Report to City of Newark and the White Clay Wild and Scenic Steering Committee (Mar 2023)

"Fecal Indicator bacteria monitoring and continued molecular source tracking for Bogy Run, City of Newark, Delaware"

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PROJECT BACKGROUND

Existing data dating back to 2012 indicates high fecal indicator bacteria (FIB) (i.e., *E. coli* and *Enterococcus*) in base flow during the recreational season (June-August) throughout the headwaters at White Clay watershed in Pennsylvania and Delaware. The data also consists of multiple dates where bacterial counts exceeded safe standards by an order of magnitude or more. In order to reduce bacterial pollution entering the watershed, we need to determine the potential sources of bacteria, which is the first step towards the most cost-effective clean up strategies to target the sources. Human sewage (both sanitary sewer and privately owned small wastewater treatment plants or septic) can be one of the significant sources for these FIB.

Starting in 2016, Stroud Water Research Center set up molecular source tracking at White Clay Creek in Pennsylvania and Delaware. Taq-man qPCR was employed at several selected sites exhibiting some of the highest concentrations to look at relative abundances of contributing sources (e.g., human, cows etc.). Expected sources, such as human sources of FIB were found at multiple sites during summer time including WCD site at Bogy Run in Newark, Delaware (Fig. 1). The City of Newark is interested in conducting additional testing at the Bogy Run to help determine the best course of action to address the human cause which is likely coming from their sewer lines that run through and along this tributary to White Clay Creek.

In 2021, Stroud Center collected samples in October and November (when the students are in session) to monitor FIB counts. Biomass were also collected on 0.2 µm filters and subjected to molecular source tracking. Samples from previous years (2018, 2019, and 2020, collected by Shane Morgan, Kristen Travers, and Univ of Delaware) were also included in the analysis. The results indicated the presence of human fecal contamination at Bogy Run from multiple sites. In 2022, we refined our study design based off information we learned from the City of Newark about their sanitary sewer improvements and needed repair locations, as well as peak flow information along Bogy Run. While some sanitary sewer repairs were made in 2022, several remaining fixes are to be completed in 2023. In order to test the effectiveness of repaired sanitary sewer at improving water quality and reducing human fecal contamination, additional sampling was conducted in summer and fall 2022. In summer, we continued our routine bacterial monitoring and added sampling in October and November 2022, when University of Delaware students are in session. Based on flow information provided by City of Newark, two time points were included in our sampling regime: peak (8-9 AM) and non-peak (early PM). We also added one extra sampling site (MID), which is located at downstream of manhole 776.

In this report, we include the data from all 4 sites at Bogy Run, as shown in Figure 1: (1) GWC, a further upstream site that near George Wilson Center; (2) NLB, the site right below New London Bridge; (3) MID, downstream of manhole 776, the middle point between New London Bridge and White Clay Drive; and (4) WCD, White Clay Drive, downstream of manhole 817. WCD is the furthest downstream site before Bogy Run meets the White Clay Creek. The WCD is also our routine monitoring site from 2016.

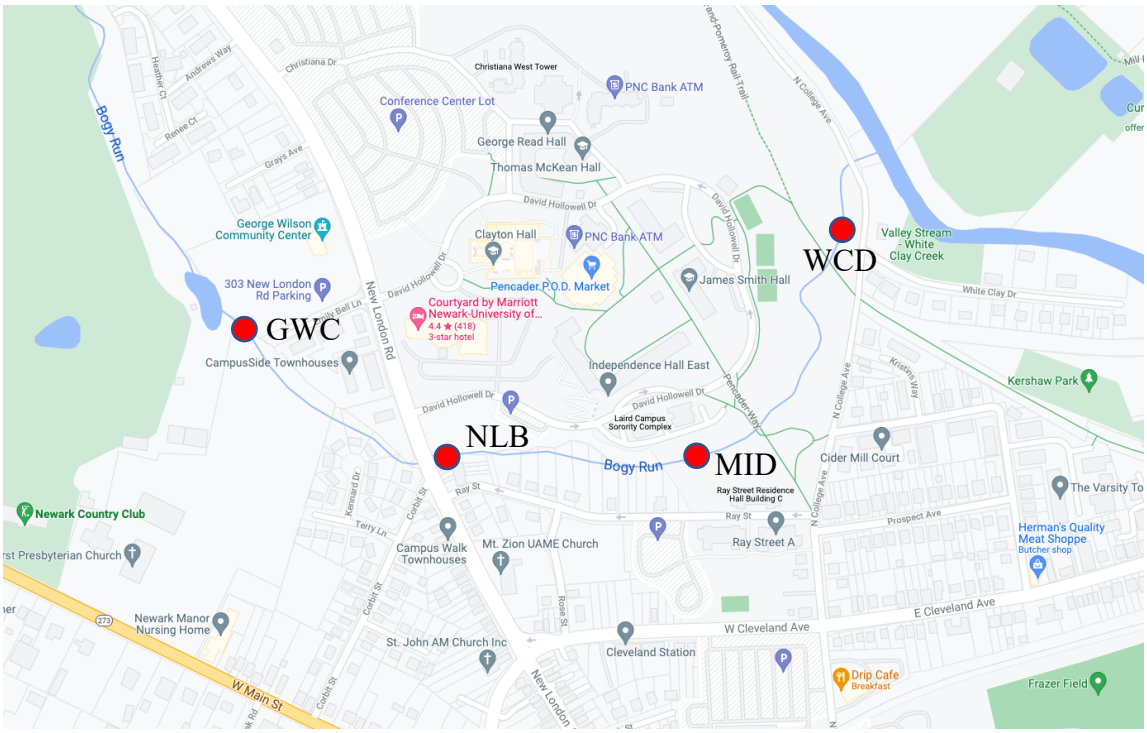

Fig. 1. Sampling sites at Bogy Run, Newark Delaware ( ). Four sites from upstream to downstream: GWC (George Wilson Center), NLB (New London Bridge), MID (mid point between NLB and WCD), and WCD (White Clay Drive).

SAMPLING AND ANALYSIS IN 2022

In addition to our routine summer sampling, we collected samples from 3 sites (NLB, MID, and WCD) in late October to November (Fig. 1). We followed the EPA sampling protocol for recreational seasons: five times within 30 days from each site, during baseflow conditions at peak flow (between 8-9:30am) and non-peak flow on the same day. Water temperature, dissolved Oxygen, and conductivity were recorded at each site using handheld meters. Counts of both *Enterococcus* and *E. coli* were analyzed using the IDEXX system at Microbiology Lab at Stroud Water Research Center. Molecular source tracking (MST) (Taq-man real time PCR) was used to test for human contamination on samples collected during the fall and summer of 2022 from all Bogy Run sites.

RESULTS AND OBSERVATIONS

FIB counts in Bogy Run

Fecal indicator bacteria (FIB) counts varied with season (Fig. 2 and Table 1). Total coliform (A), *E. coli* (B) and *Enterococcus* (C) all showed strong similar seasonality, in which recreational season (June-July) samples contained higher numbers of FIB than fall (October-November). This observation is consistent with our results on seasonal FIB patterns across big geographic span including White Clay Creek, Red Clay Creek and Delaware River Watersheds. For both total coliform and *E. coli*, the counts at upstream site (NLB) are generally higher than downstream site (WCD), except the samples from Oct-Nov 2022. Notably, the MPN counts for *E. coli* from Fall 2022 are below the EPA standard (126 counts/100ml), but *Enterococcus* counts are higher than the EPA standard (35 counts/100ml) (Fig. 2, A-C).

When we compared the FIB counts taken at peak flow time (8-9 AM) vs. non-peak time (early afternoon), mixed patterns were observed and t-test showed no significant differences were found between sampling times. At the furthest downstream site WCD, likely higher numbers of *E.coli* and *Enterococcus* counts were shown at peak time than non-peak time (excepted *Enterococcus* counts on 10/31/2022), but no such trend was visualized for total coliforms (Table 1). In contrast, no clear patterns were observed at upstream site (NLB) or mid-point (MID). Further, no significant differences were found between sampling sites or sampling times in environmental parameters including water temperature, pH, conductivity and dissolved oxygen (DO). Due to the limited sampling time and location, the samples from this study did not show any significant correlations between FIB counts with environmental parameters. However, a multi-year seasonal bacterial surveillance dataset across the White Clay, Red Clay, and Delaware River watersheds shows that FIB counts are responding to environmental factors such as water temperature and land use (Price et al., in preparation). All these results suggest that (1) FIB monitoring can be influenced by variety of environmental factors; (2) consistent and frequent sampling is needed to verify any patterns/trends that we observed. The detailed FIB counts, environmental condition measured on site, and paired t-tests were attached and shown in Table 1.

Molecular source tracking

In 2022, we collected more samples for molecular source tracking by including 4 sites from upstream to downstream Bogy Run, along the sanitary sewage pipeline (Fig. 1). Samples from peak and non-peak time at each sampling site were included based on the flow data that provided by City of Newark. We also included the samples that collected and saved during the summer 2022. Some of these samples are used as positive controls and rerun along with the samples collected in 2022. All results from molecular source tracking are listed in Table 2.

Among all the sampling sites at the Bogy Run, we have the most data from downstream site WCD. Encouragingly, none of the water samples collected in 2022 showed positive result for human contamination, even those collected in summer time with higher FIB counts (Fig. 2). Compared to previous years' results, possibility of human contamination has been greatly reduced. For instance, from 2018 to 2020, averagely about 50% samples collected in summer were tested positive for human contaminations but none of them was tested firmly positive for 2022. However, samples collected from NLB site may still contain human contamination in both 2021 and 2022 (Table 2), in which five samples

collected in summer 2022 were all tested positive. In contrast, none of the samples collected at GWC and MID was tested positive in 2022.

Suggestions and recommendations

The data showed an encouraging pattern of improvement in bacterial contamination at the Bogy Run. In 2022, no human FIB has been detected at the downstream site WCD, suggesting a significant reduction of pathogen contamination from 2018. This reduction is likely due to sewer repair and improvements made by the City of Newark. However, we also noticed that wide variations occurred in the positive samples, which represents our biggest challenge in bacterial monitoring: variation in sampling time, day, month and season. In fact, the bacterial monitoring has been facing the problem of data scarcity and sporadicity. Addressing this issue requires a more frequent and comprehensive bacterial monitoring program, which of course requires significant effort and cost. Based on our results, we recommend:

1. Continue monitoring the Bogy Run for 2023 to confirm the reduction of human contamination in both Summer and Fall. We understand City of Newark is completing several remaining fixes in the sanitary sewer lines along the Bogy Run in 2023.

2. Pay close attention to upstream site NLB to find out potential sources of human contamination. In addition to close and frequent monitoring, it is important to understand water intake and output, sewage and storm infrastructure, as well as non-point sources.

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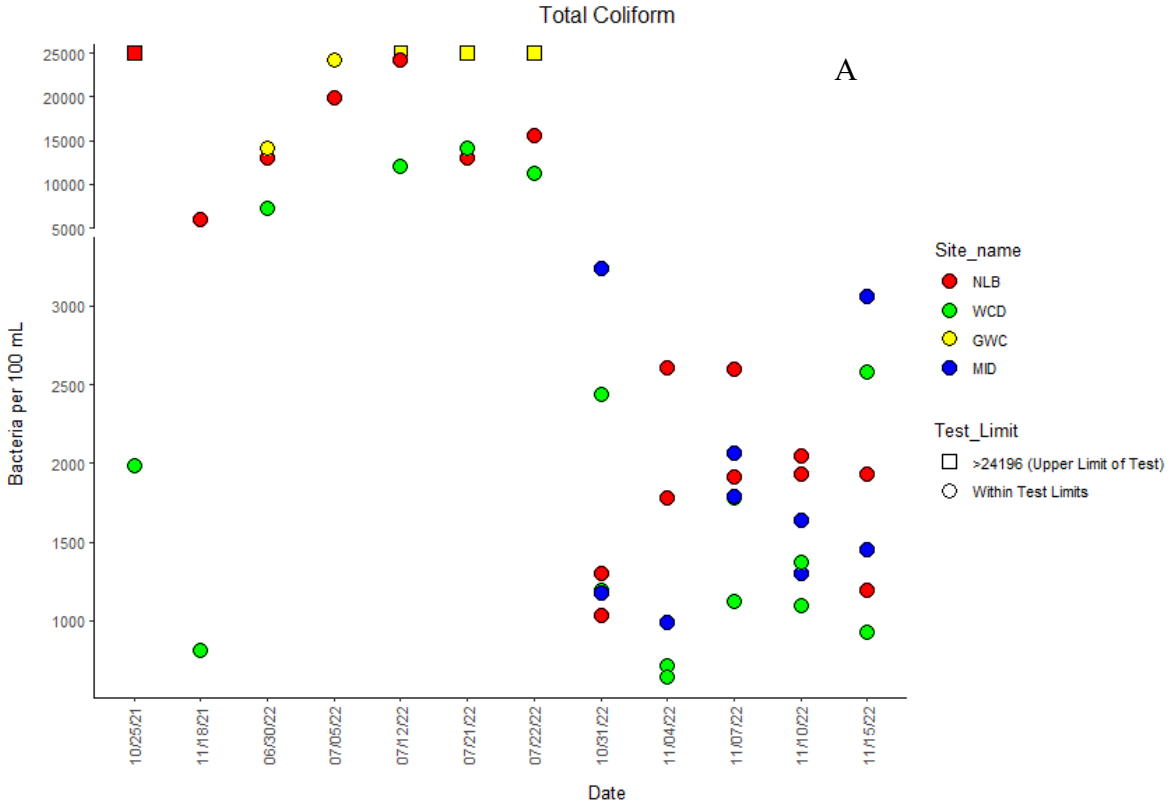
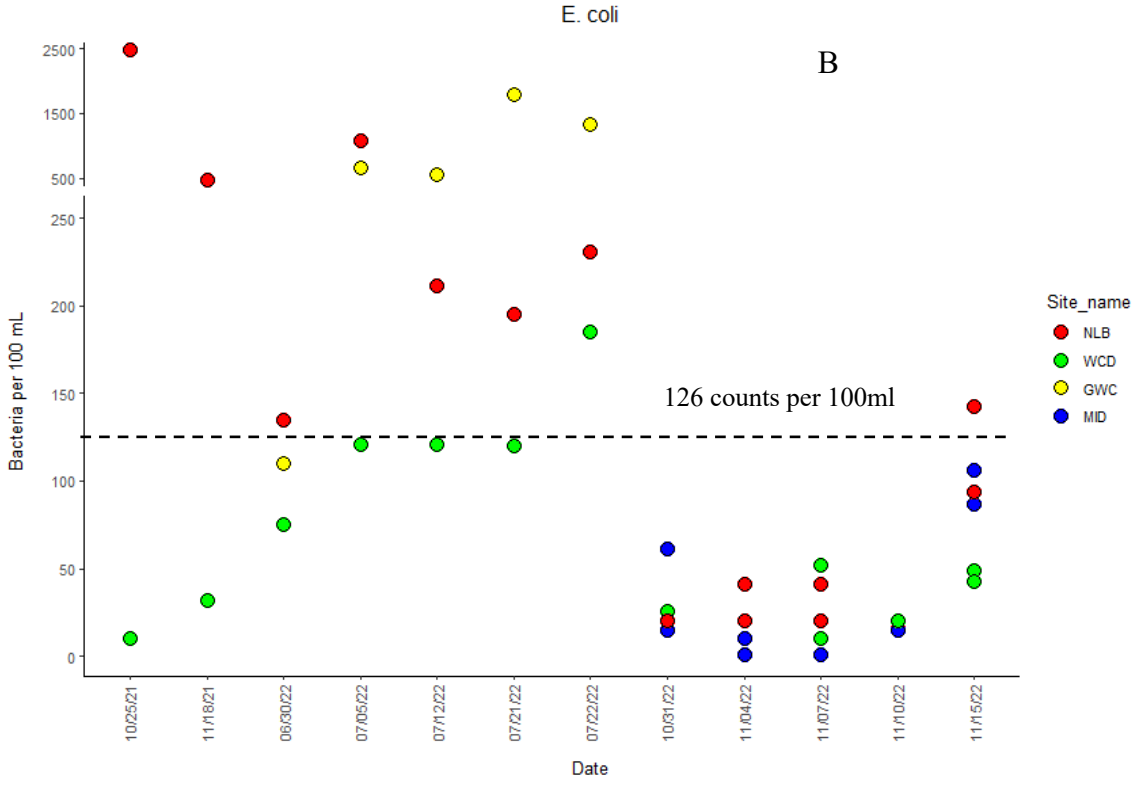

Total Coliform
A
Bacteria per 100 mL
25000
20000
15000
10000
5000
3000
2500
2000
1500
1000
10/25/21
11/18/21
06/30/22
07/05/22
07/12/22
07/21/22
07/22/22
10/31/22
11/04/22
11/07/22
11/10/22
11/15/22
Date
Site_name
NLB
WCD
GWC
MID
Test_Limit
>24196 (Upper Limit of Test)
Within Test Limits


E. coli
B
Bacteria per 100 mL
2500
1500
500
250
200
150
100
50
0
126 counts per 100ml
10/25/21
11/18/21
06/30/22
07/05/22
07/12/22
07/21/22
07/22/22
10/31/22
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11/15/22
Date
Site_name
NLB
WCD
GWC
MID

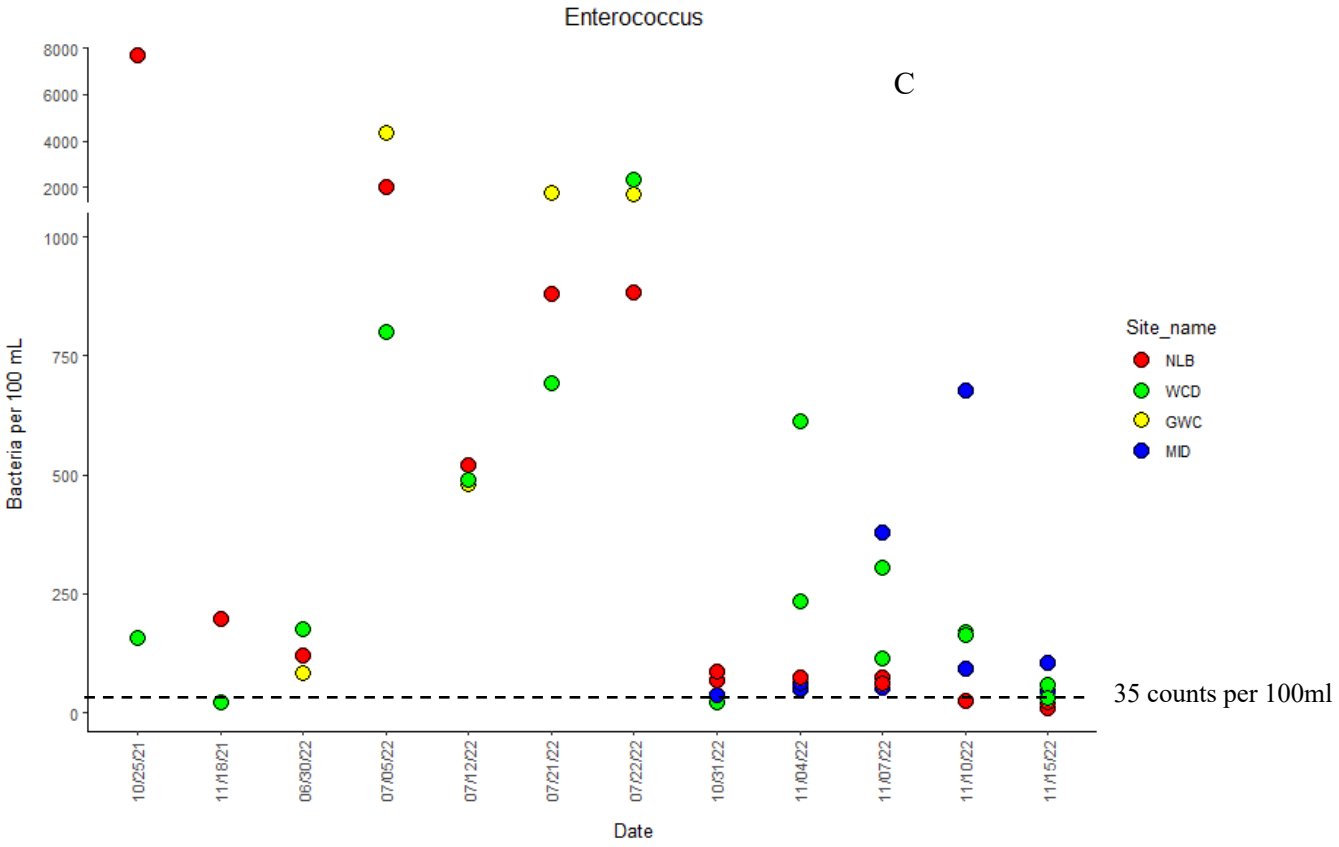

Fig. 2. FIB counts (per 100 ml) for Bogy Run. Samples were collected from Oct. 2021 to Nov. 2022. A, total coliform; B, *E. coli* and C, *Enterococcus*. Dash lines represent the EPA standards for E. coli (126 counts/100ml) and Enterococcus (35 counts/100ml). Note: samples with high total coliform counts exceeded the upper limit of detection were shown in squares (A).

Table 1. FIB counts for samples collected at Bogy Run. Data from 2021 were also included.

Site name	Site location	Site ID	Date Collected	Final Total Coliform	Final E. coli	Final Enterococcus	Time	Temp	pH	Cond	DO (mg/L)	T-test on sampling time
Bogy GW	George Wilson Center	GWC	6/30/22	14136	110	84	10:27	19.3	6.64	337	1.17	between peak vs. non-peak
Bogy GW	George Wilson Center	GWC	7/5/22	24196	663	4366	10:53	23.1	6.99	167.6	4.94	
Bogy GW	George Wilson Center	GWC	7/12/22	>24196	565	480	9:31	22.5	6.88	142.7	6.46	
Bogy GW	George Wilson Center	GWC	7/21/22	>24196	1785	1782	9:07	23.4	5.3	308.3	1.15	
Bogy GW	George Wilson Center	GWC	7/22/22	>24196	1334	1722	9:06	23.1	6.77	337.2	0.83	
Bogy Mid	Mid point between NLB and WCD	MID	10/31/22	3244	61	37.5	8:39	10.4	7.08	387.7	6.54	MID Ecoli: NS, p=0.6585
Bogy Mid	Mid point between NLB and WCD	MID	10/31/22	1179.5	15	36	14:33	12.9	7.04	366.4	9.1	
Bogy Mid	Mid point between NLB and WCD	MID	11/4/22	988	10	50	8:44	10.4	6.73	330.2	9.24	MID Entero:NS, p=0.2462
Bogy Mid	Mid point between NLB and WCD	MID	11/4/22	987	<10	61	13:10	12.5	7.2	349.6	9.13	
Bogy Mid	Mid point between NLB and WCD	MID	11/7/22	2064	<10	380	8:46	15.6	6.71	400.3	6.68	
Bogy Mid	Mid point between NLB and WCD	MID	11/7/22	1789	<10	52	13:55	16.6	6.51	407	7.7	
Bogy Mid	Mid point between NLB and WCD	MID	11/10/22	1641	15	677	8:48	8.2	6.83	340.6	8.64	
Bogy Mid	Mid point between NLB and WCD	MID	11/10/22	1301.5	15	91.5	13:49	11.2	7.66	364.6	8.7	
Bogy Mid	Mid point between NLB and WCD	MID	11/15/22	1454.5	86.5	47.5	9:03	5.5	7.77	271.6	11.4	
Bogy Mid	Mid point between NLB and WCD	MID	11/15/22	3065.5	106.5	105.5	13:27	7.3	6.69	289.9	11.1	
Bogy NL	New London bridge	NLB	6/30/22	12997	135	121	10:16	19.6	7.58	490	8.58	
Bogy NL	New London bridge	NLB	7/5/22	19863	1076	1989	10:41	20.9	7.67	353.1	8.63	
Bogy NL	New London bridge	NLB	7/12/22	24196	211	520	9:19	20.1	7.43	299.9	8.53	
Bogy NL	New London bridge	NLB	7/21/22	12997	195	880	8:58	23	6.74	524.5	7.97	
Bogy NL	New London bridge	NLB	7/22/22	15531	231	882	8:58	22.7	7.87	507.9	8.14	
Bogy NL	New London bridge	NLB	10/31/22	1037.5	20.5	67.5	8:49	10.2	7.19	242.1	6.26	NLB Ecoli: NS, p=0.4543
Bogy NL	New London bridge	NLB	10/31/22	1301.5	20.5	85.5	14:43	12.8	6.58	260.4	6.06	
Bogy NL	New London bridge	NLB	11/4/22	1785	41	63	8:54	10.6	6.25	240.4	4.88	NLB Entero:NS, p=0.3419
Bogy NL	New London bridge	NLB	11/4/22	2613	20	75	13:20	13.3	6.67	255.7	5.43	
Bogy NL	New London bridge	NLB	11/7/22	1918	20	75	8:57	16	7.62	284.9	3.04	
Bogy NL	New London bridge	NLB	11/7/22	2603	41	63	14:05	16.8	6.56	285.2	3.87	
Bogy NL	New London bridge	NLB	11/10/22	1936.5	15.5	26	8:58	8.6	6.36	237.9	5.25	
Bogy NL	New London bridge	NLB	11/10/22	2053	15.5	26	14:00	11.7	7.19	257.2	5.73	
Bogy NL	New London bridge	NLB	11/15/22	1936.5	142.5	10	9:12	5.4	7.4	202.8	9.42	
Bogy NL	New London bridge	NLB	11/15/22	1191	93.5	20.5	13:35	7.3	6.22	213.9	9.03	
Bogy upstream	New London bridge	NLB	10/25/21	>24196	2481	7701						
Bogy upstream	New London bridge	NLB	11/18/21	6016.5	466.5	196.5	12:43pm					
Bogy downstream	White Clay Drive	WCD	10/25/21	1989	10	156						
Bogy downstream	White Clay Drive	WCD	11/18/21	812	31.5	20.5	12:38pm					
Bogy WCD	White Clay Drive	WCD	6/30/22	7270	75	175	9:50	18.4	7.43	584	8.15	
Bogy WCD	White Clay Drive	WCD	7/5/22	19863	121	801	10:16	20	7.39	498.1	8.49	
Bogy WCD	White Clay Drive	WCD	7/12/22	12033	121	491	8:55	18.9	7.44	495.5	8.72	
Bogy WCD	White Clay Drive	WCD	7/21/22	14136	120	693	8:38	21.9	7.41	566.1	7.54	
Bogy WCD	White Clay Drive	WCD	7/22/22	11199	185	2333	8:37	22.1	7.48	583.1	7.45	
Bogy WCD	White Clay Drive	WCD	10/31/22	1191	26	20.5	8:27	11	7.66	448.2	8.73	WCD Ecoli: NS, p=0.1251
Bogy WCD	White Clay Drive	WCD	10/31/22	2442	20.5	37	14:18	12.7	7.08	472.4	8.82	
Bogy WCD	White Clay Drive	WCD	11/4/22	717	41	613	8:35	11.5	6.74	441.7	8.38	WCD Entero: NS, p=0.1918
Bogy WCD	White Clay Drive	WCD	11/4/22	644	20	233	13:00	13.2	7.68	460.6	8.72	
Bogy WCD	White Clay Drive	WCD	11/7/22	1126	52	305	8:35	15.7	7.43	513.8	6.81	
Bogy WCD	White Clay Drive	WCD	11/7/22	1785	10	115	13:45	17.2	6.46	529.5	6.93	
Bogy WCD	White Clay Drive	WCD	11/10/22	1093.5	20.5	171	8:38	9.4	6.51	463	8.77	
Bogy WCD	White Clay Drive	WCD	11/10/22	1377.5	20.5	163.5	13:37	12.6	7.5	491.1	15.38	
Bogy WCD	White Clay Drive	WCD	11/15/22	2586	48.5	57.5	8:54	6.5	7.66	367	12.16	
Bogy WCD	White Clay Drive	WCD	11/15/22	930	43	30.5	13:16	7.9	6.71	382	11.4	

Table 2. Taq-man qPCR results for molecular source tracking of human contaminations at Bogy Run. For easy comparison, results from previous years were included.

	2018			2019			2020			2021			2022		
Site ID	Sample Date	Gene Copy Number	Positive for Human Signal	Sample Date	Gene Copy Number	Positive for Human Signal	Sample Date	Gene Copy Number	Positive for Human Signal	Sample Date	Gene Copy Number	Positive for Human Signal	Sample Date	Gene Copy Number	Positive for Human Signal
WCD	6/14/18	58.51	Positive?	7/5/19	0.00	Negative	6/30/20	975.56	Positive				6/30/22	0.00	Negative
	6/22/18	30121.93	Positive	7/9/19	425.51	Positive	7/6/20	0.00	Negative				7/5/22	0.00	Negative
	6/27/18	3301.93	Positive	7/15/19	0.00	Negative	7/15/20	66.17	Positive?				7/12/22	1.14	Positive?
	7/2/18	79.69	Positive?	7/22/19	167.72	Positive	7/22/20	2744.42	Positive				7/21/22	0.00	Negative
	7/12/18	0.00	Negative	7/29/19	0.00	Negative	7/27/20	180.94	Positive				7/22/22	0.00	Negative
	7/17/18	23170.54	Positive							10/25/21	0.00	Negative	10/31/2022 am	0.00	Negative
										11/18/21	0.00	Negative	10/31/2022 pm	0.00	Negative
													11/4/2022 am	0.00	Negative
													11/4/2022 pm	0.00	Negative
													11/7/2022 am	0.00	Negative
													11/7/2022 pm	0.00	Negative
													11/10/2022 am	0.00	Negative
													11/10/2022 pm	0.00	Negative
													11/15/2022 am	0.00	Negative
													11/15/2022 pm	0.00	Negative
NLB													6/30/22	108.85	Positive
													7/5/22	348605.03	Positive
													7/12/22	195.38	Positive
													7/21/22	269.17	Positive
													7/22/22	59.32	Positive
										10/25/21	27120.06	Positive	10/31/2022 am	0.00	Negative
										11/18/21	3699.31	Positive	10/31/2022 pm	0.00	Negative
													11/4/2022 am	132.55	Positive
													11/4/2022 pm	0.00	Negative
													11/7/2022 am	0.00	Negative
													11/7/2022 pm	1.98	Positive?
													11/10/2022 am	0.00	Negative
													11/10/2022 pm	0.00	Negative
													11/15/2022 am	53.56	Positive
													11/15/2022 pm	0.00	Negative
GWC													6/30/22	0.00	Negative
													7/5/22	0.00	Negative
													7/12/22	0.00	Negative
													7/12/22	0.00	Negative
													7/21/22	0.00	Negative
													7/22/22	0.00	Negative
MID													10/31/2022 am	0.00	Negative
													10/31/2022 pm	0.00	Negative
													11/4/2022 am	0.00	Negative
													11/4/2022 pm	0.00	Negative
													11/7/2022 am	0.00	Negative
													11/7/2022 pm	0.00	Negative
													11/10/2022 am	0.00	Negative
													11/10/2022 am	0.00	Negative
													11/10/2022 pm	0.00	Negative
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													11/15/2022 pm	0.00	Negative
													11/15/2022 pm	0.00	Negative