

Report to City of Newark and the White Clay Wild and Scenic
Steering Committee (Mar 2024)

“Continued fecal indicator bacteria monitoring and molecular source
tracking for Bogey 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 baseflow conditions 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 EPA 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 and 2023. In summer, we continued our routine bacterial monitoring including Bogy Run and added extra sampling in October and November 2022 and 2023, when University of Delaware students are in session. Same as year 2022, two time points were included in our 2023 sampling regime: peak (8-9 AM) and non-peak (early PM).

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 (only summer samples were collected for this site); (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 this site was added in 2022; 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 in summer from 2016. During our sampling in 2023, continuous water flow was observed from a pipe above NLB (Figure 1) as well as the noticeable smell and warm water temperature. We added this site (Pipe) to our sampling (supported by the Stroud endowment fund) and the data showed pipe water contained high levels of FIB counts with potential human contamination.

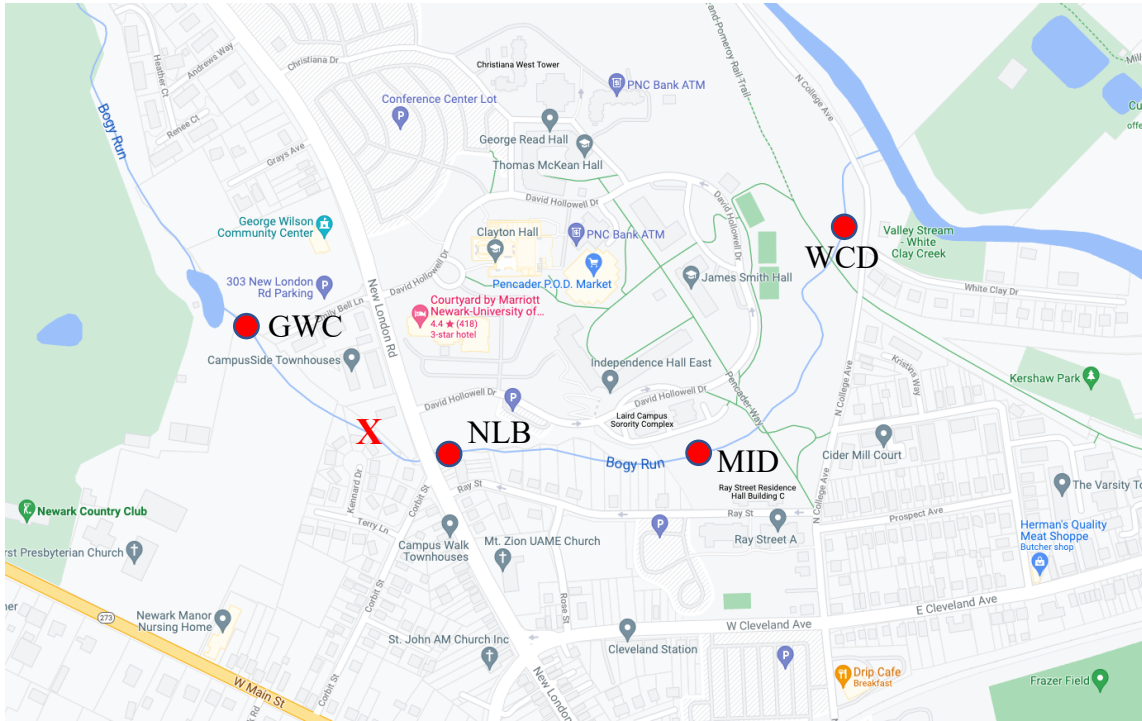


Figure 1. Top: Sampling sites at Boggy Run, Newark Delaware (●). Four sites from upstream to downstream: GWC (George Wilson Center), NLB (New London Bridge), MID (mid point between NLB and WCD), and WCD (White Clay Drive). Bottom: An extra sampling site (pipe) above NLB was added to our sampling in fall 2023 (X).

SAMPLING AND ANALYSIS IN 2023

In addition to summer routine bacteria monitoring, extra samples in 2023 were collected in November at WCD, MID, NLB and the unknown pipe (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 (PM) on the same day. Water temperature, pH, dissolved Oxygen, and conductivity were recorded at each site using handheld meters. Counts of total coliform, *E. coli* and *Enterococcus* were obtained 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

Similar to the results from 2022, 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 seasonality: recreational season (June-August) samples contained significantly 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. In general, FIB counts at upstream sites (NLB or MID) are higher than downstream site (WCD). Notably, the MPN counts for total coliform and *E. coli* from Fall 2023 are higher than those in 2022, but *Enterococcus* counts showed an opposite trend. However, these differences are not statistically significant ($P>0.05$) (Figure. 2, A-C). The pipe water we collected contained significantly higher numbers of FIB and *E. coli* and *Enterococcus*, suggesting potential contamination input to the Bogy Run. Molecular source tracking data confirmed the presence and super high human signals in the water samples that we collected (see below Table 2). Further information and investigation are needed and recommended for this site and any other flowing pipes along the Bogy Run.

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 between sampling times across all the sampling sites. Similar pattern was observed in 2022. Further, no significant differences were found between sampling sites or sampling times in environmental parameters including water temperature, pH, conductivity and dissolved oxygen (DO). 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 (in preparation). All these results suggest that (1) FIB counts can be influenced by variety of environmental factors; (2) certain sources (e.g., pipes) may increase bacteria counts in the streams especially the headwaters; (3) consistent and frequent survey and sampling is needed to verify any patterns/trends that we observed. The detailed FIB counts and environmental condition measured on site were attached and shown in Table 1 (only data from 2023 are included in this report).

Molecular source tracking

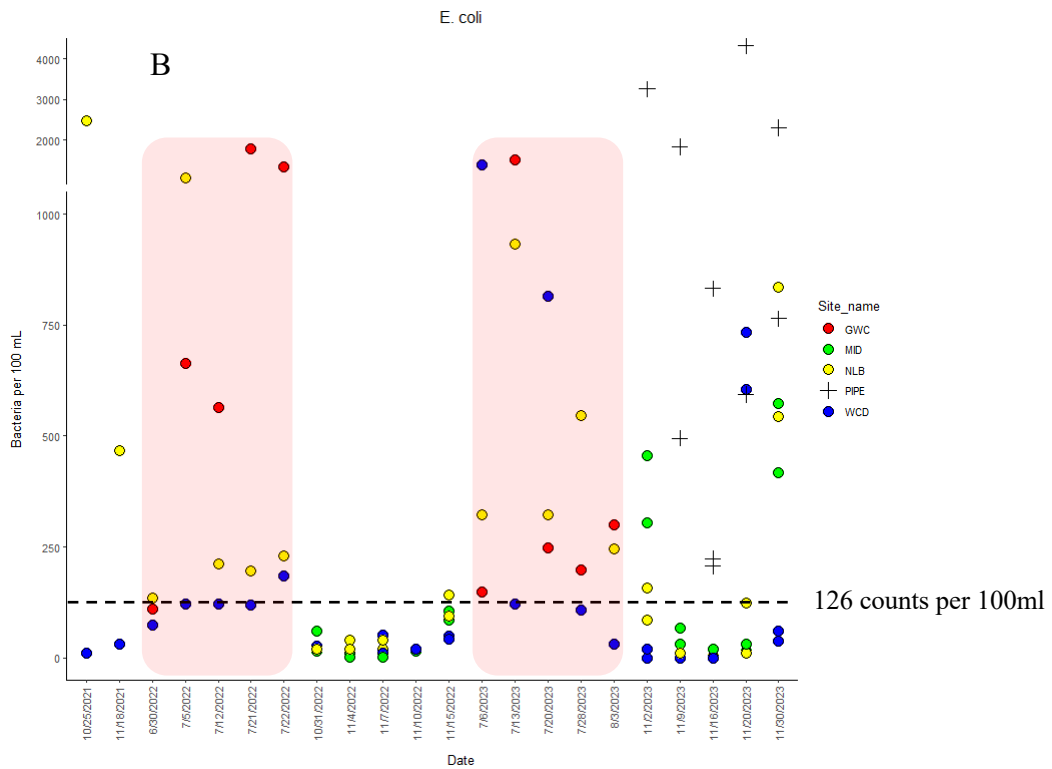
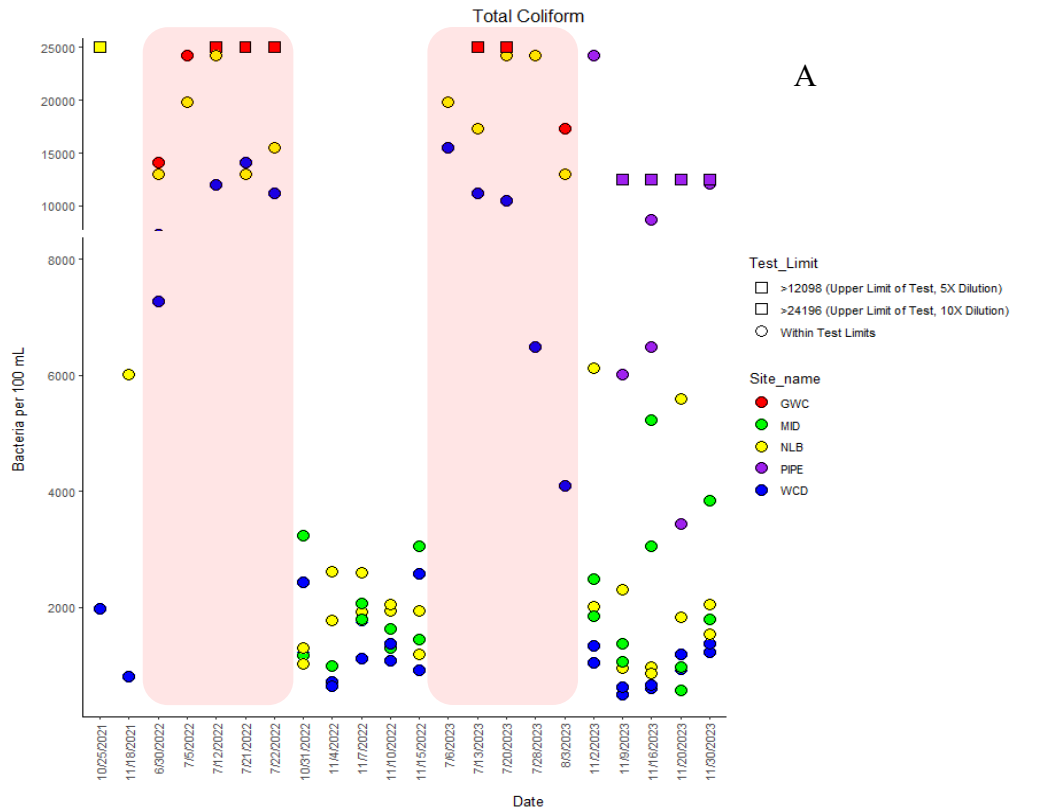
In 2023, we repeated the 2022 sampling protocol, and collected samples for molecular source tracking by including 4 sites from upstream to downstream Bogy Run, as well as the water samples from a flowing pipe (Fig. 1). Samples from peak and non-peak time at each sampling site were included based on the flow data that provided by City of Newark. The samples collected and saved during the summer 2023 were also included in the analysis. All results from molecular source tracking were listed and reported here, and data from previous years were also included (Table 2).

Source tracking data from 2023 were significantly different from 2022. More positive human signals were found in samples collected in 2023. In 2022, only water samples collected from NLB site showed positive human signals but in 2023, water samples from WCD, NLB and MID were all tested positive (Table 2). Among them, the majority of summer samples from WCD and NLB (9 out of 10) were tested positive for human contamination, but none for GWC, the furthest upstream site. In fall 2023, samples collected in late November (Nov. 30th) were all test positive from WCD, NLB and MID. Water from the pipe were also tested positive with very high human signals (range from 1,728 to 236,215 gene copies with an average at 50,422 copies per 100 ml). Based on high FIB numbers and human signals in the pipe water, we suspect this might be, at least partially, the cause of human contamination in Bogy Run. Compared to 2022 results, possibility of human contamination has been greatly increased, and the data for site WCD in 2023 are more comparable to the results that we obtained from 2018-2022 (Table 2).

Suggestions and recommendations

The data showed a different pattern from 2022 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, in 2023 more water samples were tested positive human signals across three sites along the main stem of Bogy Run. The mixed patterns from the last two years deserve further investigations and explanation. Based on our results, we recommend:

1. Continue future monitoring of the Bogy Run to verify the observation of potential human contamination in both Summer and Fall. We understand the City of Newark is repairing the sanitary sewer lines along the Bogy Run, but our bacteria data suggest a thorough inspection of the sewer lines and detection of potential leaks may be necessary.
2. Pay close attention to the pipes or other potential point sources along Bogy Run and investigate the infrastructure, age, and status for these pipes. Information on these pipes are critical to future study design due to the spatial and temporal variations of bacteria data.
3. If we are able to identify potential sources (septic outlets, storm runoff or other point sources) along Bogy Run, frequent monitoring of these specific site and molecular source tracking is highly recommended.



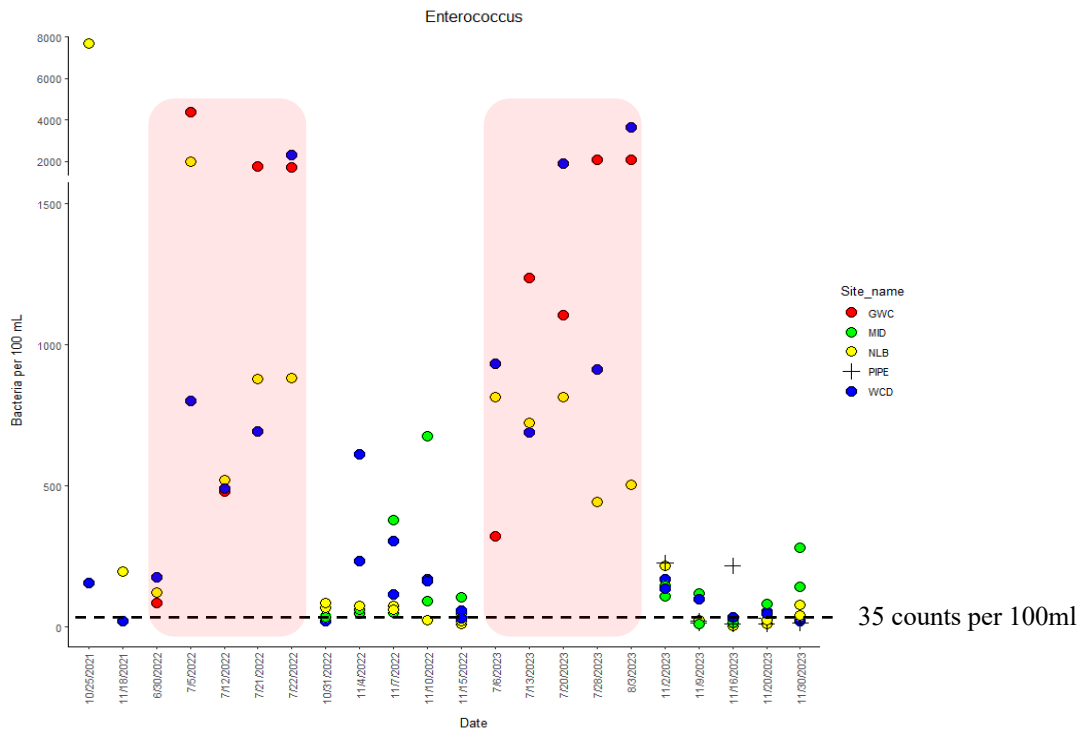


Fig. 2. FIB counts (per 100 ml) for Bogy Run. Samples from 2021, 2022 and 2023 are included. A, total coliform; B, *E. coli* and C, *Enterococcus*. Dash lines represent the EPA standards for *E. coli* (126 counts/100ml) and *Enterococcus* (35 counts/100ml). Strong seasonality of FIB counts is observed and the data from summer months are highlighted. Note: samples with high total coliform counts exceeded the upper limit of detection are shown in squares (A).

Table 1. FIB counts for samples collected at Bogy Run in 2023.

Site name	Site ID	Date	Time	Total Coliform	E. coli	Enterococcus	Temp	pH	Cond	DO (mg/L)
Bogy GW	GWC	7/6/23	9:30 AM	15531	148	323	23.4	7.44	NA	4.98
Bogy GW	GWC	7/14/23	11:08 AM	>24196	1515	1236	21	7.1	NA	2.8
Bogy GW	GWC	7/20/23	10:25 AM	>24196	249	1106	22.2	7.44	NA	4.8
Bogy GW	GWC	7/28/23	10:25 AM	24196	199	2098	22.7	6.8	228.3	3.04
Bogy GW	GWC	8/3/23	11:05 AM	17329	301	2098	21.2	6.87	194.5	3.11
Bogy Mid	MID	11/2/23	8:53 AM	1860	455	108	6.2	7.38	297.9	10.08
Bogy Mid	MID	11/2/23	1:50 PM	2489	305	145	7.9	7.29	310.5	8.52
Bogy Mid	MID	11/9/23	9:00 AM	1377.5	67.5	10	9.2	7.22	363.9	7.88
Bogy Mid	MID	11/9/23	2:55 PM	1071	31.5	120.5	13.1	7.13	393.2	7.82
Bogy Mid	MID	11/16/23	8:53 AM	5231	20.5	15.5	5.2	7.53	319.9	8.58
Bogy Mid	MID	11/16/23	3:00 PM	3065.5	<1	20.5	8.3	7.39	346.3	8.51
Bogy Mid	MID	11/20/23	9:25 AM	572.5	15.5	10	4.6	7.36	318.2	8.46
Bogy Mid	MID	11/20/23	1:58 PM	967.5	31.5	80.5	5.7	7.38	329.8	9.81
Bogy Mid	MID	11/30/23	9:34 AM	1804.5	418	281.5	1.7	7.57	217.5	13.22
Bogy Mid	MID	11/30/23	1:57 PM	3850.5	572.5	142.5	4	6.66	234.7	12.33
Bogy NL	NLB	7/6/23	9:10 AM	19863	323	816	21.5	7.5	NA	8.43
Bogy NL	NLB	7/14/23	11:00 AM	17329	932	723	21.9	7.67	NA	8.74
Bogy NL	NLB	7/20/23	10:15 AM	24196	323	816	21.7	7.53	NA	8.4
Bogy NL	NLB	7/28/23	10:15 AM	24196	546	443	23.1	7.54	391.6	8.46
Bogy NL	NLB	8/3/23	10:56 AM	12997	246	504	20.4	7.77	369.6	9.43
Bogy NL	NLB	11/2/23	9:06 AM	2014	158	216	6.8	7.17	217.9	6.64
Bogy NL	NLB	11/2/23	2:01 PM	6131	85	166	8.8	7.11	233.1	6.66
Bogy NL	NLB	11/9/23	9:12 AM	2305.5	10	26	9.7	6.77	268.5	4.68
Bogy NL	NLB	11/9/23	3:04 PM	959	<1	10	12.9	6.94	290.6	3.81
Bogy NL	NLB	11/16/23	9:01 AM	861.5	5	5	7.5	6.8	257.4	5.9
Bogy NL	NLB	11/16/23	3:12 PM	984	5	10	9.6	6.85	263.8	5.24
Bogy NL	NLB	11/20/23	9:35 AM	1827	10	10	5.5	7.02	236.9	6.25
Bogy NL	NLB	11/20/23	2:09 PM	5599.5	123	25.5	7.4	7.68	354.5	7.49
Bogy NL	NLB	11/30/23	9:42 AM	2053	835	79	2.7	6.76	265.4	13.54
Bogy NL	NLB	11/30/23	2:07 PM	1538	543	43	7.1	7.18	195.7	10.84
Bogy Pipe	Pipe	11/2/23	9:09 AM	24196	3255	228				
Bogy Pipe	Pipe	11/9/23	9:15 AM	>12098	1827	15.5				
Bogy Pipe	Pipe	11/9/23	3:10 PM	6016.5	495	20.5				
Bogy Pipe	Pipe	11/16/23	9:01 AM	>12098	832	217.5				
Bogy Pipe	Pipe	11/16/23	3:12 PM	6498.5	207	10				
Bogy Pipe	Pipe	11/20/23	9:35 AM	>12098	4332	31				
Bogy Pipe	Pipe	11/20/23	2:09 PM	3433.5	593.5	10				
Bogy Pipe	Pipe	11/30/23	9:42 AM	12098	764.5	15				
Bogy Pipe	Pipe	11/30/23	2:09 PM	>12098	2305.5	15.5				
Bogy WCD	WCD	7/6/23	8:50 AM	15531	1376	933	21.2	7.27	NA	7.48
Bogy WCD	WCD	7/14/23	10:40 AM	11199	121	689	21	7.39	NA	7.56
Bogy WCD	WCD	7/20/23	10:00 AM	10462	816	1892	21.1	7.34	NA	7.66
Bogy WCD	WCD	7/28/23	9:55 AM	6488	109	914	22.4	7.42	458.4	7.56
Bogy WCD	WCD	8/3/23	10:40 AM	4106	31	3654	19.2	7.44	510.6	8.81
Bogy WCD	WCD	11/2/23	8:41 AM	1046	20	135	7.9	7.31	425.3	8.56
Bogy WCD	WCD	11/2/23	1:38 PM	1334	<1	169	9.9	7.34	441.9	8.44
Bogy WCD	WCD	11/9/23	8:50 AM	629.5	10	98.5	10.1	7.17	481.7	7.71
Bogy WCD	WCD	11/9/23	2:43 PM	493.5	<1	25	13.7	7.05	518.8	7.23
Bogy WCD	WCD	11/16/23	8:43 AM	666.5	<1	36	7	7.25	435.7	9.45
Bogy WCD	WCD	11/16/23	2:47 PM	618	5	15	10	7.5	466.6	9.1
Bogy WCD	WCD	11/20/23	9:15 AM	936	605	54.5	6.4	6.42	436.4	9.37
Bogy WCD	WCD	11/20/23	1:44 PM	1191	733.5	48	7.8	8	449.6	9.16
Bogy WCD	WCD	11/30/23	9:24 AM	1240.5	60.5	20.5	2.9	7.46	293.1	13.25
Bogy WCD	WCD	11/30/23	1:45 PM	1377.5	37	42.5	5.8	8.27	318.9	11.97

Collected flowing pipe water, can't use meter

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.

SiteID	2023			2022			2021			2020			2019			2018			
	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	Sample Date	Gene Copy Number	Positive for Human Signal	
WCD	7/6/2023	15031.48	Positive	6/30/22	0.00	Negative	10/25/21	0.00	Negative	6/30/20	975.56	Positive	7/5/19	0.00	Negative	6/14/18	58.51	Positive?	
	7/14/2023	33.92	Positive	7/5/22	0.00	Negative	11/18/21	0.00	Negative	7/6/20	0.00	Negative	7/9/19	425.51	Positive	6/22/18	30121.93	Positive	
	7/20/2023	185.23	Positive	7/12/22	1.14	Positive?				7/15/20	66.17	Positive?	7/15/19	0.00	Negative	6/27/18	3301.93	Positive	
	7/28/2023	19.32	Positive	7/21/22	0.00	Negative				7/22/20	2744.42	Positive	7/22/19	167.72	Positive	7/2/18	79.69	Positive?	
	8/3/2023	7.09	Positive?	7/22/22	0.00	Negative				7/27/20	180.94	Positive	7/29/19	0.00	Negative	7/12/18	0.00	Negative	
	11/2/2023 am	0.00	Negative	10/31/2022 am	0.00	Negative										7/17/18	23170.54	Positive	
	11/2/2023 pm	5.84	Positive?	10/31/2022 pm	0.00	Negative													
	11/9/2023 am	0.00	Negative	11/4/2022 am	0.00	Negative													
	11/9/2023 pm	0.00	Negative	11/4/2022 pm	0.00	Negative													
	11/16/2023 am	0.00	Negative	11/7/2022 am	0.00	Negative													
	11/16/2023 pm	0.00	Negative	11/7/2022 pm	0.00	Negative													
	11/20/2023 am	0.00	Negative	11/10/2022 am	0.00	Negative													
	11/20/2023 pm	0.00	Negative	11/10/2022 pm	0.00	Negative													
	11/30/2023 am	133.00	Positive	11/15/2022 am	0.00	Negative													
	11/30/2023 pm	261.86	Positive	11/15/2022 pm	0.00	Negative													
	NLB	7/6/2023	18102.26	Positive	6/30/22	108.85	Positive	10/25/21	27120.06	Positive									
		7/14/2023	30308.35	Positive	7/5/22	348605.03	Positive	11/18/21	3699.31	Positive									
7/20/2023		2094.87	Positive	7/12/22	195.38	Positive													
7/28/2023		4817.13	Positive	7/21/22	269.17	Positive													
8/3/2023		6631.87	Positive	7/22/22	59.32	Positive													
11/2/2023 am		15.62	Positive?	10/31/2022 am	0.00	Negative													
11/2/2023 pm		16.96	Positive?	10/31/2022 pm	0.00	Negative													
11/9/2023 am		30.19	Positive	11/4/2022 am	132.55	Positive													
11/9/2023 pm		2.71	Positive	11/4/2022 pm	0.00	Negative													
11/16/2023 am		0.00	Negative	11/7/2022 am	0.00	Negative													
11/16/2023 pm		0.00	Negative	11/7/2022 pm	1.98	Positive?													
11/20/2023 am		0.00	Negative	11/10/2022 am	0.00	Negative													
11/20/2023 pm		256.18	Positive	11/10/2022 pm	0.00	Negative													
11/30/2023 am	60.40	Positive	11/15/2022 am	53.56	Positive														
11/30/2023 pm	26.41	Positive	11/15/2022 pm	0.00	Negative														
GWC	7/6/2023	0.00	Negative	6/30/22	0.00	Negative													
	7/14/2023	0.00	Negative	7/5/22	0.00	Negative													
	7/20/2023	0.00	Negative	7/12/22	0.00	Negative													
	7/28/2023	0.00	Negative	7/12/22	0.00	Negative													
	8/3/2023	1.48	Positive?	7/21/22	0.00	Negative													
				7/22/22	0.00	Negative													
MID	11/2/2023 am	71.92	Positive	10/31/2022 am	0.00	Negative													
	11/2/2023 pm	29.63	Positive	10/31/2022 pm	0.00	Negative													
	11/9/2023 am	238.19	Positive	11/4/2022 am	0.00	Negative													
	11/9/2023 pm	38.49	Positive	11/4/2022 pm	0.00	Negative													
	11/16/2023 am	8.72	Positive?	11/7/2022 am	0.00	Negative													
	11/16/2023 pm	0.00	Negative	11/7/2022 pm	0.00	Negative													
	11/20/2023 am	0.00	Negative	11/10/2022 am	0.00	Negative													
	11/20/2023 pm	7.93	Positive?	11/10/2022 pm	0.00	Negative													
	11/30/2023 am	227.97	Positive	11/10/2022 pm	0.00	Negative													
	11/30/2023 pm	322.74	Positive	11/10/2022 pm	0.00	Negative													
				11/15/2022 am	0.00	Negative													
			11/15/2022 am	0.00	Negative														
			11/15/2022 pm	0.00	Negative														
			11/15/2022 pm	0.00	Negative														
Pipe	11/2/2023 am	236215.50	Positive																
	11/9/2023 am	65887.10	Positive																
	11/9/2023 pm	4103.96	Positive																
	11/16/2023 am	69512.62	Positive																
	11/16/2023 pm	4489.13	Positive																
	11/20/2023 am	6068.45	Positive																
	11/20/2023 pm	1727.96	Positive																
	11/30/2023 am	4144.51	Positive																
11/30/2023 pm	61648.52	Positive																	