wore polyvinyl chloride (PVC) or latex gloves. Polypropylene (PP) or high-density polyethylene (HDPE) bottles were autoclaved and identified as sterile with autoclave tape before being used for sample collection. Sampling started on the left side of the swimming beach area (when facing water) on the hour with the sampler entering the water down-current or down-wind of the collection site and in a manner to avoid disturbing sediments. Samples were collected where the lake was approximately three feet deep, and 10 feet from nearest swimmers. The bottle was held near its base and plunged opening downward 8 to 12 inches below the water surface and turned underwater and away from the sampler to fill with lake water, leaving ~1 in. air space so the sample could be shaken just before analysis. All sample containers were transported to the King County Environmental Laboratory (KCEL) on ice, stored at 4oC, filtered and analyzed within 24 hours following KCEL SOP # 05-03-001-000 (King County 2002).

Samples were collected and analyzed for *E.coli* according to SOP # 506v (King County 2002).To provide a check on citizen generated data collection in Thornton Creek, additional bacteria samples were collected in Thornton Creek by the environmental group, Thornton Creek Alliance (TCA) on August 28, 2018. Bacteria analysis for these samples was done using Coliscan Easygel ® using 3 ml of sample water. Synoptic sampling by TCA occurred on at SB434 and at the middle of the swimming area.

Samples for the human-specific Bacteriodales analysis were collected synoptically with *E.coli* samples. Quantitative polymerase chain reaction (qPCR) technique was used to isolate and identify human-specific, Bacteroidales bacteria SOP#570v0, 2014 (King County, 2014; Table 1). This gene is carried by many Bacteroides species (Order level: Bacteroidales). Many of these bacteria have not been cultured, so the calibrators for this assay are derived from a cloned product. To make a clone product, the gene fragment (or target) is inserted into a plasmid and placed in a vector (in this case *E. coli*) and then used as positive control, and this human biomarker is designated as HU-2 and results are reported as DNA copies per\100ml (KCEL, pers comm 2019). In recent blind challenge studies, this biomarker was been found to be very specific to human waste and only found in animal waste at low numbers (as transient carriage). This human biomarker has demonstrated a 95% specificity and a prevalence of 100% in sewage samples (Shanks et al. 2009).

Temperature/Number of Swimmers

Water temperature of the swimming beach was collected at the middle beach sampling site and the stream site using a certified hand-held, digital thermometer. A count of swimmers (anyone in the water) for the entire swimming area was made before completing water sampling. No count of swimmers occurred between hourly sampling events. Data on waterfowl within the swimming area but not grazing on the beach or outside of the roped swimming area was also collected.

Table 1. Microbiology parameters, sample collection containers and detection limits.

	analyte	**methodology**	**container**	**Holding times**	**MDL**[1]
King County	*Escherichia coli*	SOP # 506v	500 ml PP[2] or HDPE[3], sterile	24 hrs.	1 CFU/100ml
TCA[4]	*Escherichia coli*	Coliscan Easygel	10 ml PP, sterile	24 hrs.	33[5] MPN/100ml
King Count	Human (Hu2) Bacteriodales	SOP # 506v	500 ml PP or HDPE, sterile	24 hrs.	350 DNA copies/100ml

1. method detection limit
   polypropylene
2. high density polyethylene
3. Thornton Creek Alliance citizen science bacteria monitoring
4. 3 ml sample collected

Results

The *E.coli* data collected in this study provides an initial estimate of the temporal and spatial viability at this swimming beach (Table 2; Figure 2). There were differences in Matthews Beach FIB counts between sampling days, between sampling locations and hour to hour (Figures 2 and 3; Table 2) that provide an estimate of the variability in bacteria data which could influence the decision to open or close the swimming beach. The maximum and minimum *E.coli* counts collected between 11 AM and 6 PM at each of the north, middle and south sampling locations were frequently one to two orders of magnitude different, depending on when during the monitoring period the samples were collected and there were large differences in the daily *E.coli* averages from one day to the next (Table 2). There were insufficient daily sampling events to investigate whether the day of the week influenced results. There was no apparent common daily temporal pattern in the FIB results (Figure 3). Most Bacteriodales counts in the swimming area were below the proposed method detection limit (MDL) of 350 DNA copies/100ml and rarely <MDL and frequently high in Thornton Creek (Table 3).

Temporal and spatial differences in FIB counts make estimating exceedances of bacteria criteria problematic. Temporal differences occurred at hourly, daily and weekly scales. Hourly changes in *E.coli* and Bacteriodales data show that single grab samples or averages of triplicate samples were subject to large changes in FIB counts depending on both the hour and the day samples were collected (Figure 3). Hourly means and SE were calculated from the north, middle and south swimming areas for each hourly sample between 11 AM and 6 PM (Figure 2). No consistent pattern in the hourly results between sampling days was apparent.

Additionally, there were large differences between sampling days that also did not follow a discernable pattern. Average *E.coli* counts collected on August 28 were 62 +/-15 CFU/100ml (n=24), the next day August 29 average *E.coli* counts increased to 396 +/- 133 CFU/100ml (n=3) and then on August 30 average counts were back down to 8 +/-3 CFU/100ml (n=3; Figure 2; Table 2). On Tuesday August 28 *E.coli* counts were below the proposed WDOE criteria (WAC 173-201A; *E.coli* <100 CFU/100ml; n=24), then on Wednesday August 29 *E.coli* counts exceed this criteria (n=3), and on Thursday August 30 *E.coli* counts (n=3) were well below criteria. No precipitation or wind events occurred during this period. The high *E.coli* counts on August 29 were collected once at 14:30 and no other data is available to determine if this was a short duration, localized or persistent event. Increased sampling did decrease the SE around the August 28 FIB estimate compared to days when only a single set of samples was collected (Figure 2).

Table 2. *E.coli* (CFU/100/ml) in the north, middle and south swimming area of Matthews Beach and SB434 near the mouth of a Thornton Creek collected on August 19, 21, 22, 23, 26, 28, 29 30 and September 3, 2018.

***E.coli* (CFU/100ml) sample collection time**											
LOCATOR	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	**average**	**SD**	**n**
August 19, 2019											
SB434	670	1200	720	930	730	680	1000	870	850.0	66.1	8
0818SB north	18	120	9	20	15	13	15	60	33.8	13.6	8
0818SB middle	9	25	8	26	34	15	27	45	23.6	4.5	8
0818SB south	25	32	69	66	140	170	34	33	71.1	19.4	8
average[1]	17.3	59.0	28.7	37.3	63.0	66.0	25.3	46.0			
								all beach samples[2]	42.8	8.8	24
August 21, 2019											
SB434				850							
0818SB north				130							
0818SB middle				210							
0818SB south				290							
average				210.0					210.0	46.2	3
August 22, 2018											
SB434				660							
0818SB north				110							
0818SB middle				84							
0818SB south				17							
average				70.3					70.3	27.7	3
August 23, 2018											
SB434				750							
0818SB north				15							
0818SB middle				19							
0818SB south				100							
average				44.7					44.7	27.7	3
August 26, 2018											
SB434	820	550	570	700	560	480	490	660	603.8	40.8	8
0818SB north	48	20	11	5	47	150	22	42	43.1	16.3	8
0818SB middle	32	15	38	85	280	81	230	250	126.4	38.4	8
0818SB south	59	66	28	50	88	220	91	360	120.3	40.0	8
average	46.3	33.7	25.7	46.7	138.3	150.3	114.3	217.3			
								all beach samples	96.6	20.0	24
August 28, 2018											
SB434	430	490	550	460	600	650	500	600	535.0	27.3	8
0818SB north	10	25	55	96	90	1	55	20	44.0	12.7	8
0818SB middle	25	43	110	110	350	16	62	4	90.0	39.8	8
0818SB south	20	5	95	44	120	62	60	11	52.1	14.4	8
average	18.3	24.3	86.7	83.3	186.7	26.3	59.0	11.7			
								all beach samples	62.0	14.7	24

LOCATOR	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	average	SD	n
***E.coli* (CFU/100ml) sample collection time (table 2 cont.)**											
August 29, 2018											
SB434				410							
0818SB north				520							
0818SB middle				540							
0818SB south				130							
average				396.7					396.7	133.5	3
August 30, 2018											
SB434					460						
0818SB north					4						
0818SB middle					5						
0818SB south					14						
average					7.7				7.7		
September 3, 2018											
SB434	400	290	300	310	210	290	250	270	290.0	19.4	8
0818SB north	5	4	2	6	13	22	25	23	12.5	3.4	8
0818SB middle	8	4	3	14	14	68	58	14	22.9	8.9	8
0818SB south	49	1	7	3	19	18	53	28	22.3	7.0	8
average	20.7	3.0	4.0	7.7	15.3	36.0	45.3	21.7			
								all beach samples	19.2	3.9	24

1. spatial FIB average from north, middle, south sample sites (n=3), not including creek data.
2. statistics calculated on FIB samples collected at all 3 beach sites all 8 hours (n=24), does not include creek data.

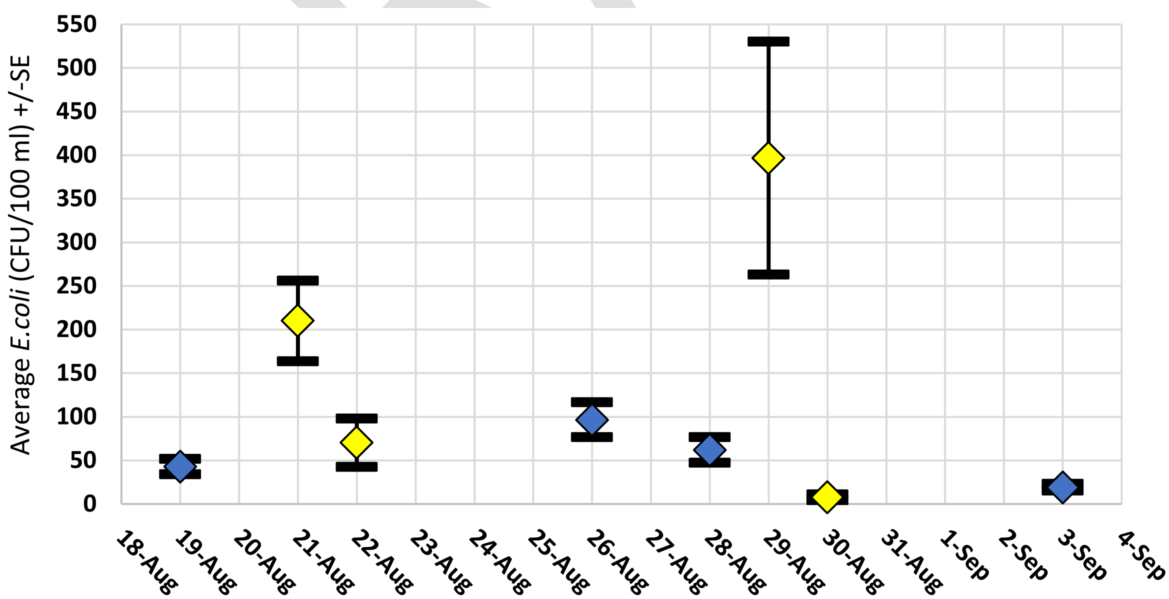

Figure 2. Average *E.coli* (CFU/100ml) +/-SE from all three sampling locations in the swimming area. Blue symbols are average counts collected on days with hour sampling (n=24) and yellow symbols are average counts collected at the three sampling locations 1X sampling day (n=3).

Spatial variability for *E.coli* counts between the north, middle and south sites in the swimming area was estimated but not for Bacteriodales as most of the samples were <MDL. *E.coli* averages and SE were calculated separately for the three swimming area sites for each hourly sample, and for the entire day by pooling all 8 hourly samples between 11 AM and 6 PM for each sampling event (Table 2). Previously, the swimming beach monitoring protocol called for the collection a single grab sample in the middle of the swimming area, typically around the middle of the day. Starting in 2019 the protocol was modified to collect three FIB samples evenly spaced across the swimming area (north, middle, south) and take the arithmetic average (King County 2018). The hour to hour differences in *E.coli* counts were frequently greater than the spatial variability between the sampling sites (Table 2; Figure 3). The *E.coli* average (+/-SE) was inconsistent on all days when hourly data was collected, and the variability was greater when *E.coli* counts were higher (1500 – 1800h on August 26) and small when *E.coli* counts were low (September 3).

Spatial differences between the north, middle, south sampling locations varied with the time of sampling with a weak general pattern of south ≥ middle > north (Figure 5). Exceptions to this pattern where the north site, furthest from the mouth of Thornton Creek, had slightly higher counts than the middle or south sites were (1200h August 19) for *E.coli* and had higher Bacteriodales counts on September 3 (Table 4), at 1600h and <MDL at all other hourly samples. On September 3 the swimming area had the highest Bacteriodales counts of all sampling days (Table 4), while synoptic *E.coli* counts where the lowest of any sampling events, all of which were below the WDOE water quality criteria of 100 CFU/100ml (Figure 4). The middle site had short duration elevated counts relative to the other hourly samples on August 26 and 28 (Figures 3 and 4) but was typically very similar to the south sampling location. The new sampling protocol of averaging these sites will miss these spatial differences, but those differences in this data set were not large or consistent (Figure 4;Table 2).

The range of *E.coli* results (Table 3) is an indication of how different sampling results could be depending upon the time of day samples were collected. Samples collected at the middle site on August 26 1600h *E.coli* sample (<100 CFU/100ml) and the average of the three samples was 150 CFU/100ml. These samples had some of greatest spatial differences between sites on days data was collected (Table 2, Figure 4). At 1700h the same day the middle site was above the WDOE criteria (230 CFU/100ml) while the average was 114 CFU/100ml (Figure 3; Table 2). Neither of these results would have much influence on a beach closure decision but combined with the range in samples (Table 4) show the potential change sampling time could have on reported values. Based on this data, the recent change in sampling protocol from single middle site grab samples to the average of three north, middle and south grab samples would not influence a beach closure decision nearly as much as when during the day samples were collected. There does not appear to be a consistent pattern in the hourly data or a consistent time of day when the highest FIB counts would occur.

The hourly patterns in *E.coli* counts did not show an obvious influence from either swimmer counts (Figure 5) or waterfowl counts (Figure 6). However, swimmer and waterfowl counts were only collected at the time of water sampling not continuously and not recorded on days when hourly samples were not collected which decreased the accuracy of the swimmer and waterfowl data. Additionally, days this data was collected at Matthews Beach coincided with a period of cool late summer weather with persistent extremely poor air quality from regional wildfires that potentially reduced swimmer numbers. August 19 and 26 and September 3 were <60^{o} F for most of the day and only August 28, the day with the highest swimmer use (Figure 5), had consistent temperatures >70^{o} F. No differentiation was made as to whether swimmers were in the north, middle or south section of the swimming area, number of infants in diapers nor were counts taken between hourly sampling events. Waterfowl data in the swimming area was also collected inconsistently and both swimmers and waterfowl moved constantly throughout the beach.

Table 3. Bacteriodales (DNA copies/100ml) in the north, middle and south swimming area of Matthews Beach and SB434 collected on August 19, 21, 22, 23, 26, 28, 29 30 and September 3, 2018. Counts greater than method detection limit (350 DNA copies/100ml) in yellow shading.

		sample collection time							
	LOCATOR	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00
19-Aug	0818SB north	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	0818SB middle	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	0818SB south	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	SB434	1200	1900	6800	740	1200	1100	<MDL	530
21-Aug	0818SB north				<MDL				
	0818SB middle				<MDL				
	0818SB south				<MDL				
	SB434				<MDL				
22-Aug	0818SB north				<MDL				
	0818SB middle				<MDL				
	0818SB south				<MDL				
	SB434				<MDL				
23-Aug	0818SB north				<MDL				
	0818SB middle				<MDL				
	0818SB south				<MDL				
	SB434				<MDL				
26-Aug	0818SB north	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	0818SB middle	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	0818SB south	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	SB434	<MDL	<MDL	1700	470	<MDL	<MDL	<MDL	<MDL
28-Aug	0818SB north	<MDL	<MDL	<MDL	690	<MDL	<MDL	<MDL	<MDL
	0818SB middle	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	0818SB south	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	SB434	75000	14000	5800	3700	4700	2300	44000	7300
29-Aug	0818SB north				<MDL				
	0818SB middle				<MDL				
	0818SB south				<MDL				
	SB434				1300				
30-Aug	0818SB north					<MDL			
	0818SB middle					<MDL			
	0818SB south					<MDL			
	SB434					<MDL			
3-Sep	0818SB north	<MDL	<MDL	<MDL	<MDL	<MDL	1300	<MDL	<MDL
	0818SB middle	<MDL	<MDL	<MDL	<MDL	<MDL	2100	450	390
	0818SB south	<MDL	<MDL	<MDL	<MDL	<MDL	540	770	320
	SB434	7100	1300	2400	460	<MDL	<MDL	2700	9200

The Thornton Creek site (SB434) is in the Matthews Beach park upstream of the lake backwater near the mouth at Lake Washington. Because of its proximity to the swimming beach and history of elevated bacteria counts in the creek, this site is sampled as part of the routine swimming beach monitoring program and is assumed to be a likely bacteria source to the beach. While this site is outside of the lifeguarded area, the creek is unofficially used extensively for water contact activities and waders were observed downstream of the sampling location on multiple sampling days. Although just over 500 ft. from the swimming area, this site was not included when calculating the swimming beach descriptive statistics (Table 2). All creek samples had *E.coli* counts (Figure 7) above the proposed *E.coli* <100 CFU/100 ml WDOE proposed recreational use criteria. There was hourly variability but no consistent hourly patterns on the different sampling days other than a general pattern of *E.coli* counts sequentially decreasing from the highest on August 19 to the lowest on September 3 (Figure 6). *E.coli* counts collected as part of the citizen science effort by TCA on August 28 (Table 5) were similar to counts from KCEL. All creek *E.coli* counts exceeded WDOE criteria and were higher than counts in the swimming area.

Table 4. Range of hourly *E.coli* counts collected between 11 AM and 6 PM.

	sample site	**north**	**middle**	**south**	**whole beach[1]**	**SB434 (creek)**
19-Aug	**maximum**	120	45	170	170	1200
	minimum	9	8	25	8	670
	range	**111**	**37**	**145**	**162**	**530**
26-Aug	**maximum**	150	280	360	360	820
	minimum	5	15	28	5	480
	range	**145**	**265**	**332**	**355**	**340**
28-Aug	**maximum**	96	350	120	350	650
	minimum	1	4	5	1	430
	range	**95**	**346**	**115**	**349**	**220**
3-Sep	**maximum**	25	68	53	68	400
	minimum	2	3	1	1	210
	range	**23**	**65**	**52**	**67**	**190**

1- combined north, middle and south sample site data, does not include creek data

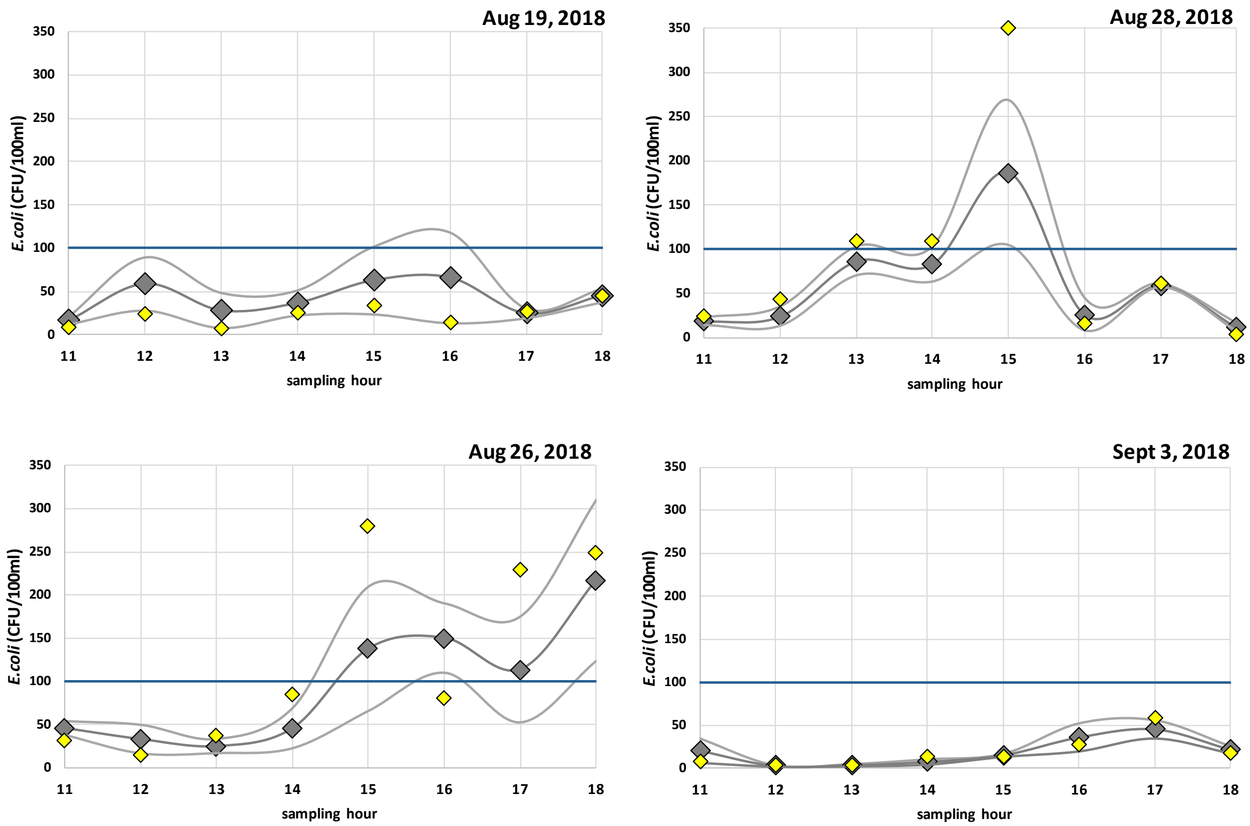

Figure 3. Matthews Beach north, middle, south hourly averages (n=3) *E.coli* (CFU/100ml) +/-SE, collected between 11 AM and 6 PM on August 19, 28, 26 and September 3, 2018. Yellow diamonds are the single grab *E.coli* count collected at from the middle of the swimming area (current swimming beach monitoring protocol), Adjacent Thornton Creek data is not included.

Table 5. Thornton Creek Alliance Citizen Science E.coli (MPN/100ml) collected on August 28, 2018.

sample site	Time	*E.coli* MPN/100ml)[1]			average	KCEL Result
TCA	14:00	500	666	666	611	460
TCA	16:00	567	533	167	422	650
0818SB middle	16:00	0	0		0	16

1- coliscan data using 3 ml samples (+/- 33 MPN)

The synoptically collected human-source Bacteriodales counts were much higher in Thornton Creek than in the swimming area on all days hourly samples were collected with the highest counts collected on August 28 (Table 4; Figure 8). The Bacteriodales and *E.coli* counts in the creek did not follow a discernable or consistent daily pattern and were not correlated. On the days when single grabs were collected (August 21, 22, 23, and 30, 2018; Table 4) Bacteriodales in both the creek and swimming area were <MDL, except for the August 29 (1300 DNA copies/100ml) in the creek. These single grab results were inconsistent with the consistently high creek Bacteriodales data collected on previous and following days when samples were collected hourly.

Table 6. King County temperature and fecal coliform (CFU/100ml) data collected as part of the routine swimming beach monitoring program and routine streams monitoring program

Sample Date	0818SB[1]		SB434[2]		0434[3]	
	Temperature (°C)	Fecal Coliform (CFU/100ml)	Temperature (°C)	Fecal Coliform	Temperature (°C)	Fecal Coliform (CFU/100ml)
8/13/2018	25.0	14	17.0	700		
8/15/2018					17.8	220
8/20/2018	24.7	49	17.0	730		
8/27/2018	23.0	49	15.0	[illegible]		
9/4/2018	22.0	<MDL	14.0	300		
9/10/2018	21.0	360	16.0	960		
9/12/2018					15.4	360
9/17/2018	20.0	31	15.0	390		

1. Thornton Creek sampling location synoptic with swimming beach monitoring

2. routine swimming beach sampling location in middle of swimming area

3. routine King County streams long-term monitoring location

The *E.coli* and Bacteriodales data from Thornton Creek show temporal changes on hourly, daily and weekly scales (Figures 7,8 and 9) but these patterns are not synchronized. The lack of correlation frequently observed between *E.coli* and Bacteriodales counts also occurred during each sampling event during this study and was highlighted in the August 28 Thornton Creek data (Figures 7 and 8). The creek *E.coli* counts were moderately high but continued a decreasing longer daily-weekly temporal trend from earlier sampling events (Figure 7;Table 2). On August 28 when Bacteriodales counts in Thornton Creek were as high as 80,000 DNA copies/100ml, only one Sept 3 had the most Bacteriodales samples >MDL of any of the sampling dates. Bacteriodales sample in the north swimming area >MDL (1400h 690 DNA copies/100ml), one of the few samples >MDL except for September 3 samples. The synoptic August 28 Bacteriodales creek counts were the highest of any collected in the study, and the hourly differences in the Bacteriodales counts were greater and followed a different hourly pattern than observed in the *E.coli* counts. The highest counts were collected at 11 AM and 5 PM (Figure 8; Table 3) temporal peaks not observed in the *E.coli* counts.

Bacteriodales counts in the swimming area on almost every sampling day were typically <MDL (320 DNA copies/100ml). The major exception was on September 3 when hourly samples had the highest and most frequently detected Bacteriodales swimming area counts (Table 3) in the middle and south sites in the swimming area closest to the mouth of Thornton Creek. Synoptic September 3 *E.coli* counts in Thornton Creek were not as high as on August 28. When the September 3 Bacteriodales counts were collected in the swimming area the Bacteriodales count in the creek was <MDL and the number of swimmers was less than observed on several other sampling days. The only other Bacteriodales swimming area sample >MDL was collected at 1400h on August 28 (Table 4) when Thornton Creek had the highest Bacteriodales counts (Figure 8) and the highest number of swimmers recorded (Figure 5).

On September 3 swimming area *E.coli* counts were low, swimming area Bacteriodales counts were high and counts of both indicators in Thornton Creek moderate. These high September 3 swimming area Bacteriodales counts were collected synoptically with *E.coli* counts in the swimming area that met WDOE criteria and were the lowest of any of the hourly sampling days. *E.coli* counts in the creek September 3 were ~400 - 600 CFU/100ml and continued the decreasing longer daily-weekly temporal trend from all earlier sampling events (Figure 7;Table 2).

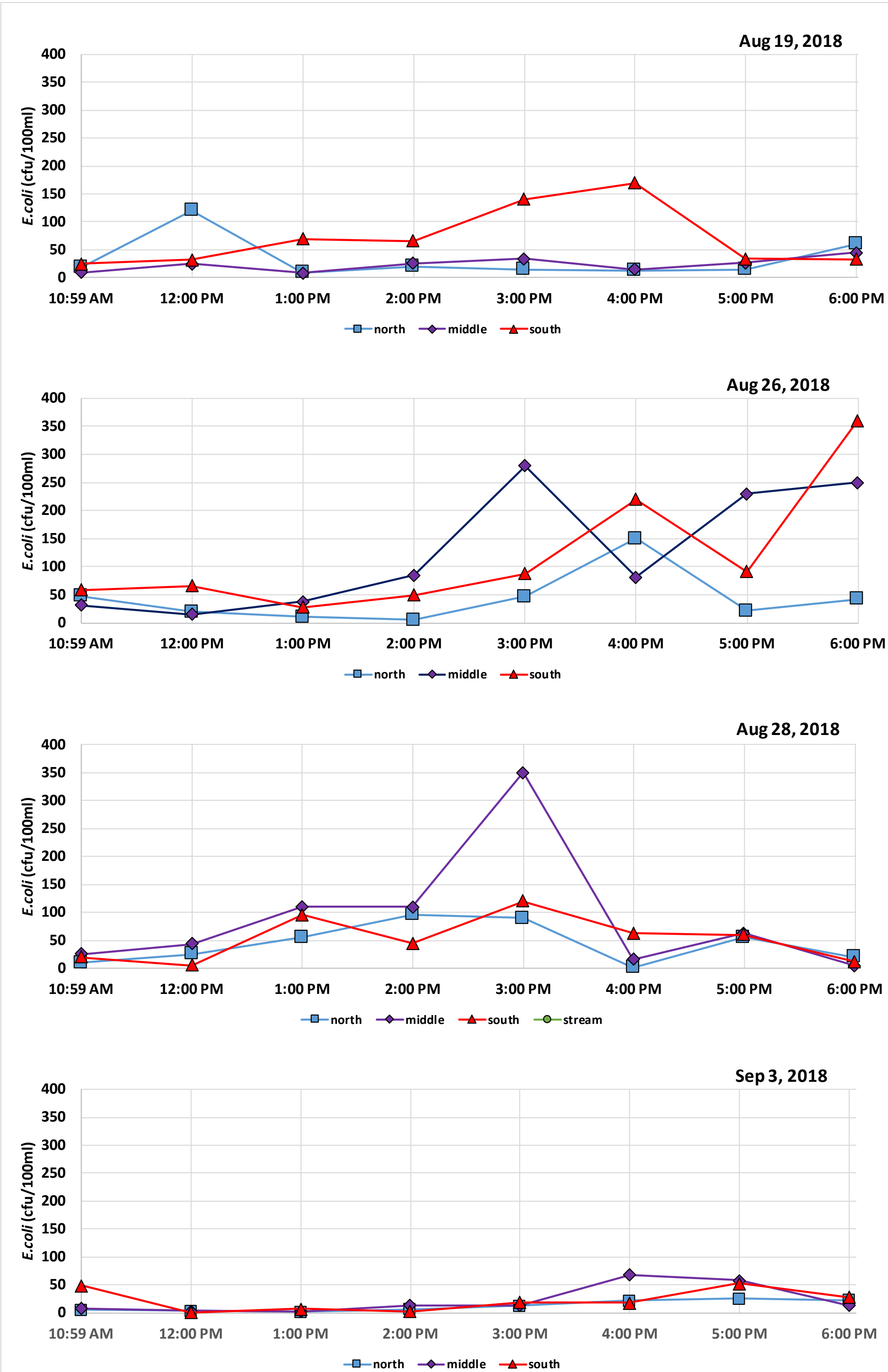

Figure 4. *E.coli* (CFU/100ml) collected at Matthews Beach north, middle and south locations on August 19, 26, 28 and September 3, 2018 hourly between 11 AM and 6 PM.

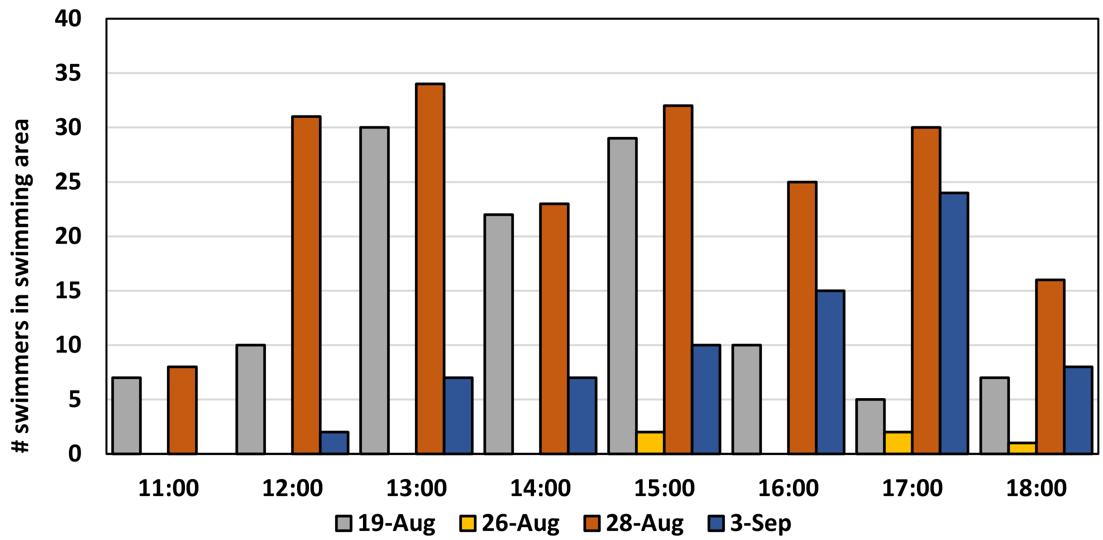

Figure 5. Number of swimmers counted immediately prior to hourly sampling events in the Matthews Beach swimming area.

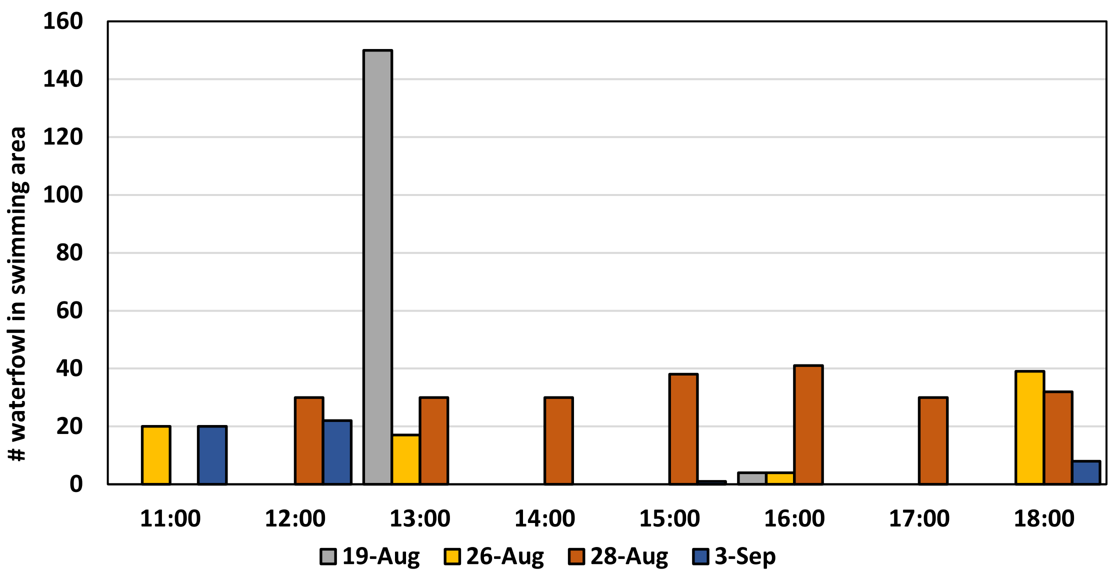

Figure 6. Number of waterfowl counted immediately prior to hourly sampling events in the Matthews Beach swimming area.

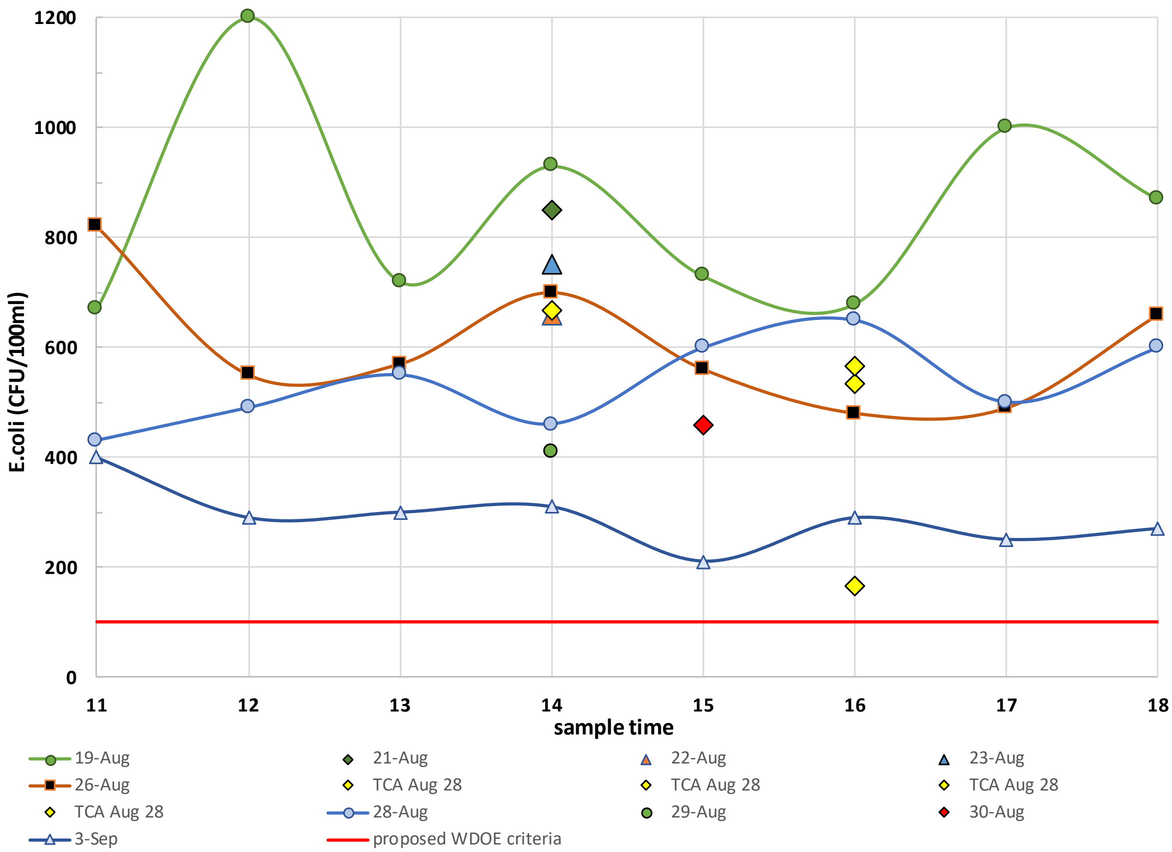

Figure 7. Thornton Creek (SB434) *E.coli* (CFU/100ml) collected between 11 AM and 6 PM on August 19, 26, 28, and September 3, 2018. Single grab samples were collected August 21, 22, 23, 29, 30, 2018 and citizen collected grabs samples (yellow) collected on August 28 Proposed WDOE *E.coli* bacteria criteria (100 CFU/100ml) is shown in red.

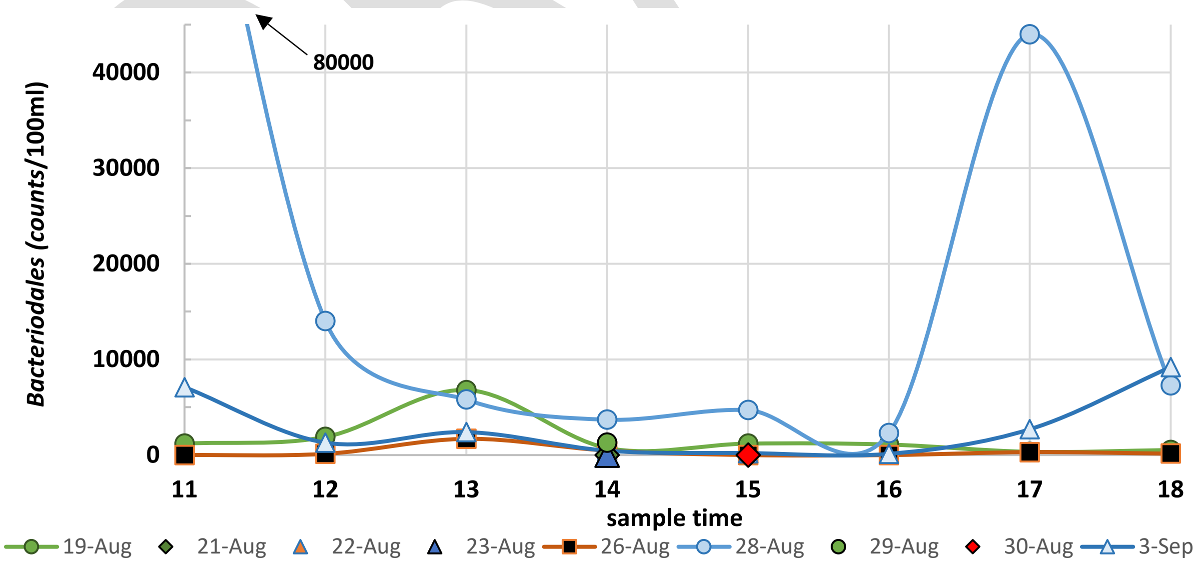

Figure 8. Thornton Creek (SB434) Bacteriodales (DNA copies/100ml) collected between 11 AM and 6 PM on August 19, 26, 28, and September 3, 2018. Additional single grab samples were collected August 21, 22, 23, 29 , 30, 2018.

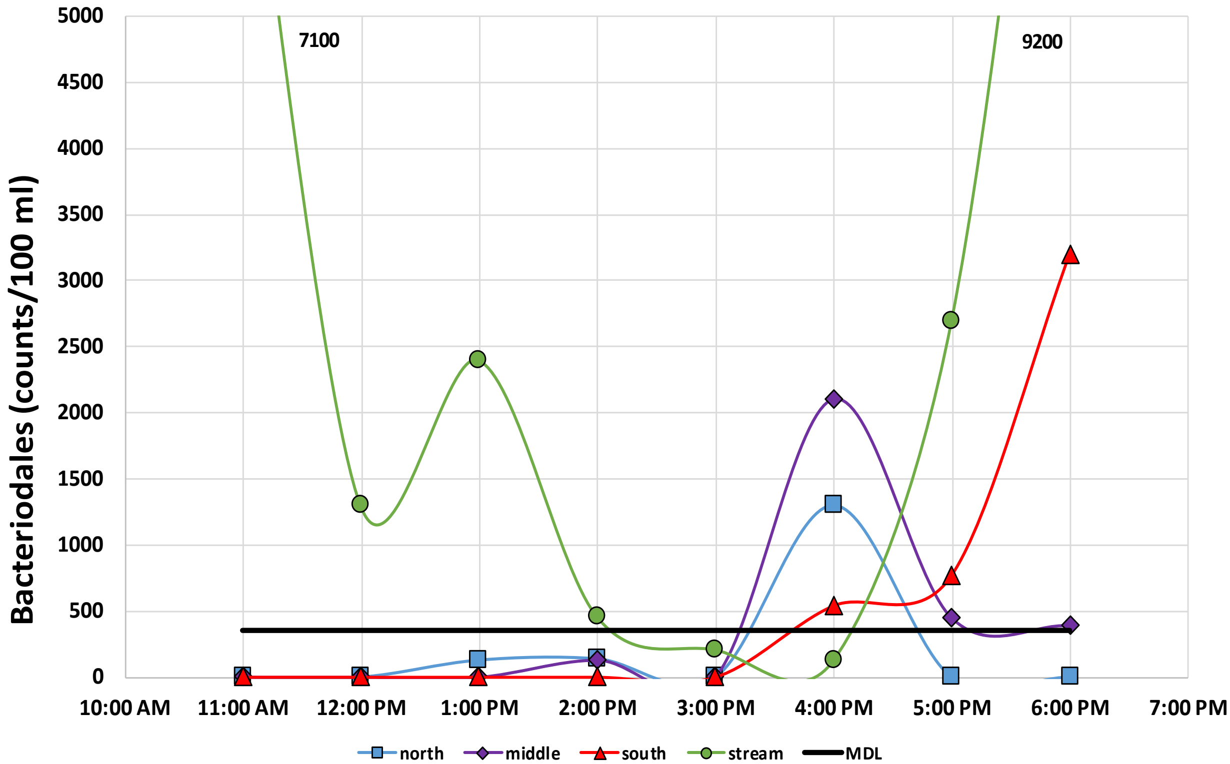

Figure 9. Bacteriodales counts/100 ml in the north, middle and south sites at Matthews Beach and Thornton Creek (SB434) collected on September 3, 2018. Data points below the 350 counts/100 ml MDL line are not quantitative (<MDL) and are included to show that Bacteriodales samples were collected synoptically with the samples >MDL. The only other Bacteriodales sample >MDL was on August 28 at 1300h (690 DNA copies/100ml).

The King County swimming beach and routine streams monitoring sampled only for fecal coliform bacteria (Table 6) based on the previous WDOE criteria that was based on fecal coliform. The Thornton Creek stream sampling site is located further upstream of SB434 and closer to the site of the homeless encampment (Figure 1). Assuming fecal coliform was $\geq$ *E.coli* data collected in the routine monitoring program shows a similar pattern of high bacteria in Thornton Creek with counts over standards. Fecal coliform counts in the beach from August and September were all well below criteria except one sample collected on September 10 after the intensive beach sampling concluded. Bacteriodales counts in swimming beach samples throughout the study were <MDL with exceptions on August 28 and September 3 (Table 3).

Discussion

The effectiveness of FIB criteria should be measured by an ability to correctly identify conditions when water exposure will lead to unacceptable exposure risk. There is consensus that the EPA 1986 criteria which relies on single grabs and long-term geometric means did not accomplish this (Kim & Grant 2004). How well a swimming beach sampling design provides the necessary information for decisions on health risk depends on how well the data collected represents the swimming water environment and swimmers' exposure. Most studies including this one concludes that a single-sample of water reveals little about the water quality of an entire recreational site (Boehm 2007) and emphasize why understanding the temporal and spatial variability in FIB is important. Olyphant and Whitman (2004) estimated that with sampling once per week there was a 75% chance of missing exceedances.

Fortunately, when the source of the bacteria is not small, isolated or ephemeral, but rather a large persistent source, such as an on-going sewage spill or leak, the temporal variability will be less important. Unfortunately, during this type of event, the potential human health risk is high. The bacteria sampling in Thornton Creek and Matthews Beach was conducted to obtain estimates of variability and investigate the representativeness of the current beach monitoring design and was not conducted in response to a specific event. Past specific events such from broken side-sewers and leaks from bathhouses have been identified and corrected using the previous monitoring protocols. The identification of these events was based on single grabs samples of waters with consistently high FIB and the temporal variability less of a problem in collecting a representative sample. The decision to close the beach in these cases was obvious. This study assumes these results provide initial estimates for the entire swimming season at multiple beaches, but this assumption remains untested.

Armstrong et al. (1996) recognized that loading of fecal pollution at monitored beaches was episodic, despite a relatively constant flux of indicator organisms in the presumptive sources (outfalls) of beach indicators. Both the *E.coli* and Bacteriodales data collected in the Matthew Beach swimming area was also episodic, with long and short-term temporal differences, while the spatial differences between the north, middle and south areas of the beach were not pronounced. The bacteria data collected at Matthews Beach and in Thornton Creek, and previously in this watershed (Frodge 2013) show FIB counts are episodic, which contributes to these sources remaining elusive of detection or prediction and difficult to monitor effectively. Matthews Beach lacks what Armstrong identified as 'presumptive sources' as there are no stormwater outfalls adjacent to the beach and the elevated FIB detected in Thornton Creek was not likely to be stormwater derived as sampling in the creek occurred during a prolonged dry period.

Temporal viability at Matthew Beach occurred on multiple time scales. *E.coli* samples at Matthew Beach frequently had order of magnitude hourly differences with similar variability observed between one day and the next. Single FIB grab samples, the average of three samples across the swimming area, and the hourly data collected over multiple sampling days at the three sampling locations did not appear to fully describe the variability of the bacteria or potential exposure swimmers may have experienced at this beach. Nor was there a consistent pattern in the hourly *E.coli* or Bacteriodales counts. The temporal variability in this bacteria data was too high and too inconsistent with current data to predict a time of day that would be optimal to sample for a representative FIB counts to determine the exposure risk to swimmers.

The variability in the daily and hourly bacteria data indicate that the FIB counts at this beach result from short and long-term temporally variable episodic events making it extremely difficult to design a sampling plan that will collect a representative FIB sample for estimating exposure risk to swimmers. The problem with this approach is there most likely is not a representative FIB sample except when FIB counts are extremely high or the water extremely clean. Given the variabilities and uncertainties associated with sample collection and analysis, there is a high probability for misclassification of water quality for samples whose indicator level is near the water quality standard (EPA 2010). The current monitor design will likely catch large persistent events, but the smaller ephemeral events will frequently be missed.

Temporal variability in indicator density, at time scales ranging from minutes to months, has been observed in time series analyses of indicator density. Variations with time scales on the order of minutes are important because such considerations influence the number of samples needed to accurately characterize microbial water quality and the confidence with which to ascribe results of sampling events (EPA 2010). Boehm (2007) noted very high variability in enterococci density at time scales less than 1 hour, and that 70 percent of single sample exceedances had durations of less than 1 hour, and 40 percent had durations of less than 10 minutes (Boehm et al. 2002).

Several studies have discussed the temporal variability of FIB in swimming beach sampling, although few studies discuss how to design a monitoring program to account for the temporal variability other than on-going sampling at frequencies as short as 10 minutes or less (EPA 2010; Boehm et al. 2002). To achieve a coefficient of variation of 50 percent around the one-hour mean, the number of samples at four sampling points was estimated to be 6, 5, 4, and 4, respectively. Boehm (2007). Considering the hourly variability, the authors note the need for a warning system that operates semi-continuously, although this warning system is undescribed. It is unlikely that Matthews Beach, let alone the other twenty swimming beaches monitored by King County, could be routinely sampled at a frequency or replication adequate to describe the FIB variability observed by these authors.

Two strategies proposed by EPA (2010) for overcoming short time-scale variability, such as that observed at Matthews Beach, when assessing bacteriological water quality are to select sample sites with less variability (e.g., sites at greater water depth) or to use composite samples if sampling at locations with high variability cannot be avoided or is required. Avoiding locations with high variability seems to avoid areas that are exposed to ephemeral inputs of FIB and assume these sources are a lower public health risk, which may not be the case. Arithmetic averages of the north, middle and south samples has been implemented in the current sampling protocol for the King County swimming beach program which is probably adequate for the small spatial variability in the swimming area, although this sampling is still once per day which does less to characterize the temporal variability.

Several studies state fecal indicator bacteria demonstrate a predictable pattern of highest density in the morning and a decrease of indicator bacteria during daylight hours results from inactivation of organisms by incident solar radiation (Sinton et al. 2002; EPA 2010, Roser et al. 2007). This pattern was also not observed at Matthews Beach. While time of day is not a direct determinate factor, but a correlate for ambient factors such as wind and insolation intensity and dosage, it is a critical element for sampling and management strategy (EPA 2010). Based on data collected in this study, the recent change in sampling protocol from single middle site grab samples to the average of three north, middle and south grab samples would not influence a beach closure decision nearly as much as when during the day samples were collected, as there does not appear to be a consistent pattern in the hourly data or a consistent time of day when the highest FIB counts would occur.

Day to day temporal differences in FIB counts also make estimating exceedances of bacteria criteria problematic and provide little insight as to which day of the week may be the best to sample. Olyphant and Whitman (2004) determined there was virtually no correlation in *E. coli* density for successive days for *E. coli* collected on the prior day at the same time for samples taken at a Great Lakes beach, correlation was, however, observed between *E. coli* density in samples taken at different times on the same day. Similar daily differences occurred at Matthews Beach when on Tuesday August 28 *E.coli* counts (62) were below the proposed WDOE; the next day Wednesday August 29 *E.coli* counts exceed criteria (397), and on Thursday August 30 *E.coli* counts were well below it (8). The high *E.coli* counts on August 29 were collected once at 14:30 and no other data is available to determine if this was a short duration, localized or persistent event. Increased sampling did decrease the SE around the August 28 FIB estimate compared to days when only a single set of samples was collected.

Spatial variability for *E.coli* counts between the north, middle and south sites in the swimming area was estimated but not for Bacteriodales as most of the samples were <MDL. Spatial differences between the north, middle, south sampling locations varied with the time of sampling with a weak general pattern and

a few exceptions of south > middle > north. A potential explanation for the spatial differences that were observed between these sites is a combination of water from Thornton Creek moving through the swimming area in relatively discreet plugs carrying the bacteria from the creek into the swimming area. Brenniman et al. (1981) did not find significant differences in indicator concentration between transects at the center and edges of two Lake Erie beaches. In a similar study of 10 Lake Michigan and Lake Superior beaches, Kleinheinz et al. (2006) also found no significant variation in indicator density between horizontal (along-shore) samples. While the bacteria results support the creek as a presumptive source of bacteria, no data on water movements is available that would support this hypothesis. A preliminary evaluation of local wind was inconclusive as well.

Another hypothesis is the swimmers or waterfowl were the source of the observed spatial FIB differences. However, swimmer and waterfowl counts were not collected continuously, only at the time of water sampling and not recorded on days when hourly samples were not collected. Additionally, days swimmer and waterfowl counts were collected coincided with a period of cool late summer weather with persistent extremely poor air quality from regional wildfires that potentially reduced swimmer numbers. August 19 and 26 and September 3 were <60º F for most of the day and only August 28, the day with the highest swimmer use, had consistent temperatures >70º F. The highest *E.coli* counts in the swimming area occurred in the afternoon on August 26 and August 28. No differentiation was made as to whether swimmers were in the north, middle or south section of the swimming area, number of infants in diapers nor were counts taken between hourly sampling events. Waterfowl data in the swimming area was also collected inconsistently and both swimmers and waterfowl moved constantly throughout the beach.

However, swimmer and waterfowl counts were only collected at the time of water sampling not continuously and not recorded on days when hourly samples were not collected which decreased the accuracy of the swimmer and waterfowl data. Additionally, days this data was collected at Matthews Beach coincided with a period of cool late summer weather with persistent extremely poor air quality from regional wildfires that potentially reduced swimmer numbers. August 19 and 26 and September 3 were <60º F for most of the day and only August 28, the day with the highest swimmer use (Figure 5), had consistent temperatures >70º F. No differentiation was made as to whether swimmers were in the north, middle or south section of the swimming area, number of infants in diapers nor were counts taken between hourly sampling events. Waterfowl data in the swimming area was also collected inconsistently and both swimmers and waterfowl moved constantly throughout the beach.

Because the water quality at many beaches is adversely impacted by inputs of contaminated stormwater runoff, many states report that they have developed standards for issuing preemptive rainfall advisories based on rainfall intensity or some other rain-related factor for at least some of their beaches (EPA 2010). Some states also issue standing advisories warning the public to avoid beach water contact after heavy rainfall or when storm drains are running (NRDC 2017).

While Matthew Beach has been impacted by rain events in the past, in the late summer of 2018 there was little rainfall that could explain the FIB counts at this beach. In 2005 Matthews Beach was closed on July related to the discharge of urban stormwater from Thornton Creek. A rainstorm a few days prior to bacteria sampling caused increased flows and discharge of bacteria in many area streams. The stormwater from Thornton Creek was the apparent source for the high counts at Matthews Beach, and as flows decreased, so did the bacteria counts (King County2004). During the present study, Seattle was in the driest May-September on record (KOMO 2018) with only two very light rain events during the study. Summer rain events impact FIB at this beach, but lack of rain during this study makes rainfall an ineffective predictor of FIB counts at Matthews Beach.

While there are no stormdrains in the vicinity of Matthew Beach, Thornton Creek discharges into Lake Washington just 500 feet south of the swimming area. In inland lakes, the configuration of influent streams and the difference between influent water temperature and ambient lake temperature might influence the distribution of indicators in the water column (EPA 2010). Thornton Creek has been listed on the 303(d) list for several years as water quality limited waterbody for fecal coliform bacteria, with mean fecal coliform bacteria of 862 CFU/100 ml during non-storm flows and 4793 CFU/100ml during storm events. (King County 2019). Because of the frequent and high bacteria in the creek and proximity to the swimming area, Thornton Creek is sampled both as part of the King County routine ambient monitoring program and each week along with the collection of swimming beach samples at Matthews Beach. The *E.coli* and Bacteriodales counts in Thornton Creek collected in this study were almost continuously above water quality criteria and appear to result from a more consistent, less variable bacteria source than in the open lake waters of the Matthews Beach swimming area. All creek samples had *E.coli* counts above the proposed Washington Department of Ecology (WDOE) proposed recreational use criteria (100 CFU/100 ml), as were the independent samples collected as part of the TCA citizen science project.

There are currently no criteria for Bacteriodales, but the consistently high Bacteriodales counts in Thornton Creek indicated human fecal material in the creek on all hourly sampling days. Rapid methods, such as quantitative polymerase chain reaction, may help in identifying beaches impacted by an undetected sewage spill. However, rapid detection technologies are not likely to be appropriate for all management situations due to equipment costs, skill level of technicians in small agencies, and other logistical aspects.

The synoptically collected human-source Bacteriodales counts were much higher in Thornton Creek than in the swimming area on all days hourly samples were collected and highest on August 28. The Bacteriodales and *E.coli* counts in the creek did not follow a discernable daily pattern or similar patterns and were not correlated. On the days when single grabs were collected Bacteriodales in both the creek and swimming area were <MDL, except for one sampling day on August 29. These single grab results were inconsistent with the high creek Bacteriodales data collected on previous and following days when samples were collected hourly. This discrepancy either resulted from high day-to-day variability or is an artifact of missed temporal variation in the creek when sampling was only once per day. What was not missed in the sampling was human fecal material was in Thornton Creek on most days that the creek was sampled, and Bacteriodales was observed in the swimming area, but much less frequently and at lower counts. Hourly changes in FIB is likely due to a plug-flow nature of flow in the creek and ephemeral inputs from unidentified sources upstream.

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Short-term variability (time scales of less than 1 hour) has also been observed in streams. Event-scale and diurnal variability are generally greater than short-term variability in streams; although, sudden loading can result in rapid changes in stream indicator density. Because short time-scale variability in streams is less significant than other variabilities, short time-scale fluctuations are not a significant factor in developing sampling plans for stream sites (EPA 2010). Additionally, morning samples are reported to yield conservative results relating water quality to human health effects when using culture methods, whereas the use of qPCR methods yields results that are relatively stable throughout the day (EPA 2010; EPA 2019). This was true for the September 3 swimming beach sample, but not for the Bacteriodales in the creek. The diurnal changes in the creek most likely result from the downstream movement of water in the creek and potentially non-constant inputs of bacteria. The most likely source of Bacteriodales in the swimming area is packets of creek water moving into the swimming area by wind or wave action that was not measured in this study.

Wade et al. (2006) states that indicator density diurnal variation for qPCR methods is lower, with relatively stable indicator density reported for samples taken throughout the day. This is apparently due to the different persistence and sensitivity to light molecular material versus viable culture cells. Those differences result in differences in diurnal variation in indicator densities when measured by the two techniques. Differences between *E.coli* and Bacteriodales hourly samples occurred in both Thornton Creek and in the swimming area although much higher in the creek and there were differences in the diurnal patterns between *E.coli* and Bacteriodales.

A potential source of the consistently high *E.coli* and Bacteriodales counts in the Thornton Creek during this study was unsanctioned long-term encampment of ~five tents lacking formal sanitary facilities immediately adjacent to the creek ~1400 feet upstream of the creek monitoring site. This camp was documented to have human waste on site and was cleaned up by the City of Seattle Encampment Response Team on July 23, 2018 just over three weeks prior to this sampling. No documentation on collection of the human waste was recorded, and no post collection method of disinfection was employed (City of Seattle Site Journal, pers com. 2018).The highest Bacteriodales counts in Thornton Creek were collected on August 28 two days after the only substantial precipitation event during the study which could have mobilized fecal material into the creek. The only locally recorded rain during the study monitoring period was 0.01 in. August 23, and 0.11 in. on August 26 https://www.wunderground.com/dashboard/pws/KWASEATT1933#history/s20180828/e20180828/mdaily .

No *E.coli* or Bacteriodales samples were collected above the camp site during the study which decreases the confidence that this location was a primary source of the fecal pollution. However, the persistently high *E.coli* and Bacteriodales counts in Thornton Creek downstream of this site showed a persistent presence of human fecal material in this section of creek and an increased exposure risk in this water body and there is a high probability that a source of human fecal material to the creek was upstream.

Identification of fecal contamination sources is necessary for hazard analysis and accurate assessment of the risk posed by pathogens. Once hazards are identified within a watershed, strategies can be developed to reduce fecal contamination thereby reducing the risk to human health (Meays et al. 2006). The location of un-sanctioned camps without sanitary facilities adjacent to waterbodies is both a pathogen hazard as well as a public policy issue in Seattle and much of the US. Reduction of human fecal exposure in this public park used for water contact recreation requires a policy discussion of the risk of allowing camps without sanitary facilities in riparian areas adjacent to parks.

Conclusions

Stay out of the water!

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