Title: Investigation and Implications of Bacterial Contamination in the Pigg River.

Introduction

Background of Study

The Pigg River, located in south central Virginia, was named after an early settler John Pigg who laid claim to 400 acres of land opposite the mouth of Snow Creek (Clement 1929). Throughout its history, the river drained predominately forested land but more recently land clearing for agricultural production has increased. By best estimates, the watershed currently consists of 65% forest, 26% agriculture (crops and cattle) and 5% urban development (Ries 2008; Benham et al. 2006).

Land Cover and Water Quality

The land cover and land use (LCLU) in a watershed can be very predictive of water quality. In watersheds predominately covered by forest, we find relatively good water quality while in watersheds increasingly plagued by human alteration, we find greater and greater concentrations of water pollutants (Baker 2003; Buck et al. 2004; Tong and Chen 2002). Direct quantification of the impact of specific land use on resultant water quality continues to be difficult. However, generalizations and risk development models are continually in development. One such model - The model of impervious cover (Schueler et al. 2009) - relates degrading water quality to the increase in impervious surfaces associated with development. Sediment moving from deforested or disturbed areas into rivers has been further developed into a concept of the urban sediment cascade (Taylor and Owens 2009). These models are useful when correlating land use to any type of land disturbance. Agricultural impacts on water quality have also been generalized (Stone et al. 2005; Cuffney et al. 2000). Land development tends to degrade water quality when not controlled or mitigated.

More specifically, agricultural land use and associated practices have been pinpointed as a specific source of river microbial loading (Tong and Chen 2002; Stein et al. 2008; Petersen et al. 2018; Petersen and Hubbart 2020). This land use when associated with precipitation events that cause rivers to rise is now better understood as a driving mechanism for elevated *E. coli* concentrations in these rivers (Haramoto et al. 2006; Pandeya et al 2012; Rodrigues et al. 2018). Rainfall events directly after the application of manure or upon cattle-impacted lands can cause even greater elevated bacteria in receiving streams (Soupir et al. 2006; Guber et al. 2007). Crop lands can be an additional source for elevated bacterial loadings (VanderZaag et al. 2010) but this is dependent upon specific land management strategies. Finally, wildlife in associated riparian forest are an additional source of contamination (Cox et al. 2005).

Finally, the urban environment presents another set of contamination concerns. Pathogens (bacteria, parasites, protozoans, viruses) are harbored in urban river environments entering from stormwater, wastewater and overflowing or leaking sanitary sewer systems (Olds et al. 2018). Unlike agricultural concerns, stormwater over impervious surface directly impairs urban rivers due to increased inflow of water volume and pollutant loads that correlate with disease risk (Arnone and Walling 2007). Increased river discharge has caused extensive eroding of river banks coating river beds with sediment and organic material and nutrients creating an ideal environment for bacteria such as *E. coli* to survive until the next storm re-suspension. We know that sediment loading as a direct result of erosion of exposed land or streambank failures contains heavy loads of bacteria (Chen and Lui 2017). *Escherichia coli* survive in sediment much longer than in overlying waters (Pachepsky and Shelton 2011). This sediment acts as a sink with the potential of continued bacterial contamination long after any contamination event (Mallin et al. 2007). Thus, it is concerning that impacted rivers may harbor extensive beds of bacteria, potential pathogens and other pollutants that will be resuspended continually when waters rise due to increased flow.

Bacterial Contamination and Health Risk

While concern over land use and river contamination can be documented, the translation of bacterial contamination to public health risk and disease is more elusive. Using climate and epidemiological records, Rose et al. (2000) found statistical evidence suggesting a correlation between storm events and disease outbreak in cities. DeFlorio-Barker et al. (2018) estimated recreational waterborne illness on United States surface waters is significant and costly. An estimated 4 billion surface water recreation events occur annually, resulting in an estimated 90 million illnesses with a cost of \$2.2-\$3.7 billion annually. Illnesses of moderate severity (suggested by a visit to a health care provider) were responsible for over 65% of the economic burden while severe illnesses (result in hospitalization or death) were responsible for approximately 8% of the total economic burden.

In response, criteria for contamination levels to minimize disease risk have been formulated (Kay et al. 2004). In these scenarios, researchers examined how successive increases in fecal bacterial concentrations generated a concurrent risk of illness. Water low in fecal contamination (FC) or a concentration of < 40 FC per 100 ml generated a minimal risk or less than 1% chance of contracting some form of illness. Subsequent incremental increases in fecal contamination were studied and quantified. Concentrations greater than the 500 FC per 100 ml were associated with (>10%) potential illness rick.

Translating these established risks into credible regulatory standards is even more difficult. The United States Clean Water Act calendar month geometric mean standard of 126 *E. coli* (CFU per 100 mL) was suggested to provide a minimal disease risk (<1%) (Dufour and Ballentine 1986). Further research suggested that maintaining *E. coli* below 200 CFU per 100 mL as a geometric mean and lower than 400 CFU per 100 mL as an instantaneous standard helps keep the rate of gastrointestinal illness from exposure to approximately 1–2 % (Tobin and Ward 1984). These microbiological standards are not based on robust epidemiological data but represent best estimates of associated risk (Kay and Fawell 2007). The best preventative approach is to maintain bacterial and pollutant concentrations as low as possible.

Legacy Pollution and Sediment

With changes occurring to the natural landscape and the problem of sedimentation so pervasive, controlling bacterial contamination in rivers is becoming more difficult. Current trends including removal of dams, alterations to river water courses, flooding, farming, urbanization and deforestation all contribute more and more sedimentation into water resources. Compounding this are alterations in precipitation driven by changes in climate. We now see greater intensification in precipitation and widespread variability in its distribution. This creates what many authors describe as a "wicked" problem (Shortle and Horan, 2017). Our freshwater streams and rivers flow through an altered landscape driven by an intensifying water cycle. Improvement in water quality is a very difficult task.

To tackle such problems, an understanding of sources and sinks along with areas of intensification or "hot spots" must be clear (Fleming et al. 2019). Spreading management expenses and fixes throughout a region may not produce observable or necessary improvements in water quality other than money spent. Reductions in pollutant runoff or improvements in stormwater treatment applied to farms or urban landscapes may be offset by "legacy" pollutants stored in the system from previous disturbances. These legacy pollutants exist in streambank soils and throughout the stream bed creating the primary underlying material that streams flow through and move during storm events. Intense storms work to activate these pollutants by resuspending sediment and pulling deposited material from stream banks back into the water. With each storm, sediment moves down the channel while upstream erosion resupplies more and more easily movable soil. As we know these sediments harbor bacteria that are activated from movement, sediment hotspots in any river may be the primary problem in stream channels – causing most of the problems. This disconnect between watershed management practices and observable stream water quality improvement will take decades to resolve and only when the systems flush existing sediments and reach an equilibrium with erosion and streambank stability.

Further, our rivers have been historically altered by dams (Walter and Merritts 2008). Each of these structures is characterized by a particular volume of water impounded and a watershed drainage network above. The current trend of dam removal while admirable and with certain positive attributes is also unleashing even more legacy sediments into the system. This sediment must be processed, distributed and eventually washed through into our bays and oceans. Hence, adding additional sediment stored behind dams to a river system already burdened by legacy sediment and land use changes severely compounds an already difficult problem.

Our investigations were initiated in the summer of 2018 to evaluate water quality conditions in the Pigg River, which is an important tributary to Leesville Lake. This lake is an important recreational, environmental, and power generation reservoir in Central Virginia. While the initial focus was on water quality, greater focus and scrutiny began in 2019 to determine origins of the bacterial contamination (assessed by *E. coli* concentrations) discovered throughout the 2018 study. In 2020, we intensively evaluated water quality in areas of urban, agricultural and forested land use to

determine the impact of these as sources of contamination on the river. Further study has looked at the impact of dam removal and legacy sediment causing greater bacterial contamination during storm events.

Regulatory Background

Within the Pigg River, which flows through Franklin County and Pittsylvania County in Virginia, continuous observations of high levels of *E. coli* and other elevated water quality measures has prompted concern over changing land use and a need to control it. Several documented concerns have been observed recently. In 2017, an obsolete power dam near Rocky Mount Virginia was removed. While dam removal has become a popular river improvement strategy, in this instance removal of the dam occurred without significant sediment management in place. This delivered substantial loads of bacteria and turbidity downstream.

The Virginia Total Maximum Daily Load (TMDL) Program, which addresses waters with bacteria levels exceeding state standards, published a report in 2006 on waters around Leesville Lake (Benham et al. 2006). This report addressed bacteria levels flowing from the lake's two main tributaries, Pigg River and Old Woman's Creek, placing them on Virginia's 303(d) list of impaired waters. The Pigg River has been listed as impaired since 1998. Snow Creek (another tributary to Leesville Lake-Pigg River) and Old Woman's Creek have been listed as impaired since 2002.

The TMDL report identified three point-sources discharging bacteria into the Pigg River basin, with one located in the Story Creek watershed area. There were no permitted dischargers in the Old Woman's Creek watershed. The TMDL reporting specifies nonpoint sources as the primary source for high bacteria levels; including agriculture, land-applied animal waste, and livestock manure. The report also specifies that cattle and wildlife directly dumping feces into streams causes a heavy bacteria load. Nonpoint sources from residential areas include straight pipes, failing septic systems, and pet waste (Benham et al. 2006) are also contributors.

Pigg River and Old Woman's Creek TMDL Implementation Plan published by Virginia Department of Environmental Quality in 2009 identified work necessary for *E. coli* reductions in the watershed to bring violation rates below 10% per year (VADEQ IP Progress Report). The majority of the need suggested controlling pasture runoff with livestock fencing and point source reductions. Of concern for Leesville Lake is the elevated *E. coli* concentrations entering from Pigg River discharge. (Notably since 2013, water entering Leesville Lake from Old Woman's Creek has contained *E. coli* levels well below state standards for water used for recreational purposes.)

The total cost-share payments for Best Management Practices installed throughout the Pigg River Watershed are \$1,588,908. Eighteen on-site sewage disposal practices were installed in the watershed in FY13, these included replacement of 16 failing septic systems and the repair of two septic systems. A total of 38 miles of livestock stream exclusion fencing has been installed through TMDL practices, constituting 60% of the

fencing goal in the TMDL implementation plan (VADEQ IP Progress Report). Funding for this program ended in 2015.

Virginia Standards

The code of Virginia, 9VAC25-260-170 outlines the following standards for the waters of Virginia. Bacteria criteria (counts/100ml) shall apply to protect primary contact recreational uses in surface waters. In freshwater, *E. coli* bacteria shall not exceed a geometric mean of 126 counts/100ml and shall not have greater than a 10% excursion frequency of a statistical threshold value (STV) of 410 counts/100 ml, both in an assessment period of up to 90 days.

Geometric means shall be calculated using all data collected during any calendar month with a minimum of four weekly samples. If there is insufficient data to calculate monthly geometric means in freshwater, no more than 10% of the total samples in the assessment period shall exceed 1173 E. coli CFU/100 ml. If there is insufficient data to calculate monthly geometric means in transition and saltwater, no more than 10% of the total samples in the assessment period shall exceed 519 enterococci CFU/100 ml.

Methods

Study Design and Rational

For this study, we sampled multiple sites along the main stem of the Pigg River (Figure 1). Each river site was chosen for accessibility to facilitate direct sampling for bacteriological and chemical analyses.

In 2018 we conducted a comprehensive sampling of the river establishing stations and analyzing data. In 2019 and 2020, we continued to monitor the river but included potential "hot spots" to include Bacterial Source Tracking (BST). A hot spot was considered a sampling station where excessive concentrations of *E. coli* were measured in 2018 using the Colilert methodology. Excessive was considered over 1000 counts/100ml during low flow conditions and in excess of 2400 counts/100ml during flood conditions. The excessive classification for hot spots was determined through

observations of the data collected in 2018 along with statistical analysis looking at clusters in the data.



Figure 1 – Sampling sites on the Pigg River. Samples were collected throughout the watershed beginning on the Lower Pigg just below the City of Rocky Mount and finishing at headwaters of Leesville Lake.

Additionally, in 2019 we sampled during low flow and storm flow conditions. Low flow was defined as conditions that did not meet the criteria for stormwater flow. Stormwater flow was defined as conditions where high turbidity (>50 NTU) and appreciable rain (> 20 mm) occurred in the watershed within 24 hours of sampling. Low flow sampling was designed to pinpoint exact location of bacterial contamination. The second sampling was designed to identified contamination during a stormwater event.

In 2020, we continued sampling of the river and increased our efforts to identify hot spots near Rocky Mount Virginia. In 2021 and 2022 we concentrated efforts on the lower Pigg trying to quantify the potential areas impacted most significantly by sedimentation and bacteria.

Site Descriptions and Land Use Impact

Each river segment was characterized using aerial maps and quantification of land use adjacent and directly along the river. A land use or buffer was considered along when that land use abutted the river. Adjacent was identified as within 100 feet of the riverbank. River segments were measured, and land use identified. Using the aerial maps, forest vs. pasture or other use was easily identified. Each land use was measured and then quantified using the following criteria to determine a land use type:

- Minimal Impact > 50% land use forested and buffered along reach. Buffer minimum of 50 feet. Minimal impact from pastureland use
- Mixed Impact approximately 50% of reach buffered or forested with remaining reach pasture with minimal buffer.
- Significant Impact < 25 % of reach forested or buffered up to 75% reach pasture with minimal buffer.
- Major Impact <10% of reach forested or buffered up to 90% reach pasture with minimal buffer.

River Segment	Designated Land Use	River Distance
C		(Miles)
Toshes to Toler	Riverine/Limnetic – Minimal	3.5
	Impact	
Museville to Toshes	Mixed Impact/Significant	10.9
	Impact	
Snow Creek Rd to Museville	Significant Impact	3.5
Truevine to Snow Creek	Significant Impact	10.5
Colonial to Truevine	Minimal Impact	5.1
Chestnut to Colonial	Mixed Impact.	14.9
Power Dam to Chestnut	Major Impact	4.8

Table 1 – River segments, land use and distance between proposed sampling sites. Sampling sites were determined by highway river crossings.

Power Dam:

The Dam sampling location is at the confluence of Power Dam Road and Pigg River (GPS coordinates: N 36° 59' 45.9", W 79° 51' 36.2"). This site was selected for sampling due to its close proximity to the site of a dam removal project in 2017 and its accessibility via Power Dam Road.

Chestnut Hill:

The Chestnut Hill sample site is located at the intersection of Chestnut Hill Road and Pigg River (GPS coordinates: N 37° 00' 11.5", W 79° 49' 34.1"). Powder Mill Creek joins

Pigg River directly upstream of this site. The 4.9 -mile river segment upstream of the Chestnut Hill Site has been classified as having major impact (Table 1).

Colonial:

The Colonial Sampling Site is located at GPS coordinates (N 36°56'24.9", W 79°46'03.0"). Doe Run Creek, a small tributary, joins Pigg River upstream of this site, potentially impacting the water quality found at this site. The 14.9-mile river segment upstream of this site has been classified as having <u>minimal</u> impact (Table 1).

Truevine:

Truevine sampling site is located at the intersection of Truevine Road and Pigg River (GPS coordinates: N 36°56'05.6", W 79°43'01.2"). The 5.1-mile river segment upstream of this site has been determined to have minimal impact (Table 1). Potential contributors for water quality at this site include Big Chestnut Creek and Walker Creek.

Snow Creek:

The Snow Creek Sampling Site can be found at the intersection of Snow Creek Rd and Pigg River (GPS coordinates of N 36°56'40.1", W 79°38'18.1"). The 10.5-mile river segment upstream of this site is known to have mixed impact closer to Truevine and significant impact near Museville (Table 1). This segment is characterized by the presence of numerous tributaries that converge into Pigg River.

Museville:

The Museville Sampling Site is located off the Museville Bridge (GPS coordinates: N 36°56'02.9", W 79°35'40.0"). The upstream segment of the river, extending 3.5 miles from this location, has been determined to have a significant impact (Table 1).

Toshes:

The Toshes Sampling Site is located at the intersection of Pigg River and Toshes Road (GPS Coordinates: N 36°59'19.9", W 79°30'56.1"). The 10.9-mile upstream segment of the Pigg River from this site has been determined to have been significantly impacted. This section of the river is characterized by the presence of numerous large tributaries that converge into the Pigg River.

Toler:

Toler Sampling site is located near the confluence of the Pigg River and Leesville Lake (GPS Coordinates: N 37°00'30.2", W79°28'41.4"). The 3.5-mile upstream segment is determined to have low impact (Table 1). This site is the site with road access closest to the mouth of the Pigg River and was utilized to estimate the quality of Pigg River water entering Leesville Lake.

Sampling Methodology

Water was collected from each bridge crossing either directly by filling water bottles and placement of the YSI multiprobe into the water or collection from the bridge using a collection bottle (Figure 2). After collection, water for laboratory analysis was immediately transferred to acid washed bottles (nutrients) and sterilized bacteriological bottles and stored in a cooler until analysis in the laboratory. *E. coli* samples were immediately processed upon return to the laboratory while nutrient samples remained refrigerated until analysis within 30 days of collection. Remaining water was analyzed using a YSI multiprobe and Turner Turbidimeter.



Figure 2 – Collection of water samples directly from a bridge crossing. A rope is lowered with a bottle attached for retrieval of the sample.

Water quality data was obtained using a YSI 556 multiprobe meter (Xylem, Yellow Springs, Ohio) following pre and post calibration QA/QC procedures in accordance with EPA protocols (EPA 2017). *E. coli* was quantified using Colilert-18 (IDEXX, Westbrook, Maine) meeting all EPA standards for testing (Warden et al. 2011). This methodology uses MPN to quantify E. *coli*, reported as MPN/100 ml of sample in accordance with federal and state standards. Total phosphorus (TP) samples were collected in acid washed Nalgene bottles and analyzed analytically using an EasyChem auto analyzer (Systea Analytical Technologies). The EasyChem analysis is compatible with Ascorbic Acid Total Phosphorus Analysis detailed in Standard Methods for Analysis of Water and Wastewater (Baird and Bridgewater 2017).

In an experimental analysis, stream segments believed to be impacted by the dam removal was studied under 3 types of flow conditions; baseline, disturbed bottom and stormwater. Under baseline flow, water samples were analyzed directly while stormwater was measured during a defined bankfull (Rosgen 1996) storm event. The disturbed bottom measures occurred during baseline flow with multiple individuals disturbing the stream bottom and banks forcing sediment into the stream that mimicked turbidity during a stormwater event (Figure 3).



Figure 3 – Sampling of the river after an artificial mixing event created by disturbing bottom and streambank sediments.

Bacterial Source Tracking (BST)

Water for bacterial source tracking was collected into sterile bottles and shipped overnight in cooled ice chests for analysis (Source Molecular Corporation, Miami Lakes, FL). There, water samples were filtered through 0.45 micron membrane filter(s) then placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. Primers employed were for generation of amplicons for human, ruminant and cattle markers. All assays were run in duplicate. Quantification was achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers. For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Statistical Analysis

The general trends observed in the study were initially displayed using descriptive statistics, including means and standard errors for all observations. The number of observations varied depending upon samples taken in the river and Leesville Lake. In general, the Pigg River was sampled three times each year (2018-2020) primarily late summer to early fall. Leesville Lake was sampled monthly from late April through October, for a total of seven sampling events.

Two types of regression analysis were used to create a best fit model to determine predictors for all of the data collected. The least squares regression analysis examined the best fit or best predictors for *E. coli* based upon all collected parameters (see Table 6). From these findings, two linear regressions were created from identified parameters as the final statistical tool to model relationships in the watershed.

Results

Bacterial and other Pollution

It is clear from the collected data that the Pigg River is significantly impaired (Table 2). Our bacteriological results suggest the river continually contains concentrations of *E. coli* in exceedance of 9VAC25-260-170 through our four-year study period. Every month, the Geometric Mean (GM) exceeded the 126/100 ml standard for a violation rate of 100%. Violation of the STV standard of 410 /100ml occurred at a rate of 82% in our study. In instances where the river enters flood stage, the concentrations of *E. coli* exceed 100x the allowable standard. The river is severely impaired by bacterial contamination.

Table 2 – Calculated Geometric Means and Standard Threshold Values for each sampling period (2018-2022) in the study (CFU/100ml). A sampling period consisted of a series of samples taken within a 90-day period on the river. Virginia Standards for recreational waters 9VAC25-260-170 set standards for *E. coli* bacteria GM 126 counts/100ml and shall not have greater than a 10% excursion frequency of a statistical threshold value (STV) of 410 counts/100 ml, both in an assessment period of up to 90 days.

Sampling Event	1	2	3	4	5	6	7	8	9	10	11
Number of	7	7	7	7	5	15	24	11	11	8	8
Samples (N)											
Geometric	1086	328	461	1040	755	441	185	1070	175	791	16851
Mean (GM)											

Standard	2442	1387	689	2231	2230	5703	373	5821	275	1202	24580
Threshold											
Value (STV)											

To examine possible host sources for the bacteria, we analyzed genetic markers contained in *Bacteroides*. Results displayed here are accompanied by the *E. coli* measures during the same period. During each sampling period, at least one sampling was accompanied by BST. Although the data were not rigorously validated for this purpose, ratios of genetic marker copy numbers were used to estimate the relative contribution of various animal hosts to the bacterial load in the water. When data were combined for all sites and for all weather conditions our bacterial source tracking studies yielded some interpretational results in the river (Table 3).

Table 3 – Results from the BST analysis for each of 4 years where this type of analysis was conducted. All data in this table represents the means +/- Standard Deviations of stations where BST was measured in a particular year.

	2019	2020	2021	2022
E coli	3207 ± 5109	252 ± 117	486 ± 466	20467 ± 2367
(CFU/100ml)				
Human	1378 ± 2810	252 ± 258	183 ± 104	923 ± 1298
(copies/100ml)				
Ruminant	42265 ± 66491	6380 ± 6642	1021 ± 1787	$261951\pm$
(copies/100ml)				407587
Bovine	2005 ± 1266	11.1 ± 22.3	1378 ± 1908	1457 ± 2234
(copies/100ml)				

Essentially, most of *Bacteroides* bacteria that were genetically tracked across all sites was ruminant in origin. However, to determine if these BST concentrations were correlated with other water quality parameters a Principal Component Analysis was conducted (Figure 4).



Figure 4 – Principal Component Analysis (PCA) of selected parameters from the study. F1 along the x-axis represents the first component and F2 along the y axis represents the second component. Each parameter is abbreviated and includes: Cond = Conductivity (ms/cm), E. coli = E. Coli Bacteria (CFU/100ml), TP = Total Phosphorus (mg/L), Turbidity = NTU, Human = BST Human (copies/100ml), Ruminant = BST ruminant (copies/100ml) and Bovine = BST Bovine (copies/100ml)

The PSA analysis suggests that *E. coli*, TP and Turbidity were the strongest predictors of water quality based on this set of parameters. Each is strongly clustered and correlated in the first component that accounts for 57.69% of the variability. Additionally, the Bovine and Ruminant were correlated with these same water quality parameters. Human BST and Conductivity do not correlate strongly with either principal component.

Building upon these findings, both *E. coli* and the ruminant concentrations were correlated using Partial Least Squares Analysis Variable in Projection Assessment (PLS-VIP). This analysis correlates all parameters to one of the selected to determine how each is related. It is generally accepted that a value greater than 1 is significantly related to the tested parameter. Hence, with this analysis we can look further into the relationship between all of the parameters for the prediction of water quality.

The PLS-VIP *E. coli* analysis suggested turbidity, TP and Bovine BST concentrations were significantly correlated to the concentrations of *E. coli* throughout the study (Figure 5). This further supports the findings from the PCA analysis suggesting these are the most important parameters of study.



Figure 5 – PLS-VIP analysis correlating various parameters with *E. coli*. A value of 1 was used in this analysis for a significant relationship to the parameter. Abbreviations of parameter are similar to those in the PCA analysis.

A PLS-VIP analysis was also conducted for ruminant concentrations in the study to determine if there was any relationship among the other parameters. Results suggested that Bovine BST concentrations and conductivity were the only related parameters (Figure 6). As Bovine BST concentrations are a subset of the ruminant BST concentrations and conductivity unrelated to the other parameters, it was interpreted that ruminant BST concentrations did not provide any interpretive value. This measure appears to be too generalized to pinpoint the source of bacterial contamination.



Figure 6 – PLS-VIP analysis for ruminant BST concentrations. Descriptions the same as in Figure 5.

Looking at how contamination was distributed in the river, each of the parameters was averaged and plotted along an axis representing the Pigg River from Toshes Road to Chestnut Hill (Figure 7). This analysis strongly suggests that much of the contamination is concentrated at Chestnut Hill, and primarily in the Colonial section of the river. Only turbidity measures do not directly support the conclusion that Colonial is the most contaminated site, but all parameters did suggest contamination is greater in the upper portion of the river.



Figure 7 – The most important predictive parameters (E. coli, TP, Turbidity and Bovine) in the Pigg River from Toshes sample site through Chestnut Hill. These measures are the means throughout the entire study period (2018-2022).

Clearly, additional levels of pollution were evident during storm flow when compared to the disturbance concentrations obtained in the study (Figs. 8 & 9). Yet, evidence could not be presented to determine if the additional pollutant loading occurred outside the stream channel from land runoff or directly from streambank erosion. Evidence did support the idea that legacy pollutants and sediments can create "hot spots" that pollute the river during storm events. It was clear from this experiment that the disturbance of sediment on the stream bed and near the banks was enough to produce significantly higher concentrations of both *E. coli* and nutrients (Figure 8). However, bacterial source tracking data did not provide evidence for the presence of significant quantities of *Bacteroides* bacteria from the host species tested (Fig. 9). Regardless of the source, this experimentation demonstrated the potential for the sediments in the streambed to be significant contributors to pollution loading and the importance of legacy sediments.



Figure 8 – Bacteria (E. coli) and nutrients (Total Phosphorus) measured at multiple locations in a river impacted by legacy sediments during three different water quality scenarios.



Figure 9 – Response of Human and Ruminant BST to the disturbance and stormflow measures.

Discussion

Results from this study yielded insights into the functioning of Pigg River and how contaminates flowing through this system may eventually impact Leesville Lake. First and foremost, the river is very polluted and constantly in violation of *E. coli* standards. This is a cause for concern. The river system has been evaluated under the Total Maximum Daily Load (TMDL) program (Benham et al. 2006) and believed to be improving based on a limited number of samples over a short period of time until the conclusion of the study in 2015. However, this study does not support this conclusion. The river is in violation of *E. coli* standards throughout all scenarios measured in this study. The problem appears to be worsening.

Secondly, pinpointing the sources of this problem are a more challenging endeavor. Certainly, agricultural land use throughout the Pigg River watershed suggest this as a cause (Benham et al. 2006). Our measures of *E. coli* correlations to Bovine markers support this conclusion. At the Colonial Turnpike site (Figure 7), all pollution parameters were elevated and coincidentally so was Bovine markers. This suggests that Bovine land in the watershed is a significant contributor to the problem. How significant or to what extent Bovine pollution may contribute to the E. coli problem is a more difficult task to quantify. Peterson et al. (2018) suggested in watersheds of mixed use that agricultural land generated the greatest *E. coli* concern to receiving waters. Further research found agricultural lands generate an average of 560 CFU per 100 ml while similar mixed land use areas generated 330 CFU per 100 ml and forested land a much lower 206 CFU per 100 ml (Petersen and Hubbart 2020). Our averages of up to 7000 CFU per 100 ml suggest other sources may contribute to this problem.

One aspect of the study revealed that ruminant markers were elevated particularly during storm events and also correlated with the other pollutant parameters (Fig. 4). This measure includes other ruminants such as deer which we suspect are significant contributors to *E. coli*. Therefore, it is difficult to separate what contributions Bovine vs. other ruminants have toward the elevation of E. coli in this watershed. Another aspect was the idea that the legacy sediments contribute a significant amount of *E. coli* and other pollutants during rain events. Our data support this conclusion looking only at *E. coli* or TP but not the BST analysis (Fig 9). This is a result that needs further study.

How this agricultural land is managed may be our greatest concern. Soupir et al. (2006) found bacteria concentrations were highest in runoff samples from plots with cowpies compared to those treated with liquid dairy manure or other litter. Derlet et al. (2012) demonstrated that cattle grazing rather than wildlife contributed most significantly to elevated *E. coli* levels measured in receiving water. Cattle were found to contribute significant quantities of manure in streams and excessive cattle density created violations of *E. coli* standards after rain events (Hansen et al. 2020). Management of cattle on agricultural land may be the decisive management consideration toward lowering of *E. coli* in this watershed and must be strongly considered.

Sedimentation and nutrient enrichment were the other important covariables as well. Increasing *E. coli* is associated with turbidity and TP. This is a very pervasive problem with multiple concerns and origins. *E. coli* has been reported to survive for extensive periods of time in manure-amended soil (Jiang et al. 2002) and may also live as long as a year as viable cells or in a dormant condition (Van Elsas et al. 2011). Once in the stream bed this sediment may accumulate for extended periods of time. Evidence suggests movement of sediment fractions larger than the median size of the bed surface material is rare and occurs only at relatively high flows (Church and Hassan 2002). Sediment may be a continual source of bacterial contamination after a storm event (Mallin et al. 2007; Olds et al. 2018). In this watershed, years of accumulation of contaminated sediment may be severe and pose the greatest health risk (Heise and Förstner 2006). Minimizing the addition of sediment throughout the watershed may be imperative to allow flushing of what is currently present. Until erosion is significantly controlled water quality may not improve and will continue to threaten the health of Leesville Lake.

This conclusion is supported by the data around Chestnut Hill and Colonial portions of the watershed. These are the most contaminated sites on the river and strongly suggest this pollution is being driven by the legacy sediment deposited in that area from the 2017 Power Dam removal. This leads to the idea that time lags and legacy sediments must be considered in the management of our water resources. A body of literature now exists (reviewed by Vero et al. 2017) suggesting that missing water quality reduction targets and resultant dead zones of major managed aquatic ecosystems (such as the Chesapeake Bay) may be from time lags and legacy pollutants flushing from the system. This forces very difficult policy positions as extensive and expensive measures implemented throughout a watershed may have minimal or non-existent results. But this phenomenon may well explain why a greater frequency of storm events accompanies a worsening of water quality. Thus, policy makers need to characterize storms as a cleansing or flushing event rather than a pollution event. Further, the temptation to correlate lower flow years with good water quality needs to be avoided. This may simply represent a greater storage of legacy pollution thus causing more difficult explanation scenarios after heavy rains (Fleming et al. 2019).

Thus, the damage to our streams and rivers may have already occurred. It is incumbent on us to manage these systems allowing them to "recover" and most importantly equilibrate with the watershed they flow through. The constant changing of hydrology by development and land cover changes may be the greatest problem and challenge to improvement of water quality. Globally, streams and rivers need time to equilibrate, flush and mend before we will see significant outcomes from our land management and treatment technologies.

Conclusions and Future Directions

The risk of disease particularly in the upper sections of Leesville Lake generated by Pigg River influx is of ultimate concern. We know reduction in runoff from agricultural land uses along with some lower wildlife contributions is needed to meet reductions necessary for compliance (Coffey et al. 2015). But it must be understood that risks associated with exposure to recreational waters containing fresh cattle feces may not be substantially different from risks associated with exposures to waters impacted by human fecal sources (Soller et al. 2010). While human sourced contamination is most problematic, the overall concentration of contamination may be of greater concern (Stoller et al. 2014). Reductions in all contributed sources of fecal contamination is necessary to provide recreational waters that are safe and enjoyable.

The Pigg River watershed is contaminated throughout the basin. The human sourced contamination in the headwater region is overshadowed by concern over agricultural and wildlife sourced ruminant contamination throughout. Concerns over sedimentation and elevated turbidity associated with both stormwater and bedload bacterial contamination are pervasive and have raised concern into Leesville Lake. This system needs evaluated and managed from the Pigg River Watershed into the upper portions of Leesville Lake.

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