Microbacterial Water Quality Assessment for Union County Conservation District
2011

Conducted by
Regional Science Consortium
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INTRODUCTION

The purpose of this study was to analyze water samples for bacterial contamination. Water samples were analyzed using U.S. EPA standard methods. All water samples were processed by vacuum filtration then cultured on nutrient appropriate agar plates or through quantitative polymerase chain reaction (qPCR). Water samples were collected and processed in duplicate to ensure quality control measures. Blanks were also processed to ensure quality control methods. Sample sites were located in Union County, Pennsylvania. Three streams were sampled at an upstream and downstream site. Water samples were collected by the Union County Conservation District and were given to the Regional Science Consortium at Presque Isle for processing. Sample sites, sample numbers, and abbreviations are listed in Table 1.

Table 1. The locations of the water sampling sites.

Sample Number	Location	Abbreviations
1A	Penns Creek – Upstream	PCU
1B	Penns Creek – Upstream (duplicate)	PCU
2A	Penns Creek – Downstream	PCD
2B	Penns Creek – Downstream (duplicate)	PCD
3A	Buffalo Creek – Upstream	BCU
3B	Buffalo Creek – Upstream (duplicate)	BCU
4A	Buffalo Creek – Downstream	BCD
4B	Buffalo Creek – Downstream (duplicate)	BCD
5A	White Deer Creek – Upstream	WDCU
5B	White Deer Creek – Upstream (duplicate)	WDCU
6A	White Deer Creek – Downstream	WDCD
6B	White Deer Creek – Downstream (duplicate)	WDCD

METHODS

Sample Collection

Samples were collected on June 24th, July 22nd, and August 26th by members of the Regional Science Consortium. Samples were kept on ice during transport and processed immediately following arrival.

DNA Isolation

100 mL of sample were filtered on 0.45 µm mixed cellulose ester pads to entrap bacteria. Bacterial DNA was released by rapid boiling for seven minutes in 600 µl of a buffered solution. Samples were frozen and then analyzed by qPCR using primers that are specific for different *Bacteroides* species.

Quantitative PCR (qPCR)

For the general (overall), human specific, and bovine *Bacteroides* primers, 5 µl of a sample was mixed with 15 µl of a master mix that has been previously described (Smith et. al., 2009). For the pig and bird specific primers, qPCR was performed in the same way except that an intercalating dye was used as the indicator in place of a specific reporter probe. To determine the

estimated concentration of *Bacteroides* in each sample, all signals were standardized to a serial dilution of known concentration of a *Bacteroides* control strain. For the general and human specific probes, this will produce an exact concentration. Since the bovine, pig, and bird primers, the curves will only estimate the concentration since the laboratory strain was not of the origin of these species. In these cases, the background of a no DNA control was subtracted from the signal to estimate the final concentration using the *Bacteroides* standard.

RESULTS & DISCUSSION

Fecal Coliform - Plating

The presence of fecal coliform bacteria was analyzed by colony forming units (CFU) on nutrient agar plates from water samples collected at the six stream locations. Penn's Creek ranged from 1.5 – 372 CFU, Buffalo Creek ranged from 291.0 – Too Numerous to Count (TNC; >600 CFU) CFU, and White Deer Creek ranged from 77.5 – 203.5 CFU (Table 2). Overall, the Buffalo Creek site had the highest counts throughout the season measuring at TNC CFU on June 24, 2011 (Figure 1). Levels were also high at the downstream site of Penn's Creek on the July 22, 2011 and August 26, 2011 sampling dates. Overall, fecal coliform levels at the White Deer Creek were low throughout the season.

Table 2. Average fecal coliform colony forming units (CFU) per 100 mL at six stream locations in Union County, PA during the 2011 season. Those values indicated as Too Numerous to Count (TNC) consist of approximately >600 colonies per 100 mL.

Sample Number	June 24	July 22	August 26
1 - PCU	146.5	1.5	173.0
2 - PCD	174.5	372.0	322.5
3 - BCU	TNC	363.0	291.0
4 - BCD	TNC	355.0	330.0
5 - WDCU	148.5	84.0	90.5
6 - WDCD	203.5	77.5	107.0

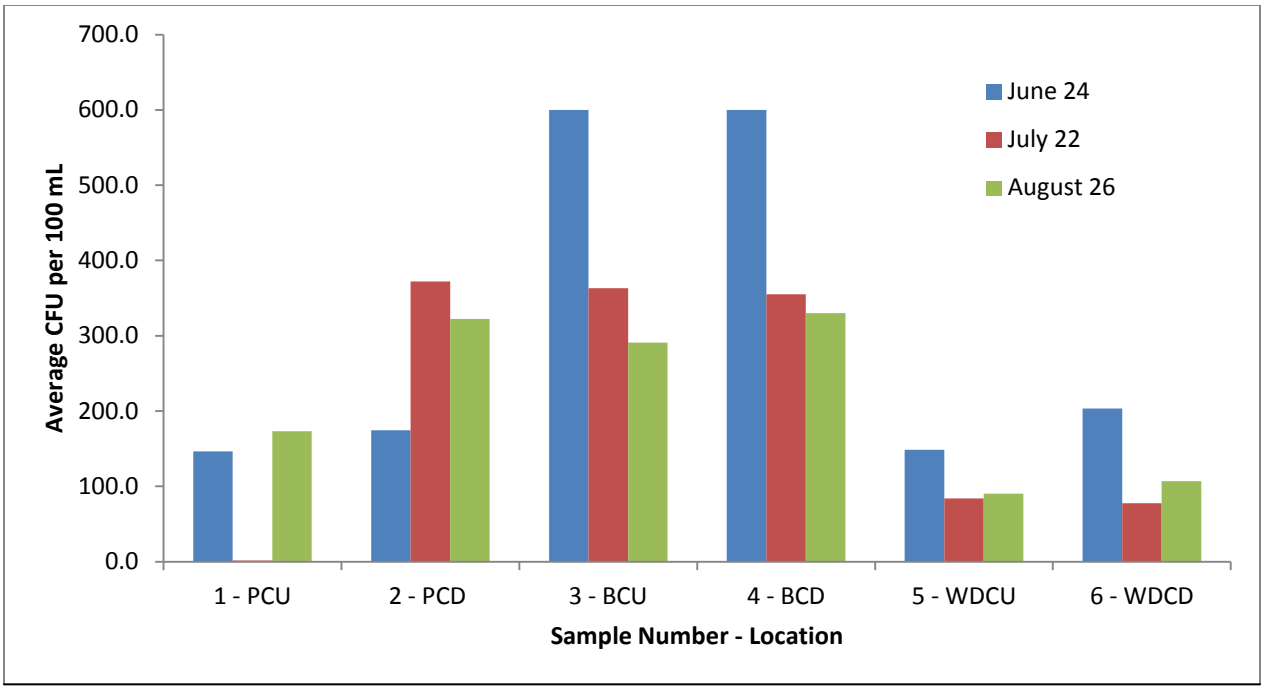

Figure 1. Average fecal coliform colony forming units (CFU) per 100 mL of water sample from six stream locations.

Fecal coliform levels were one of the first bacterial indicators to rate water quality at swimming beaches, with safe swimming waters at <200 CFU/100 mL (1976). All streams exceeded this 200 CFU / 100 mL at least once during the season. Both the upstream and downstream sample sites at Buffalo Creek exceeded this benchmark in all samples analyzed.

Enterococcus - Plating

The presence of enterococcus bacteria was analyzed by colony forming units (CFU) on nutrient agar plates from water samples collected at the six stream locations. Penn's Creek ranged from 31.0 – 171.0 CFU, Buffalo Creek ranged from 1.0 – 416.0 CFU, and White Deer Creek ranged from 0 – 157.0 CFU (Table 3). Overall, the Buffalo Creek site had the highest counts throughout the season measuring at the highest counts on all three sample dates (Figure 2).

Table 3. Average enterococcus colony forming units (CFU) per 100 mL at six stream locations.

Sample Number	June 24	July 22	August 26
1 - PCU	64.5	68.0	92.5
2 - PCD	48.5	31.0	171.0
3 - BCU	257.0	1.0	243.5
4 - BCD	416.0	209.0	91.8
5 - WDCU	62.5	0.0	84.5
6 - WDCD	139.0	157.0	153.5

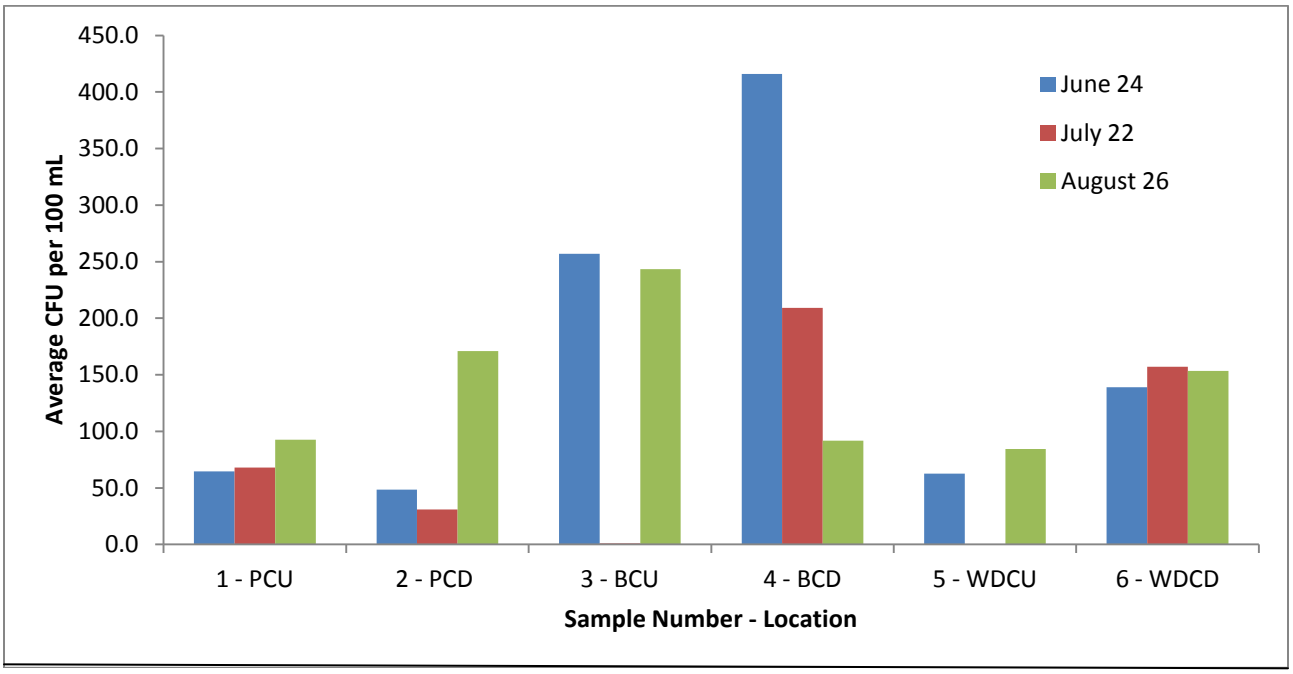

Figure 2. Average enterococcus colony forming units (CFU) per 100 mL of water sample from six stream locations.

Enterococcus levels are often used as indicators to rate water quality at salt-water swimming beaches, however they can also be used at fresh water beaches with safe swimming waters at <61 CFU/100 mL. All streams exceeded this 61 CFU / 100 mL at least once during the season.

Enterococcus - qPCR

The presence of enterococcus bacteria was also analyzed by qPCR from water samples collected at the six stream locations. Values represented in Table 4 are in cell equivalents (CE). As one reviews qPCR data, it is important to keep in mind that qPCR analysis will detect live cells and dead cells. Therefore the cells detected in qPCR analysis as CE are not all viable and capable of growing colonies on plated media or causing illness. Bacterial cells associated with fecal contamination often have a limited lifespan once they exit their host, hence creating a considerable number of non-viable cells in the environment.

Enterococcus at Penn's Creek ranged from 136,740.46 – 176,765.89 CE, Buffalo Creek ranged from 211,567.76 – 1,129,786.49 CE, and White Deer Creek ranged from 95,454.19 – 218,467.66 CE (Table 4). Overall, the Buffalo Creek site had the highest counts throughout the season, with the downstream followed by the upstream sample location measuring at the highest counts on all three sample dates (Figure 3).

Table 4. Enterococcus present in water samples collected on the three dates during the 2011 season. Values listed are the average cell equivalents (CE) of the duplicate water samples collected on each date.

Sample Number	June 24	July 22	August 26
1 - PCU	157,479.06	158,493.10	136,740.46
2 - PCD	151,529.65	176,765.89	170,087.84
3 - BCU	335,855.20	211,567.76	295,393.78
4 - BCD	358,119.06	873,966.80	1,129,786.49
5 - WDCU	124,988.92	143,945.24	168,999.62
6 - WDCD	218,467.66	122,605.21	95,454.19

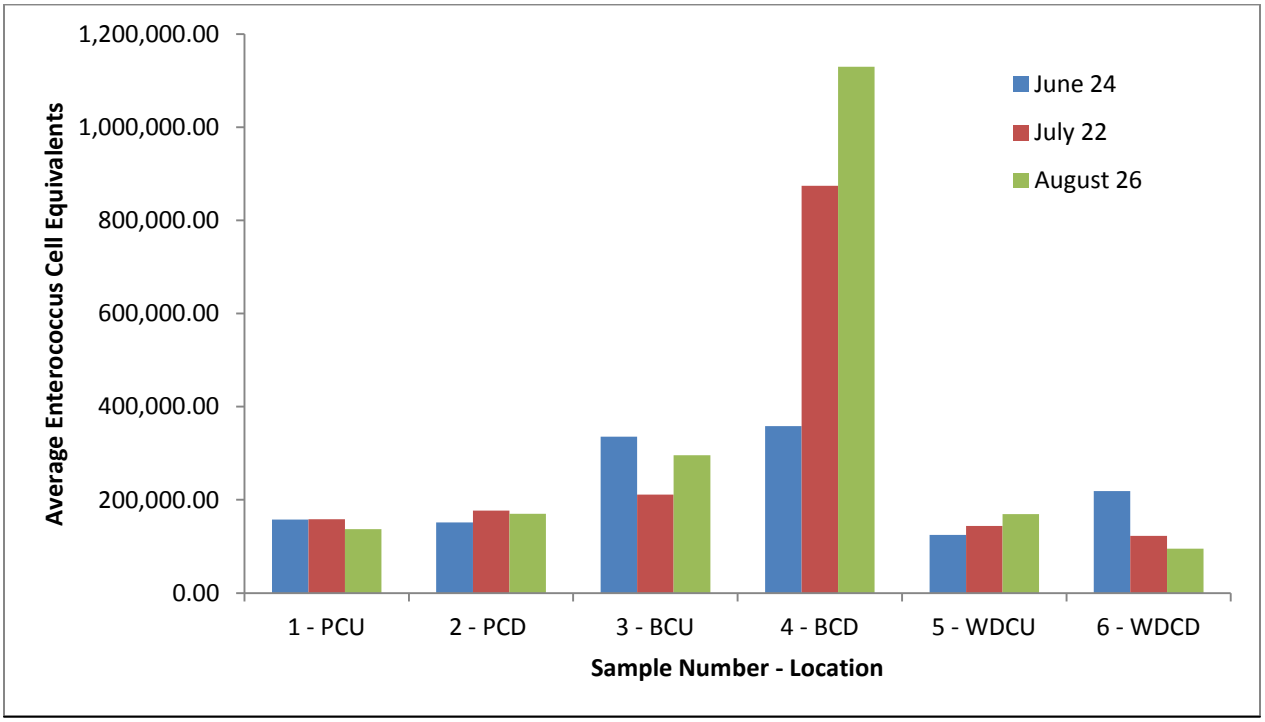

Figure 3. The average general enterococcus cell equivalents at each sample location on the three sampling dates during the 2011 season.

Bacteroides - qPCR

General *Bacteroides* cell equivalents (CE) ranged from 431.27 – 13,708.98 (June 24, 2011), 1,423.86 – 5,893.82 (July 22, 2011), and 61.25 – 4,396.68 (August 26, 2011) (Table 5). White Deer Creek at both the upstream (Location 5) and the downstream (Location 6) sites had the lowest *Bacteroides* levels detected on all three dates (Figure 4.). The highest *Bacteroides* levels were detected at the Buffalo Creek downstream (13,708.98 CE), and Penn's Creek downstream (6,423.43 CE) on June 24, 2011. Downstream *Bacteroides* levels at each site were higher at all sites except Buffalo Creek on July 22, 2011 where levels were very similar, and White Deer Creek on August 26, 2011, indicating potentially a low flow from upstream to downstream due to drought conditions during this time of the season. Flows for the upstream sites were very similar to downstream sites at Penn's Creek and Buffalo Creek on July 22, 2011. This indicates

samples were possibly collected after a storm event resulting in overall high *Bacteroides* levels as well as similarities between the upstream and downstream locations.

Table 5. General *Bacteroides* present in water samples collected on the three dates during the 2011 season. Values listed are the average cell equivalents (CE) of the duplicate water samples collected on each date.

Sample Number	June 24	July 22	August 26
1 – PCU	3,019.04	5,062.92	1,449.07
2 – PCD	6,423.43	5,288.93	4,396.68
3 – BCU	3,521.11	5,893.82	668.96
4 – BCD	13,708.98	5,860.69	2,193.68
5 – WDCU	431.27	1,423.86	311.86
6 - WDCD	2,190.88	2,048.32	61.25

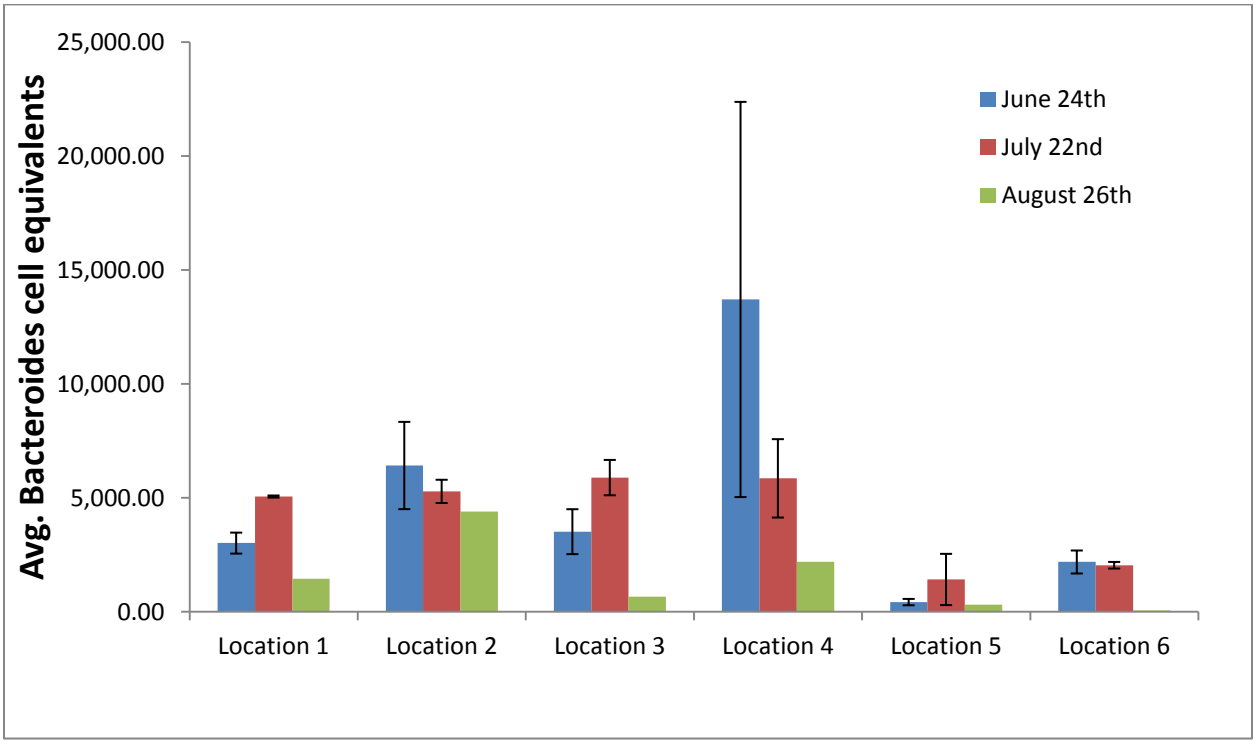

Figure 4. The average general *Bacteroides* cell equivalents (± standard error) at each sample location on the three sampling dates during the 2011 season.

When examining specific source *Bacteroides*, we were able to successfully analyze for Human, Pig, Bovine, and Bird sources. The remainder of the existing *Bacteroides* includes Horse and Other potential sources. The Horse primer was analyzed for, however the data was not valid and could not be distinguished from Other sources in the sample.

The Other source of *Bacteroides* (which may include Horse) had the highest percent contribution to the total *Bacteroides* present in the sample (Table 6). Excluding the Other source of *Bacteroides*, Pig had the highest percent contribution at all six locations.

Table 6. Percent contribution of source specific *Bacteroides* at six sampling locations.

Sample Number	Human	Pig	Bovine	Bird	Other
1 – PCU	0.88	22.06	0.11	0.00	76.95
2 – PCD	0.37	25.40	0.18	15.75	58.30
3 – BCU	4.55	34.74	11.13	0.00	49.58
4 – BCD	1.78	23.38	3.78	15.53	55.53
5 – WDCU	11.83	37.16	0.57	0.46	49.98
6 - WDCD	16.03	23.42	0.29	16.48	43.78

High contributions also included Bird at White Deer Creek downstream (16.48%), Penn's Creek downstream (15.75%), and Buffalo Creek downstream (15.53%); however Bird contribution at the upstream locations at all streams was minimal to undetected.

Human contributions of *Bacteroides* were found at all locations, with the highest percent contribution at White Deer Creek upstream (11.83%) and downstream (16.03%). Managers of this area may want to consider where this source of contamination is originating.

Bovine contamination had the greatest impact at Buffalo Creek upstream (11.13%), which appears to be diluted at the downstream sampling location (3.78%). Overall, Bovine appears to have the least contribution to *Bacteroides* contamination at the streams sampled. Bovine contribution was greatest at the Buffalo Creek location.

Contributions of *Bacteroides* by Other sources (which may include Horse) were greatest at Penn's Creek (upstream - 76.95%; downstream – 58.30%), indicating the analyzed sources (Human, Pig, Bovine, and Bird) only make up 23.05% of the total *Bacteroides* contamination in the sample. Nearly 50% of the contributions of *Bacteroides* at White Deer Creek were detected by specific sources analyzed in this study.

When examining the percent contribution, as discussed above, we are examining the composition of the bacterial pollution at each site. White Deer Creek upstream had the lowest overall *Bacteroides* pollution compared to all other sites, followed by White Deer Creek downstream (Figure 5). Although there appears to be a variety of *Bacteroides* sources contributing to White Deer Creek, they are at very minute amounts. Penn's Creek downstream and Buffalo Creek downstream had the highest *Bacteroides* contamination. Buffalo Creek downstream was positive for all sources tested, with Other, Pig, and Bird making up the majority of the contamination. Buffalo Creek upstream was not positive for Bird *Bacteroides* contamination; however the source of bird contamination may occur between the upstream and downstream sites. Penn's Creek downstream *Bacteroides* contamination was made up mostly of Other, Bird, and Pig sources. The Penn's Creek upstream site was also not positive for Bird *Bacteroides*

contamination, indication bird contamination may occur between the upstream and downstream sites (similar to Buffalo Creek).

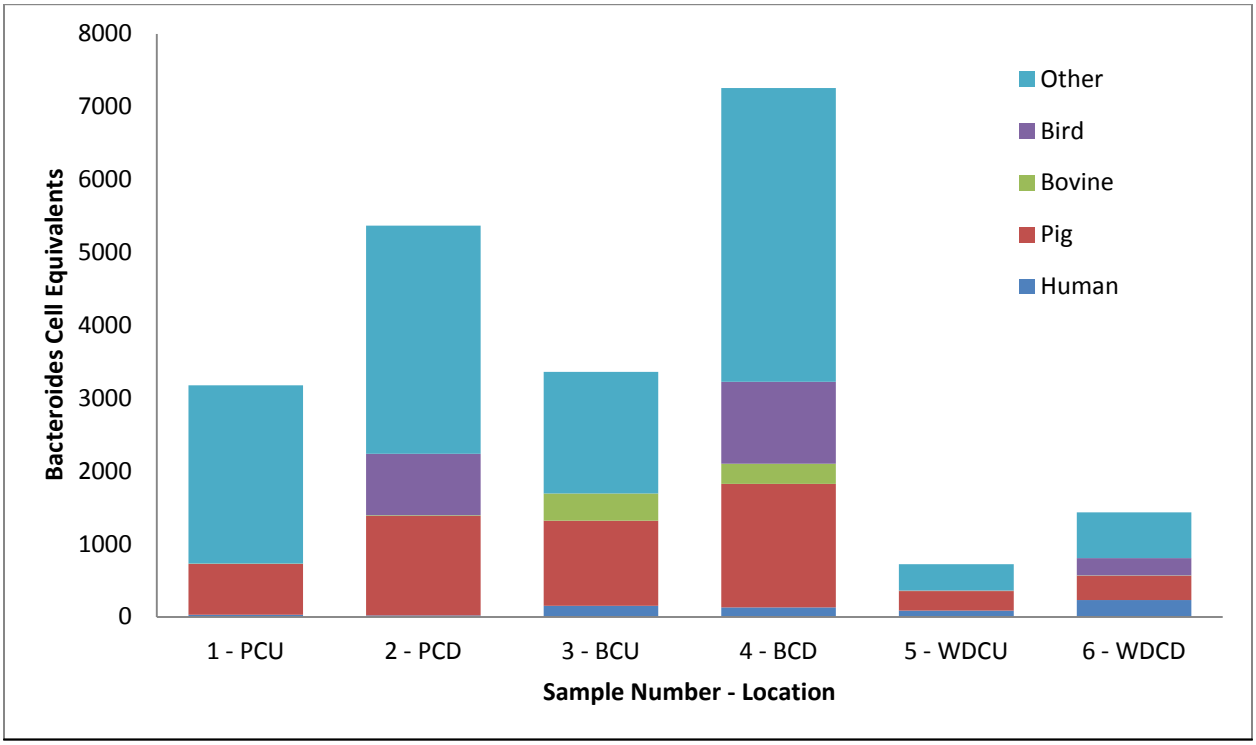

Figure 5. Cell equivalent (CE) contribution of source specific *Bacteroides* determined from six sample locations over the 2011 season.

CONCLUSIONS

The objective of this study was to analyze water samples for bacterial contamination and potential sources of contamination in upstream and downstream sites of three streams in Union County, Pennsylvania. Both fecal coliform and enterococcus were found to exceed safe swimming water quality benchmarks in each stream at least once during the season; however the sites sampled were streams that run through agriculture and forested lands and are not public swimming beaches. The dynamic of stream waters differs from that of swimming waters which are usually found in lakes, offering a greater volume of water and potential for an increase in dilution.

The *Bacteroides* data provided information as to the possible source and the extent of bacterial contamination. *Bacteroides* levels peaked at 13,708.98 CE. Previous studies on the swimming beaches of Lake Erie, Pennsylvania found that *Bacteroides* levels of 100,000 – 300,000 CE often correlated to 235 CFU / 100 mL *Escherichia coli* (U.S. EPA benchmark for safe swimming waters). Therefore, the *Bacteroides* levels found in this study do not indicate highly contaminated waters (in regard to the bacterium *Bacteroides*). When examining the amount of

each type of source contamination that contributed to the total amount of *Bacteroides* present at a site, Pig appeared to have a great influence on all streams. There was a Human source of contamination at White Deer Creek that may want to be further investigated, although the total amount of *Bacteroides* present was very low relative to the other streams sampled.

When reviewing the qPCR *Bacteroides* data, it should be noted that the percent of source contribution is as estimate. The qPCR primers used to identify specific organisms can result in false positives and false negatives. The data provided should be used as a tool to assist in identifying potential *Bacteroides* sources at specific sites; however one should note that the development of specific primers is still recent and errors in source identification do occur.

In conclusion, while the overall human contribution to bacterial pollution is low at these sites, different farm animals may be playing a role. However, the individual contribution of any one farm animal at a particular location is low, and it remains to be determined where the majority of the *Bacteroides* contamination arose from, which could include non-farm animals such as deer and small rodents, or other farm animals not included in this study.

Overall, when comparing all sites, bacteria, and dates it appears that Buffalo Creek (relative to the other streams sampled) had the highest levels of bacterial contamination. Although all the bacteria analyzed in this study are indicators of fecal contamination, they do not necessarily correlate to each other. The bacteria analyzed all have different survivorship once they exit host organism. One must also consider the water sample itself represents bacterial levels temporally and spatially, and these levels can differ at both horizontal and vertical transects of the stream.