University of Washington Bothell
North Creek Water Quality Analysis Report
Independent research spring 2013
Anne Power and Kent Parkinson

Introduction

Since October 2012, the consultant group GeoEngineers had been collecting water samples and running analysis on the quality of North Creek water. This research found that wetland depressions of North Creek were polluted with exceptionally high levels of fecal coliform and *E. coli* pathogens. As recent as April of 2013, fecal coliform counts were found in a range that exceeded 300,000 CFU's per 100 mL at one of the micro-depressions being tested. This evidence indicated that these pathogens spiked on the University of Washington Bothell Campus and that the source was unknown. Due to these findings student research began in late April and continued through June 6th. Water samples were collected and analyzed on a weekly basis. The intention of this research is to monitor over an extended period of time the water quality of North Creek as it flows through campus, and to determine if possible the source(s) of the high levels of pathogens found on site. There are a couple hypotheses as to the source. One hypothesis is a broken sewer line leaking untreated sewage into the creek. The other is the possibility of the source being crow feces deposited by the large number of crows that roost in the wetlands at different times throughout the year.

Six sites were chosen in total, five of which are in the University's wetlands (see Figure 1). Of these five sites, site one, three, four and five, samples were taken directly from North Creek. Site two is a pond (see Figure 3) near the walking path in the wetlands, however, we decided to drop site two and concentrate on the main channel of North Creek and its pathogen levels.

Site one (see Figure 2) is on the most southern edge of campus and south of the crow roosting area identified by University students and faculty. This site is next to Highway 522 and is the final out flow of North Creek as it leaves campus. This site was chosen because it is the last site available before departing the wetlands and University property. It also has a scale affixed to a permanent post to indicate water depth.
Site three (see Figure 4) is on the main channel downstream to the south from a King County sewer trunk line near the area preliminary data indicated spikes in the pathogen populations,

yet upstream from the crow roosting habitat that has been observed by the University's students and faculty. This site was chosen for two reasons. It can give information as to the water quality before entering the roosting area and it can give us data about the sewer trunk line and any possible affects it may have on North Creek water quality.

Site four (see Figure 5) is on the main channel above the King County sewer trunk line that travels under Interstate 405 and through the campus wetland. This site was chosen to get an indication as to the quality of the water before it flows over the sewer trunk line and picks up any possible contamination, should there be a leak from the pipe.

Site five (see Figure 6) is on the northern end of the University's wetland on the main channel of North Creek. This site was chosen for two reasons. Site five is the first site water could be sampled as it came onto campus after passing under Interstate 405. It is also approximately 100 yards above the secondary channel giving us a clear indication of what is in the flow without any back water flow that may result from feeding into the secondary channels mouth.

Site six (see Figure 7) was adopted later in the study and was chosen because it was off campus in the north creek business park across interstate 405 from the University campus. We chose to adopt this site to verify a quality baseline of the water flowing onto the University campus.

Please refer to the map and photos for site locations. (See Index)

Methodology in the field:

Equipment used on all sites consisted of a calibrated "YSI 85 Meter" used for detecting oxygen levels in percentages and milligrams per liter, temperature readings in Celsius and conductivity readings in micro Siemens. Readings were collected via suspension of the probe directly into the flowing water, and allowing the readings to stabilize before recording the data. A calibrated Oakton pH Tester 10 was also used to measure the pH levels of the water at all sites. The application of this device required the probe end of the device to be suspended in the flowing water for approximately two minutes, allowing the pH reading to stabilize before recording the data. Both the YSI 85 Meter and the Oaktron pH Tester 10 were calibrated every week on the same morning samples were collected. Turbidity was also measured at all sites using a 2100P Turbidimeter model number 46500-00 by manufacture HACH. To collect the data for this device, three glass viles with screw on caps were rinsed three times in North Creek water and filled at a depth of about six inches under the surface of the creek. The rinsed caps were placed on the full vials, and the exteriors dried and cleaned with clean Kimtech Kimwipes manufactured by Kimberly Clark before being placed in the device for analysis.

To produce samples of water for analysis in the lab, four samples were taken at each site. One control consisting of reverse osmosis (RO) water (until the last week, June 3 & 4, when we switched to de-ionized water) and three North Creek Water samples. The control vial (100 mL) was filled and emptied three times with RO water, then filled a fourth time and capped. The remaining three 100 mL vials were submerged in North Creek at a depth of about six inches, filled and emptied three times to rinse. They were then filled a fourth time, again at approximately six inches depth, and capped. This process was repeated at each of the sites, the only variation being at site two, where it was a small pond of standing water and not the creek. All 100 mL viles and RO water supplied by the University of Washington

Methodology in the Lab

Upon return from the field, samples were immediately prepped for filtration using the dilution methodology as per HACH Analytical Procedures and EPA Approved Method 10029. Diluted water samples were then filtered through Millipore 47mm membrane using a vacuum pump filtration device with sterilized funnel and base, and placed into Millipore single use 47mm Petri-Pad petri dishes prepped with m-Coliblue 24 Broth culture medium. Dishes were then placed in an incubator oven at 35 degrees Celsius for twenty-four hours, then removed and placed into a refrigerator. The samples were held for twenty hours in this cold state before performing count. Using a Nikon SMZ1500 microscope, *E. coli* and Total Coliform colonies were observed and counted. Calculations for *E. coli* and Total coliform densities per 100 mL were performed. All data results summarized in excel spreadsheet (see attached electronic file).

Equipment sterilization process from 4/9/13 through 5/21/13 utilized a 10% Hydrochloric Acid solution bath with a triple rinse, and repeated. Equipment was allowed to air dry. This process was changed to washing the equipment with soap and warm water using a scrub brush, rinsing with water, then following up with a 70% Ethyl alcohol bath/rinse and allowed to air dry.

Discussion:

The first week (4/09/13) of sample filtration, we did not dilute the samples and they were filtered at 100 mL. Subsequent weeks were filtered using the aforementioned dilution methodology. Dilution amounts as follows:

25 mL sample/100mL solution   Weeks 4/16/13 – 5/2/13:

10 mL sample/100mL solution   Week 5/7/13

1 mL sample/100mL solution   Weeks 5/14/13 – 6/4/13

In attempts to prevent contamination, all equipment was put through the sterilization wash/ rinse process between *every* sample, not just between sample sites. Even so, we found there to be consistent contamination of total coliform in our control samples. This caused us to switch sterilization techniques. It is unclear yet if using the ethyl alcohol technique has improved upon the problem. We believe the contamination is likely coming from our sample bottles, as they have been used multiple times and not as a single use as they were intended. However, there has been no *E. coli* contamination problem.

Although we are focusing on the *E. coli* data, it is important to remedy the total coliform contamination problem. There may be unforeseen importance to having valid total coliform data available in the future.

In addition to testing water samples from North Creek, we obtained three water runoff samples from Will Jonsson. He collected the water runoff from the soil cores he pulled from various sites in the wetland. Table 2 in the Index contains the information about *E. coli* and Total Coliform count, as well as an image (Figure 8) of the sample growth from the crow area soil.

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Figure 1. Map of UWB campus wetlands, North Creek sampling sites

Figure 2. Photograph of Site 1 (southern most site on wetland creek)

Figure 3. Photograph of Site 2 (pond)

Figure 4. Photograph of Site 3 (south/downstream of sewer line, upstream of crow area)

Figure 5. Photograph of Site 4 (upstream of sewer line)

Figure 6. Photograph of Site 5 (northernmost site on campus wetlands)

Figure 7. Photograph of Site 6 (upstream, before creek passes onto campus property)

GPS Coordinates			4/9/13	4/16/13	4/23/13	4/30/13	5/7/13	5/14/13	5/20/13	5/21/13 Rain Event	5/28/13 Sterilization	6/3/13	6/4/13
N 47.75748 W 122.18881	SITE 1	Total Coliform /100 mL (a)	tntc	548	440	1052	2950	8000		34200			8700
		(b)	519.9996	716	564	1744	3040	4600		49000			8100
		(c)	913.3332	906	580	2906.66667	3400	6700		35900			6900
		TC CONTROL		0	0	4	210	500		24200			100
		E. coli / 100 mL (a)	tntc	72	84	788	1330	1900		3800			5000
		(b)	tntc	52	72	836	1110	800		3200			4200
		(c)	333.33333	92	84	tntc	880	1900		2700			4300
		EC CONTROL		0	0	0	0	0		100			0
N 47.75906 W 122.18961	SITE 2	Total Coliform /100 mL (a)		620	2506.66666	5066.66668	5480	1800					
		(b)		760	2186.6672	7706.66668	3210	2200					
		(c)		704	2613.3328	6080.0016	1790	5700					
		TC CONTROL		24	4	12	0	2600					
		E. coli / 100 mL (a)		4	40	112	100	0					
		(b)		16	24	112	0	0					
		(c)		0	36	136	0	0					
		EC CONTROL		0	0	0	0	0					
N 47.76166 W 122.18911	SITE 3	Total Coliform /100 mL (a)	tntc	448	1360	2373.33333	2030	6500		38666.6667			7900
		(b)		640	1680	1852	2600	7500		28100			5400
		(c)	tntc	676	1304	1973.33333	2420	5200		42100			7600
		TC CONTROL		4	0	4	240	500		104666.667			2400
		E. coli / 100 mL (a)	tntc	104	860	1680	520	1800		2400			200
		(b)		52	1236	2080	530	400		2900			1000
		(c)	tntc	92	1144	280	580	600		2500			300
		EC CONTROL		0	0	0	0	100		0			0
N 47.76237 W 122.18962	SITE 4	Total Coliform /100 mL (a)	620	516	1413.33387	2400	2970	5200		109333.333			17500
		(b)	tntc	684	1813.3344	2640	3100	3500		Error			99333.3332
		(c)	746.66667	692	1320	2720	3000	6200		78666.6668			15900
		TC CONTROL		0	12	0	20	400		600			0
		E. coli / 100 mL (a)	tntc	56	1140	240	420	1000		3300			800
		(b)	tntc	72	696	348	420	1000		Error			500
		(c)	tntc	80	2293.33333	240	530	400		2400			700
		EC CONTROL		0	0	0	0	0		0			0
N 47.76404 W 122.18859	SITE 5	Total Coliform /100 mL (a)	960	460	2000.00016	2799.9984	2470			46666.6668			14100
		(b)	tntc	584	1973.33333	2960	2460			28666.6667			12000
		(c)	960	668	1840	2613.33333	2600			71333.3332			6400
		TC CONTROL		0	0	79	0			1000			900
		E. coli / 100 mL (a)	213.33333	104	1733.33333	220	280			4600			0
		(b)	tntc	76	1733.33333	284	310			4000			100
		(c)	193.33333	48	tntc	276	370			4400			300
		EC CONTROL		0	0	0	0			0			0
N 47.76864 W 122.18689	SITE 6	Total Coliform /100 mL (a)							9100			4000	
		(b)							1900			3600	
		(c)							2300			3500	
		CONTROL RO H2O							100				
		CONTROL DI H2O							800			0	
		E. coli / 100 mL (a)							200			200	
		(b)							300			400	
		(c)							300			200	
		CONTROL RO H2O							0				
		CONTROL DI H2O							0			0	

Water Quality Research Project University of Washington Bothell Campus Wetlands Anne Power, Kent Parkinson with Professor Rob Turner **Soil / water runoff samples, collection by Will Jonsson**				
GPS Coordinates			4/29/13	5/6/13
N 47.76104 W 122.18783		**Total Coliform** /100 mL	1873.33333	
	15 mL	TC CONTROL	364	
		E. coli / 100 mL	tntc	
		EC CONTROL	0	
N 47.76067 W 122.18778		**Total Coliform** /100 mL		did not count. <10 count
	10 mL	TC CONTROL		
	Adjacent to depression in "Crow Area" See photo	***E. coli*** / 100 mL		92,866.67
		Thousands of discrete blue dots. A few specs of red (TC), mostly near perimeter of filtered area, however I did not count.		
N 47.76046 W 122.18764		**Total Coliform** /100 mL		tntc
	10 mL	TC CONTROL		
	Sample from streambank, approximately 2 inches above water flow. Previously under water.	***E. coli*** / 100 mL		tntc
		Lots of confluent growth / bleeding together of entire filter		

Table 2. Water runoff *E. coli* and Total Coliform counts from wetland soil samples

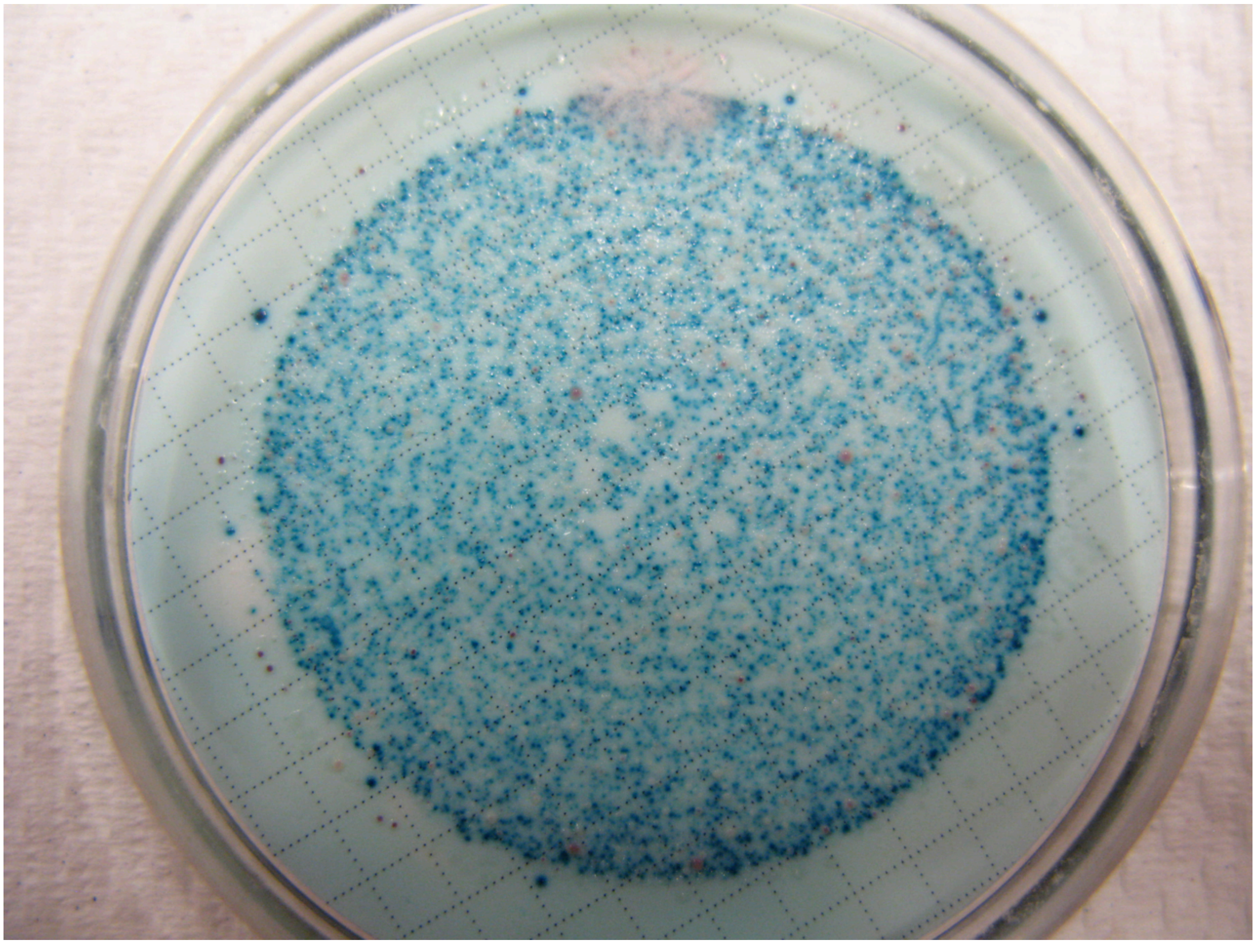

Figure 8. Water runoff sample from soil taken adjacent to depression in ‘crow area’ of wetlands

Summary of Results - ~~M~~ May 7, 2013 Samples

per 100mL

	E. coli	Total Coliforms
SITE 1		
Control	Ø	210
1A	1330	2950
1B	1110	3040
1C	880	3400
SITE 2		
Control	Ø	Ø
2A	100	5480
2B	Ø	3210
2C	Ø	1790
SITE 3		
Control	Ø	240
3A	520	2030
3B	530	2600
3C	580	2420
SITE 4		
Control	Ø	20
4A	420	2970
4B	420	3100
4C	530	3000
SITE 5		
Control	Ø	Ø
5A	280	2470
5B	310	2460
5C	370	2600

Wetlands; Samples - Surface H_2O

(@ 10mL diluted into 100mL
11mL sample H_2O + 99mL DI H_2O,
100mL filtered for a
"10 mL" sample / 100mL)

SITE 1 - 5/7/13

Control

count: E. Coli zero
Total Coliform 21

* Filter was dropped (after filtration) onto counter top briefly, likely skews control results

Sample 1A

count: E. coli 133 Total
Total Coliform 295 total (TNTC)

Sample 1B

count: E. coli 111 Total
Total Coliform 304 Total (TNTC)

Sample 1C

count: E. coli 88 Total
Total Coliform 340 Total (TNTC)

SITE 2 5/7/13

Control

count: E. coli zero
Total Coliform zero

Sample 2A

count: E. coli 10 total
Total Coliform 548 Total (TNTC)

Sample 2B

count: E. coli zero
Total Coliform 321 total (TNTC)

5/9/13

Sample 2C ~~8~~
Count: E. coli zero
Total Coliform 179 Total

SITE 3 5/7/13

Control
count: E. coli ~~Ø~~ zero
Total Coliform 24 total

Sample 3A
Counts: E. coli 52 Total
Total Coliform 203 Total (TNTC)

* Dirt in container when filtered. odd. ~~because~~ Possible not removed fully from container when washed 2° caked on side from soil samples. (?)

Sample 3B
count: E. coli 53 Total
Total Coliform 260 Total (TNTC)

Sample 3C
Count: E. coli 58 Total
Total Coliform 242 Total (TNTC)

SITE 4 5/7/13

Control
count: E. coli zero
Total Coliform 2 Total

Sample 4A
count: E. coli 42 Total
Total Coliform 297 Total (TNTC)

Sample 4B
count: E. coli 42 Total
Total Coliform 319 Total (TNTC)

Sample 4C E.coli 53 Total
Count: Total Coliform 300 Total (TNTC)

SITE 5 5/7/13

Control
count: E.coli zero
Total Coliform zero

Sample 5A
Count: E.Coli 28 total
Total Coliform 247 total (TNTC)

Sample 5B
Count: E.Coli 31 total
Total Coliform 246 Total (TNTC)

Sample 5C
Count: E.coli 37 Total
Total Coliform 260 Total (TNTC)

10mL /in 100mL

Wetland Soil Runoff Samples from Will 5/6/13

* Sample taken Adjacent to depression in "Craw Area"

See pg. 63 E.Coli TNTC Thousands of discrete tiny blue dots, like grains of sand.
Total Coliform difficult to see – tiny specs of red, mostly near perimeter of filtered area. Not nearly close to E.coli count
N47.76067
W122.18778

* Sample from Streambank, ± 2inches above H_2O flow (previously underwater)

E.coli TNTC
Total Coliform TNTC > lots of bleeding together "Confluent Growth"
N 47.76046
W 122.18764

Calculations

1 Control E. coli: ∅ — **Zero EC**
Total Coliform: (21 ÷ 10 mL) × 100 = **210 TC colonies / 100mL**

1A E.coli: (133 ÷ 10mL) × 100 = **1330 EC colonies / 100mL**
Total Coliforms: (295 ÷ 10mL) × 100 = **2950 TC colonies / 100 mL**

1B E.coli: (111 ÷ 10mL) × 100 = **1110 EC colonies / 100mL**
Total Coliform: (304 ÷ 10mL) × 100 = **3040 TC colonies / 100mL**

1C E.coli: (88 ÷ 10mL) × 100 = **880 EC colonies / 100mL**
[redacted] total Coliform: (340 ÷ 10mL) × 100 = **3400 TColiforms / 100mL**

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2 Control E.coli: ∅
Total Coliform: ∅

2A E.coli: (10 ÷ 10mL) × 100 = **100 EC colonies / 100mL**
Total Coliforms: (548 ÷ 10mL) × 100 = **5480 TC colonies / 100mL**

2B E.coli: Zero — **Zero EC**
Total Coliforms: (321 ÷ 10mL) × 100 = **3210 TC colonies / 100mL**

2C E.coli: — **Zero EC**
Total Coliforms: (179 ÷ 10mL) × 100 = **1790 TC colonies / 100mL**

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3 Control E.coli: — **Zero EC**
Total Coliform (24 ÷ 10mL) × 100 = **240 TC colonies / 100mL**

3A E.coli: (52 ÷ 10mL) × 100 = **520 EC colonies / 100mL**
Total Coliform: (203 ÷ 10) × 100 = **2030 TC / 100mL**

[3B] E.coli: (53 ÷ 10 mL)x100 = [530 EC colonies/100mL]

Total Coliform: (260 ÷ 10mL) x100 = [2600 TC colonies/100mL]

[3C] E.coli: (58 ÷ 10mL) x 100 = [580 EC colonies/100mL]

Total Coliform: (242 ÷ 10mL) x 100 = [2420 TC colonies/100mL]

[4 Control] E.coli [Zero EC]

Total Coliform: (2 ÷ 10mL) x100 = [20 TC colonies /100mL]

[4A] E.coli: (42 ÷ 10mL) x 100 = [420 EC colonies/100mL]

Total Coliform: (297 ÷ 10mL) x 100 = [2970 TC colonies/100mL]

[4B] E.coli: (42 ÷ 10mL) x 100 = [420 EC colonies /100mL]

Total Coliform: (319 ÷ 10mL) x 100 = [3100 TC colonies/100mL]

[4C] E.coli. (53 ÷ 10mL) x100 = [530 EC colonies/100mL]

Total Coliforms: (300 ÷ 10) x 100 = [3000 TC colonies /100mL]

[5 Control] E.coli Ø [Zero EC]

Total coliforms Ø [Zero TC]

[5A] E.coli: (28 ÷ 10mL) x100 = [280 EC colonies/100mL]

~~Tot~~ Total Coliforms: (247 ÷ 10mL) x100 = [2470 TC colonies /100mL]

[5B] E.coli: (31 ÷ 10mL) x100 = [310 EC colonies/100mL]

Total Coliforms: (246 ÷ 10mL) x100 = [2460 TC colonies/100mL]

5C

E. Coli: (37 ÷ 10mL) × 100 = 370 EC colonies / 100mL

Total Coliform: (260 ÷ 10mL) × 100 = 2600 TC colonies / 100mL