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Masking impact of sediment generated *E. coli*. on evaluation of environmental health

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The Pigg River, located in south central Virginia United States of America, is studied over a 6-year (2018–2023) period to better understand river function and pinpoint sources of pollution. The Pigg River is an important resource in central Virginia as it drains significant agricultural lands providing a water source for cattle and agricultural production. It also drains the City of Rocky Mount where both a municipal wastewater facility and low head hydroelectric dam are located. The obsolete low head dam is removed in 2017 prompting concerns over excessive sedimentation flowing downstream contaminating Leesville Lake that is part of a larger pump-storage hydroelectric project. In response, the Pigg River is monitored along with multiple sediment disturbance experiments at selected sites below Rocky Mount to validate these concerns. The study found areas of greatest pollution accumulation just below Rocky Mount then continuing through an 18–20 km stretch of the river. Using *Escherichia coli* (*E. coli*) as the primary pollution and health concern indicator, along with Microbial Source Tracking (MST) and other molecular techniques, we found that contamination is extremely high during storm events (>10,000 CFU/100 mL) and often remained above instantaneous contamination standards of 220 CFU/100 mL during baseline flow. Ruminants are identified as the primary stormwater source of contamination while human markers predominated during baseline flow. Most significantly, areas beyond the dam removal site are identified as 'hot spots' generating the highest readings of contamination. Without clear association between *E. coli* and MST markers at these hot spots, the study concluded growth and release of *E. coli* from sediments is the significant source of contamination. These sediments, released in 2017 and impounded since 1915, so significantly elevated *E. coli* that it masked any ability to discern health concerns otherwise associated with these markers. Hence, legacy sediments must be seen as a confounder toward the usefulness of *E. coli* as an effective environmental health indicator.

KEYWORDS

dam removal, *E. coli*, environmental health, legacy sediment, river water quality

Introduction

Water quality and environmental health

Characterizing river water quality and correlating it to environmental health can be challenging. We know that bacteria measured as *E. coli* (*Escherichia coli*) and sediment have a very complex and symbiotic relationship (Salam et al., 2021). *E. coli* tends to increase with increasing sedimentation in streams. We also know that in watersheds predominately covered by forest, we find relatively good water quality while in watersheds increasingly

plagued by human alteration such as agriculture or urban development, we find degradation (Baker, 2003; Buck et al., 2004; Tong and Chen, 2002). Sediment that is often laden with bacteria moves from deforested or disturbed areas directly into rivers. Sediment can remain for long periods of time moving slowing though these systems becoming legacy sediments (Fleming et al., 2019; Livers and Snyder, 2025). Models to describe this relationship such as the model of impervious cover (Schueler et al., 2009) or the urban cascade (Taylor and Owens, 2009) characterize this problem associating it with increasing impervious surfaces. These models are useful when correlating urban land use to degrading water quality yet difficult when trying to pinpoint direct sources of health contamination.

To pinpoint pollution, *E. coli* is widely accepted as the standard water health indicator (USEPA, 1986). Yet it lacks many desirable characteristics needed in an ideal indicator organism (Ishii and Sadowsky, 2008). Of particular concern is the ability of *E. coli* to live and reproduce in the environment becoming part of the naturalized microbial community rather than remaining an independent evaluator of pollution (Jang et al., 2017). Compounding this concern is the strong association of *E. coli* with sediment and the propensity to associate with stormwater with excessive suspended sediments (Jeng et al., 2005). Hence, sediments that often coat the bottom of streams and become resuspended during increased flow events naturally increasing the concentration of *E. coli*. This internal source of contamination confounds predictive ability needing greater research to compartmentalize what is naturally occurring and what is indicating environmental health problems.

Land use is another source of pollution. Agricultural land use is loosely associated with declining water quality (Stone et al., 2005; Cuffney et al., 2000). More specifically, agricultural land use is 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 correlates with elevated *E. coli* concentrations (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). Croplands bring 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 add an additional source of contamination (Cox et al., 2005).

Considering the urban environment, pathogens (bacteria, parasites, protozoans, viruses) enter rivers via stormwater, wastewater and overflowing or leaking sanitary sewer systems (Olds et al., 2018). Stormwater over impervious surface is another source that correlates with disease risk (Arnone and Walling, 2007). Excessive water quantity erodes river banks while coating river beds with sediment and organic material 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). *E. coli* survives 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, rivers experiencing erosion and

sedimentation harbor extensive beds of bacteria, potential pathogens and other pollutants that resuspended continually when waters rise from storms.

Further, 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 sediment stored through decades of impoundment. The current trend of dam removal while admirable and with certain positive attributes is also unleashing legacy sediments into river systems (Livers and Snyder, 2025). This sediment is eventually processed, distributed and washed through into bays and oceans. Hence, adding additional sediment stored behind dams to a river system already burdened by sediment and land use that increases erosion severely compounds an already difficult problem (Shahady and Cleary 2020).

Bacterial contamination and health risk

While impact of land use and river contamination can be documented, the translation of bacterial contamination into 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. They estimate 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 healthcare provider) are responsible for over 65% of the economic burden while severe illnesses (resulting in hospitalization or death) are responsible for approximately 8% of the total economic burden.

In response, criteria for contamination levels to minimize disease risk are 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 are studied and quantified. Concentrations greater than the 500 FC per 100 mL are associated with (>10%) potential illness risk.

Translating these established risks into credible regulatory standards produced our current regulatory framework (USEPA, 1986). The United States Clean Water Act calendar month geometric mean standard of 126 *E. coli* (CFU per 100 mL) is suggested to provide a minimal disease risk of less than 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.

While tracking *E. coli* provides indication of the total microbial loading into a water source and the risk for disease, advances in

Microbial Source Tracking (MST) now provides even greater detail into the sources for this contamination. Microbes from the genus *Bacteriodes* are used to pinpoint human and other animal origins of the contamination (Simpson et al., 2002; Job et al., 2023). This technique provides greater specificity when tracking environmental health concerns but is much more expensive potentially limiting its effectiveness as an approach.

Legacy pollution and sediment

The problem of sedimentation has become so pervasive that river flow is always accompanied by some level of turbidity. The removal of dams, alterations to river water courses, fire, flooding, farming, urbanization and deforestation all contribute to continued sedimentation of water resources (James, 2019). Compounding this are alterations in precipitation driven by changes in climate. This creates what many authors have described as a “wicked” problem (Shortle and Horan, 2017). Our freshwater streams and rivers flow through an altered human landscape driven by an intensifying water cycle (Wohl, 2019).

One approach to tackle such problems is an understanding of sources and sinks along with areas of intensification or “hot spots” (Fleming et al., 2019). Once identified, restoration may occur. Because these hot spots and legacy sediments are pervasive in watersheds, the spreading management expenses and fixes throughout a region may not produce any observable improvements other than money spent. Further, measurable results may take upward of 20+ years to be realized or never realized at all due to the slow movement of these sediments (Melland et al., 2018; Livers and Snyder, 2025). The transport longevity of sediments in river systems is poorly understood and studied (Livers and Snyder, 2025).

Legacy pollutants that exist in streambank soils and throughout the stream bed may be the primary polluting material now observed 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 by water movement. Hence, 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 may only be resolved when systems flush existing sediments reaching an equilibrium.

Our investigations were initiated in the summer of 2018 to evaluate water quality conditions in the Pigg River, an important tributary to Leesville Lake. This lake is an important recreational, environmental, and power generation reservoir in Central Virginia. While the initial focus is on water quality, greater focus and scrutiny began in 2019 to determine origins of the bacterial contamination (assessed by *E. coli* concentrations and later MST) 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. During 2021–2023, we experimented with bottom sediment

disturbance and stormwater flow to better quantify sources of bacteria. Throughout the study, the importance of sedimentation from both dam removal and legacy accumulation are evaluated to determine impact on bacterial contamination.

Methods and materials

Study design and rationale

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

In 2018, we conducted a comprehensive sampling of the river establishing stations and analyzing data. In 2019–2020, we continued to monitor the river while adding potential “hot spots” to include Microbial Source Tracking (MST). A hot spot is considered a sampling station where excessive concentrations of *E. coli* are measured using the Colilert methodology. Excessive is considered over 1,000 counts/100 mL during low flow conditions and in excess of 2,400 counts/100 mL during storm conditions. The excessive classification for hot spots is determined through observations of the data collected in 2018 along with statistical analysis examining clusters in the data.

Additionally, in 2019 we sampled during low flow and storm flow conditions. Low flow is defined as conditions with good water clarity (turbidity <1 NTU). Stormwater flow is defined as conditions where greater turbidity (>10 NTU) and appreciable rain (>20 mm) occurring in the watershed within 24 h of sampling. Low flow sampling is designed to pinpoint exact location of bacterial contamination. The second sampling is designed to identify 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 2021–2023 we concentrated efforts on the lower Pigg River trying to quantify the potential areas impacted most significantly by sedimentation and bacteria. We also conducted two separate disturbance experiments to determine the impact of bottom sediments on water quality when disturbed.

Site descriptions and land use impact

The Pigg River, located in south central Virginia, is 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 has 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 et al., 2008; Benham et al., 2006).

Each river segment is characterized using aerial maps and quantification of land use adjacent and directly along the river. Designated land use is characterized where land abutted the river out to 100 m. Using the aerial maps, forest vs. pasture or other use is

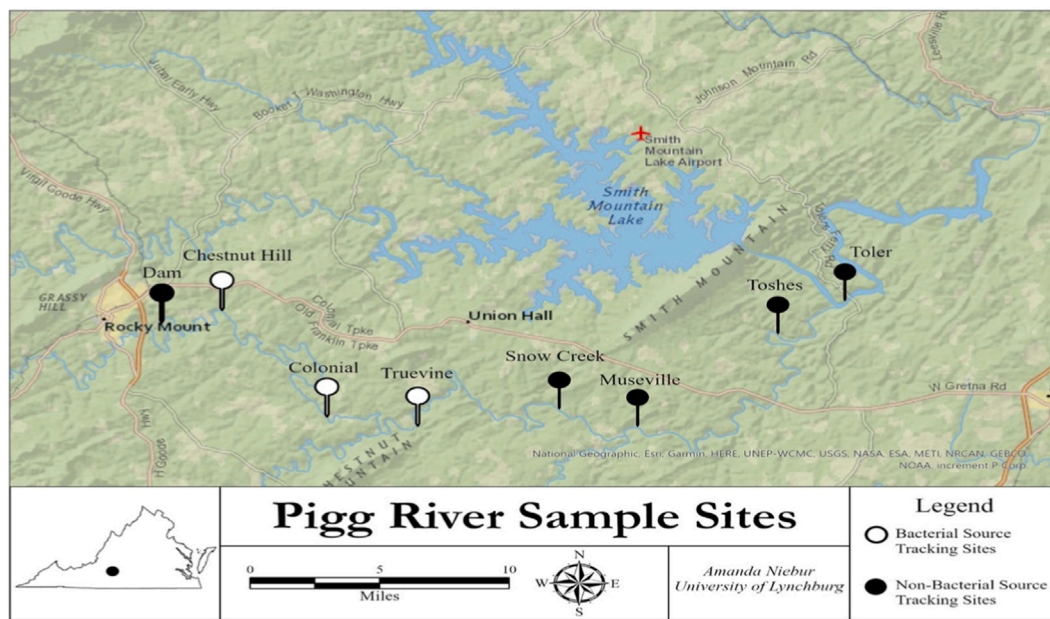


FIGURE 1
Sampling sites on the Pigg River. Samples are collected throughout the watershed beginning on the Lower Pigg just below the City of Rocky Mount and finishing at headwaters of Leesville Lake. White markers denote sites where disturbance experiments are conducted.

easily identified. Each land use is 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.

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") just below the city of Rocky Mount (Figure 1). This site is selected for sampling due to its close proximity to the site of a dam removal project in 2017 (Figure 2) and its accessibility via Power Dam Road. This site directly drains the city of Rocky Mount.

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 7.9 km river segment upstream of the Chestnut Hill Site has been classified as having major impact due

to extensive agricultural use and lack of significant buffer (Table 1). A Power Dam is removed in 2017 above this site.

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 water quality. The 14.9-mile river segment upstream of this site is classified as minimal 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 contributes 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.

TABLE 1 River segments, land use and distance between proposed sampling sites. Sampling sites are determined by highway river crossings.

River segment	Designated land use	River distance (kilometers)
Toshes to toler	Riverine/Limnetic – Minimal impact	6.5
Museville to toshes	Mixed impact/Significant impact	17.5
Snow creek Rd to Museville	Significant impact	5.6
Truevine to snow creek	Significant impact	16.9
Colonial to truevine	Minimal impact	8.2
Chestnut to colonial	Mixed impact	24.0
Power dam to chestnut	Major impact	7.7



FIGURE 2
Site of removal of the Power Dam. This structure is located approximately 8 km miles upstream of the Chestnut Hill Site.

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 5.6 km 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 17.5 km 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 5.6 km upstream segment is determined to have low impact (Table 1). This site has road access closest to the mouth of the Pigg River and is utilized to estimate the quality of Pigg River water entering Leesville Lake.

Water sampling

Water is 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. After collection, water for laboratory analysis is immediately transferred

TABLE 2 Calculated Geometric Means and Statistical Threshold Values for discrete sampling events over the study period (2018–2023) reported as (CFU/100 mL). Each sampling event (1–12) consisted of a series of samples throughout the length of the river during various flow regimes taken within a 90-day period in the river with number of samples shown in the table for each event. Virginia Standards for recreational waters 9VAC25-260-170 set standards for *E. coli* bacteria GM at 126 counts/100 mL 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	12
Number of samples (N)	7	7	7	7	5	15	24	11	11	8	8	12
Geometric mean (GM)	1,086	328	461	1,040	755	441	185	1,070	175	791	16,851	153
Statistical threshold value (STV)	2,442	1,387	689	2,231	2,230	5,703	373	5,821	275	1,202	24,580	289

to acid washed bottles (nutrients) and sterilized bacteriological bottles and stored in a cooler until analysis in the laboratory. *E. coli* samples are immediately processed upon return to the laboratory while nutrient samples remained refrigerated until analysis within 30 days of collection. Remaining water is analyzed using a YSI multiprobe and Turner Turbidimeter.

Water quality data is 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* is 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 are 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).

For the disturbance experiments, stream segments believed to be impacted by the dam removal and laden with sediments are studied under 3 types of flow conditions; baseline, disturbed bottom and stormwater. Under baseline flow, water samples are collected directly from the stream surface under low flow conditions avoiding any sediment. After the baseline collection, several individuals are deployed above this same sample location and began kicking sediment dislodging it creating flow conditions similar to stormwater flow until turbidity is greater than 50 NTU. Samples are then collected with similar methodology as baseline flow yet these samples contained the bottom disturbed sediment flowing in the river. Stormwater samples are collected during a defined bankfull (Rosgen, 1996) storm event.

Microbial Source Tracking (MST)

Water for bacterial source tracking is collected into sterile bottles and shipped overnight in cooled ice chests for analysis (Source Molecular Corporation, Miami Lakes, FL). There, water samples are filtered through 0.45 micron membrane filter(s) then placed in a separate, sterile 2 mL disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1 min 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 are run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20 μ L sample extract, forward primer, reverse primer, probe and an optimized buffer. Primers employed are for generation of amplicons for human, ruminant and cattle markers. All assays are run in duplicate. Quantification is 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, are run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Standards for bacterial water quality

The code of Virginia (promulgated through EPA and the Clean Water Act), 9VAC25-260-170 outlines the following standards for the waters of Virginia. Bacteria criteria (counts/100 mL) 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/100 mL 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.

Statistical analysis

The general trends observed in the study are 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 is sampled three times each year (2018–2020) and twice per year (2021–2023) primarily late summer to early fall.

TABLE 3 Results from the MST analysis for each of 5 years where this type of analysis is conducted. All data in this table represents the means \pm Standard Deviations of stations where MST is measured in a particular year. Although the data are not rigorously validated for this purpose, ratios of genetic marker copy numbers presented here are used to estimate the relative contribution of various animal hosts to the bacterial load measured as *E. coli*.

Parameter	2019 (n = 16)	2020 (n = 6)	2021 (n = 8)	2022 (n = 10)	2023 (n = 10)
<i>E. coli</i> (CFU/100 mL)	3,207 \pm 5,109	252 \pm 117	486 \pm 466	20,467 \pm 2,367	172 \pm 85.3
Human (copies/100 mL)	1,378 \pm 2,810	252 \pm 258	183 \pm 104	1,647 \pm 1,614	567 \pm 365
Ruminant (copies/100 mL)	42,265 \pm 66,491	6,380 \pm 6,642	1,021 \pm 1787	523,000 \pm 459,213	36.5 \pm 1.5
Bovine (copies/100 mL)	2005 \pm 1,266	11.1 \pm 22.3	1,378 \pm 1908	2,914 \pm 2,471	34 \pm 0

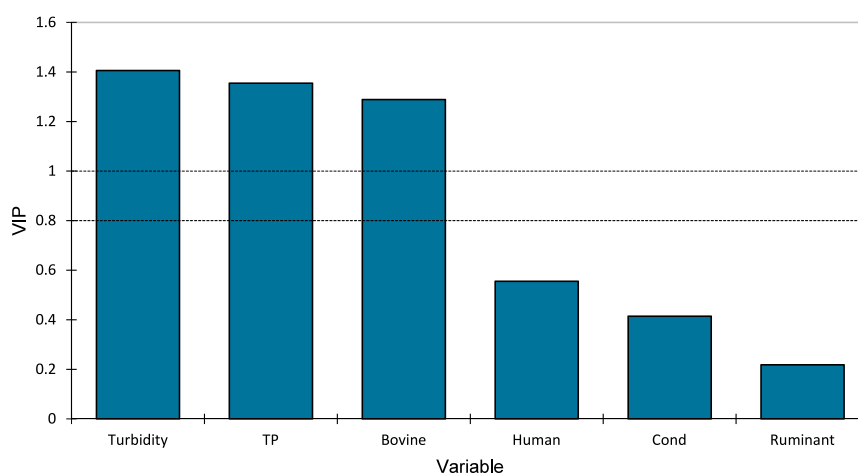


FIGURE 3 PLS-VIP analysis correlating various parameters with *E. coli*. A value of 1 is used in this analysis for a significant relationship to the parameter. Abbreviations of parameter are similar to those in the PCA analysis.

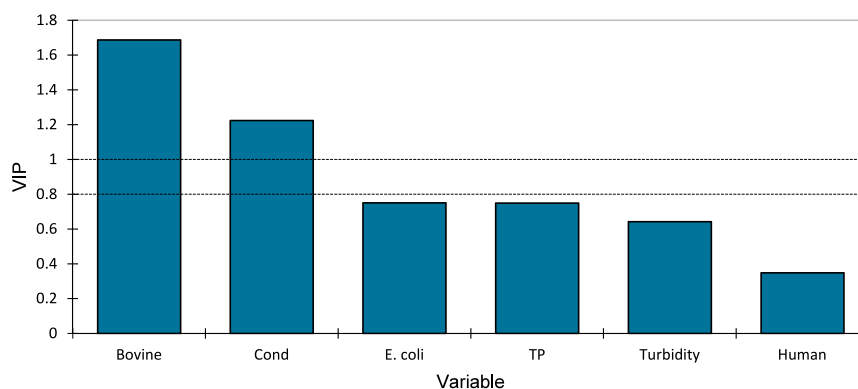


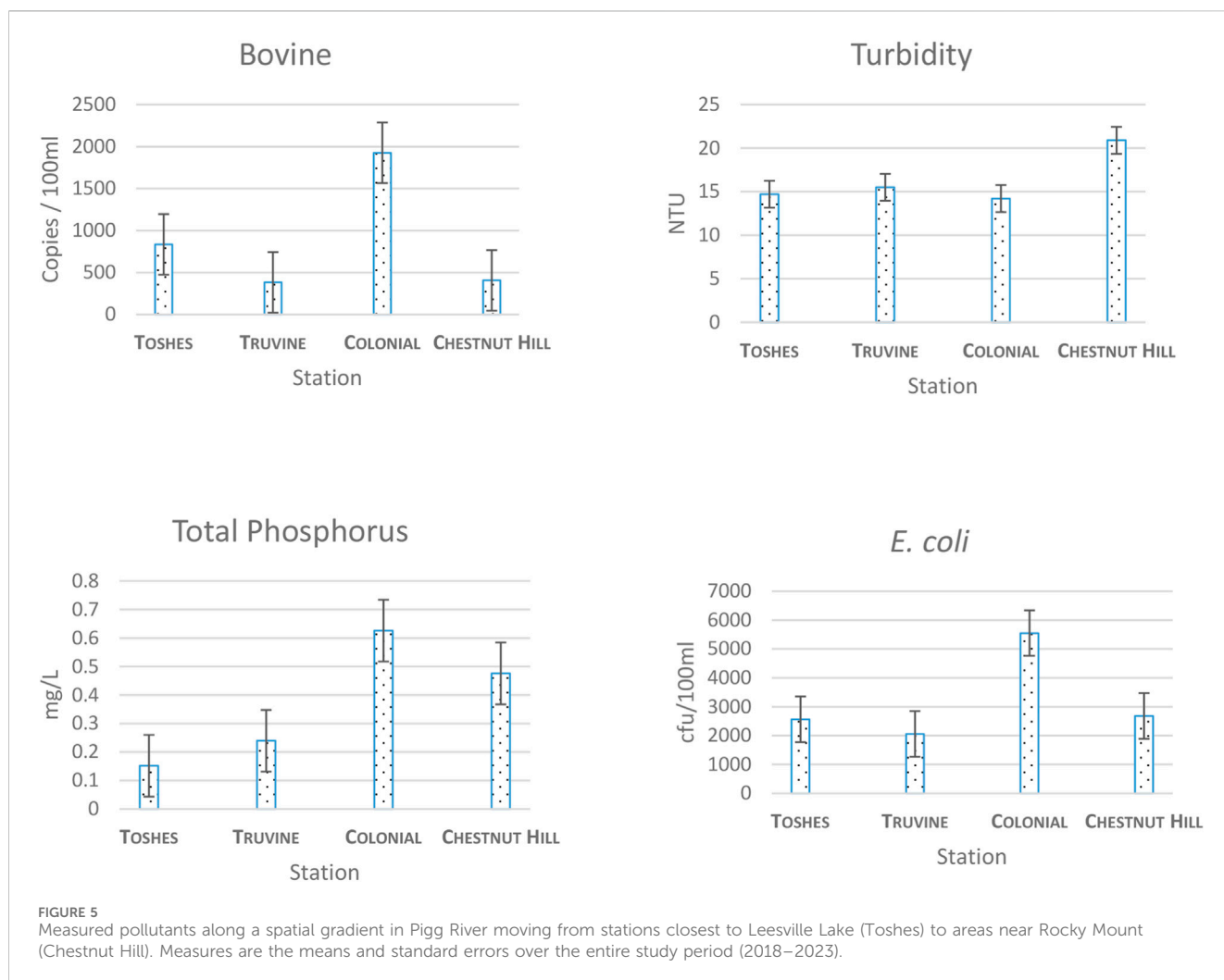
FIGURE 4 PLS-VIP analysis for ruminant MST concentrations. Descriptions the same as in Figure 3.

Two types of regression analysis are used to create a best fit model to determine predictors for all of the data collected. A partial least squares regression analysis examined the best fit or best predictors for *E. coli* and ruminant markers based upon all collected parameters. From these findings, two linear regressions are created from identified parameters as the final statistical tool to model relationships in the watershed.

Results

Bacterial pollution

It is clear from the collected data that the Pigg River is significantly impaired (Table 3). Our bacteriological results suggest the river continually contains concentrations of



E. coli in exceedance of 9VAC25-260-170 throughout the 6-year study period. During each sampling event, the Geometric Mean (GM) exceeded the 126 CFU/100 mL standard for a violation rate of 100%. Violation of the STV standard of 410/100 mL occurred at a rate of 75% in our study where the standard calls for 10% or less. 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.

To examine possible host sources for bacteria, we analyzed genetic markers contained in *Bacteroides* (Table 3). We found a majority of *Bacteroides* bacteria found across all sites are ruminant in origin and the predominant source most notably storm events. Bovine and human markers are variable but became a greater proportion of the samples when *E. coli* measures are lower at base flow conditions. Looking all data over all years, bacterial increases are considerable during storm events and predominantly from ruminant sources.

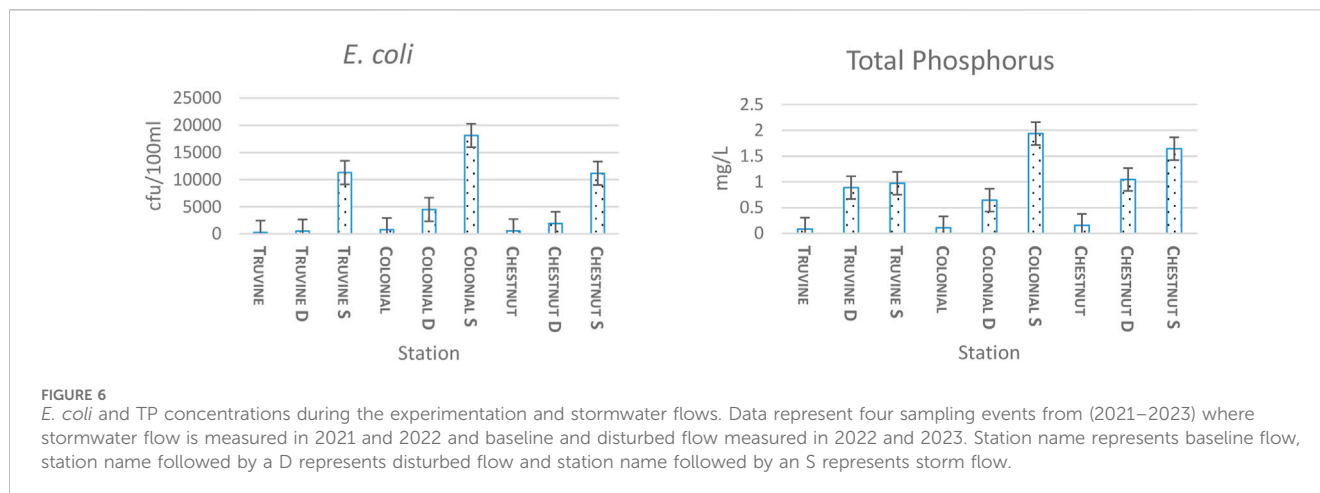
Relationships to pollution

To determine how MST measures are associated with other water quality parameters, both *E. coli* and the ruminant

concentrations are correlated with all of the other parameters (Figures 3, 4). This analysis correlates all parameters to one selected variable to determine how each is related to the other variables. 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 analysis using *E. coli* suggested turbidity, TP and Bovine MST are significantly correlated to the concentrations of *E. coli* (Figure 3). This supports the idea that stormwater runoff is a significance source of the *E. coli* found in the river. While turbidity and TP may have multiple origins in this watershed, bovine markers are specific to agriculture production and by association from this analysis suggestive of impact on *E. coli* measured. What is not understood from this analysis is the low correlation with ruminant markers. These markers while significantly increasing in abundance during storm event are not associated with other parameters such as turbidity and TP.

To further explore what may be generating the high ruminant markers during storm events, a PLS-VIP analysis is conducted comparing ruminant measures with the other parameters. Results suggested that Bovine MST concentrations and conductivity are the



only related parameters (Figure 4). As Bovine MST concentrations are a subset of the ruminant MST, and conductivity unrelated to the other parameters, it is interpreted that ruminant MST concentrations did not provide any interpretive value at least for a specific source in the analysis. This measure appears to be too generalized to pinpoint a source of bacterial contamination.

Spatial relationship with sediment and land use

To determine the importance of land use throughout the watershed and interpreting the potential impacts outlined in Table 1, significant parameters identified in PLS-VIP are averaged and plotted along an axis from Toshes Road to Chestnut Hill (Figure 5). These stations begin closest to Leesville Lake (Toshes Road) up to Rocky Mount (Chestnut Hill). This analysis strongly suggests that greater concentrations of contamination occur in the upper portions of the river near Rocky Mount, 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 among those sampled yet all other parameters did suggest contamination is greater in this section of the river.

The concentration of pollutants at the Colonial station is suggestive of internal loading in the river exacerbating observed conditions. Chestnut to Colonial is of mixed impact based on land use (Table 1) and nothing in the surrounding area is suggestive of increased pollution when compared to any other area in our analysis. To further understand this idea, we conducted the disturbance experiments. This hypothesis tested water in the river at baseline flow and then after it is manually disturbed to mimic stormwater conditions. Additionally, stormwater (precipitation event that elevated river flow and turbidity) is also measured.

The disturbance experiments supported the hypothesis that bedload or legacy sediments that coat the bottom of the river carry a pollutant load that when disturbed change water quality (Figure 6). In all instances, we increased levels of measured pollutants represented by TP and *E. coli* when we disturbed the sediment. This suggests a flow event that disturbs the bottom

sediments adds pollutants to the water irrespective of surrounding land use or incoming water flow. Additionally, it is apparent that surrounding land use adds even greater pollutant loads beyond what we could simulate with our disturbance experiments. While bedload sediment adds pollution through river flow and mechanical disturbance, overland flow and streambank disturbance are important contributors as well as seen during storm events.

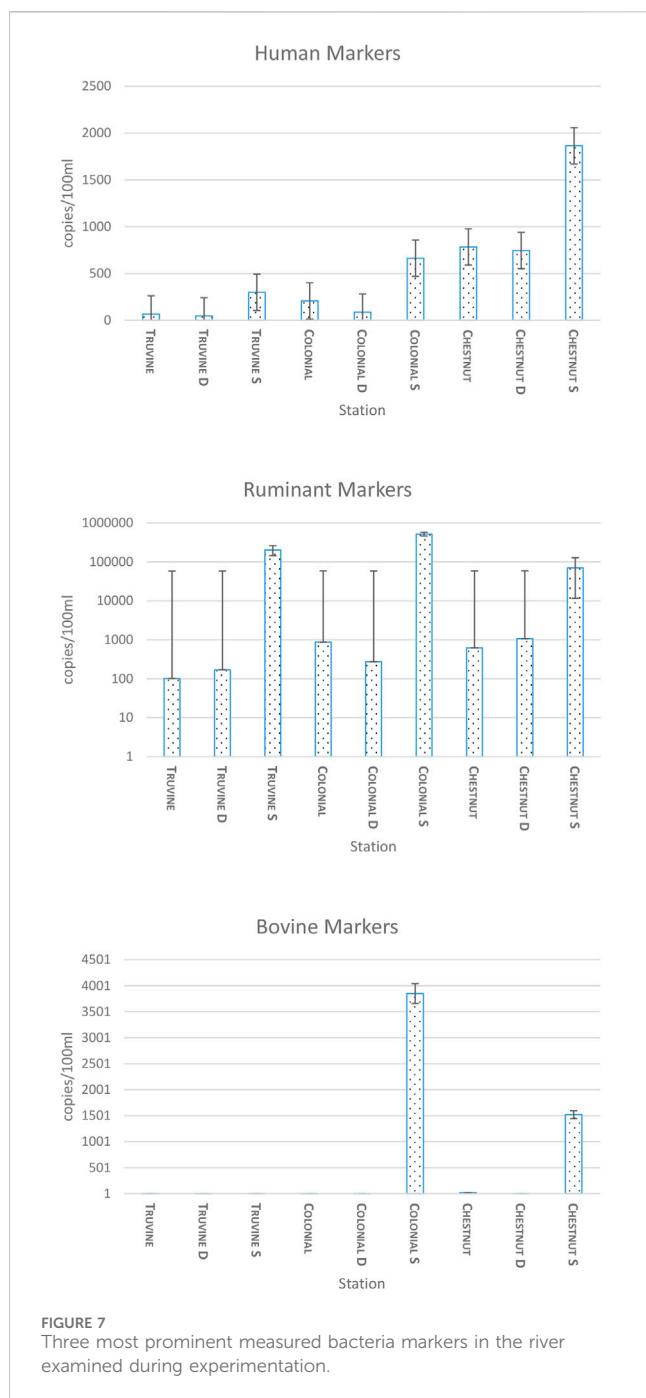
Additional evidence supports the idea that legacy pollutants and sediments can create “hot spots” that pollute the river at greater rates during storm events. It is clear from this experiment that the disturbance of sediment on the stream bed produced higher concentrations of *E. coli* at the Colonial site during both years we conducted the experiment (Figure 6). Expectations based on land use suggest Chestnut Hill should be the most impacted site. The removal of the Power Dam and subsequent sedimentation in this section of the river is the suspected source of this contamination due to river flow and deposit of those sediments.

Bacteriological characterization

The bacterial characterization of samples from the experiments demonstrated stark differences among identified markers (Figure 7). Human, ruminant and bovine markers are found in all of the samples. Human markers are found in every sample and are in greatest concentration at Chestnut Hill site. This is consistent with land use and it is likely from the houses that line the river and wastewater discharge from the treatment facility for the city of Rocky Mount.

Ruminant markers dominated observations with greatest concentrations in the Colonial area. As we could not get an increase in these ruminant markers when we disturbed the sediment yet stormwater flow caused significant increases the observation suggests this marker is watershed driven and from storm events. Bovine markers are only found in much lower concentrations and only during storm events at both Colonial and Chestnut sites. This suggest this measure is of minimal importance only occurring in isolated areas and during rain events.

Further, greatest observations for ruminant and bovine markers during storm events occurred at the Colonial site. As we could not



stimulate any of the markers in our disturbance experiments, it is assumed markers are derived from external flow. Land use not identified in our study may be the source at the Colonial site. But, we did see *E. coli* increases in our experimentation (Figure 6) consistent with the idea that internal loading is causing increases in this parameter and other pollution parameters. This suggests, that bedload sediment driven by various flow rates in rivers may drive concentrations of *E. coli* in river water a majority of the time. And the greater the concentration in the bed-load sediments the higher the elevation in overlying water. This seems to exist during most flows with the exception of storm events that exceed 1–2 years events.

Discussion

Results from this study yielded insights into the functioning of Pigg River and how pollutants flowing through this system may eventually impact water quality and health. First and foremost, the river is very polluted and constantly in violation of *E. coli* standards (Table 2). Only during baseline flow do river *E. coli* concentrations fall below violation standards yet this result is variable. To the contrary, storm flow creates highly contaminated conditions from *E. coli* and sediment. 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, the current study does not support this conclusion. The river is in violation of *E. coli* standards consistently in our sampling and particularly during increased flow events.

Secondly, pinpointing the sources of *E. coli* contamination is challenging. Certainly, agricultural land use throughout the Pigg River watershed is of concern (Benham et al., 2006). Our measures of bovine markers support this conclusion in some instances (Table 3). At the Colonial Turnpike site (Figure 5), all pollution parameters are elevated and coincidentally so are bovine markers. This suggests that Bovine land use in the watershed is potentially a significant contributor to the problem. However, the extent bovine pollution contributes to the overall *E. coli* problem is not clear as it is a minor component of storm flow and is generally not present at baseline and in our disturbance experiments. Peterson et al. (2018) suggested in watersheds of mixed use that agricultural land generated the greatest *E. coli* concern to receiving waters. Research suggests 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 are well beyond those estimates and up to 7000 CFU per 100 mL suggesting much greater contributors exist in this watershed other than agriculture. In fact, the disturbance of sediment generated much greater *E. coli* to overlying water than what the literature suggests agriculture adds.

One key to understanding such a high impact in this watershed is the elevated concentration of ruminant markers particularly during storm events and in the sediment (Figure 7). Ruminants includes many other species of wildlife including deer which we suspect are significant contributors to the *E. coli* pollution load we are measuring. Bovine markers, while also a ruminant, are a minor contributor to total ruminant markers during storm events. Another aspect is the idea that the legacy sediments contribute a significant amount of *E. coli* and other pollutants during rain events. Byappanahalli et al. (2003) found that *E. coli* not only survives in sediment but also increases in abundance. Elevated *E. coli* may be an excellent surrogate for sedimentation in these watersheds rather than an indicator of significant health risk. While wildlife stormwater contributions appear to be the significant contributor of *E. coli* into the river system it may be the seed source replenishing sediment allowing it to flourish then become resuspended during storm events.

The inability of the basic *E. coli* test to differentiate among sources of bacteria is a concern. Even using *Bacteroides* to pinpoint

sources demonstrates these are only indicator microorganisms (IMs) and as such suggest *potential* health concern when elevated (Saxena et al., 2015). We could not find specific correlation to bacteria markers in the study to associate with potential disease and as such believe these IMs may be widespread throughout the watershed and pervasive throughout the legacy sediments. Bohrerova et al. (2017) found a similar result after low head dam removal with spikes in nutrients, turbidity and *E. coli* downstream attributed to the sediment release. And movement of this material downstream may be slow and only occur during very high flooding events (Church and Hassan, 2002). Because fine sediment that often harbor nutrients and bacteria are entrained with larger bed material in the stream, bulk movement only occurs when sufficient critical force from flooding is applied. It is for this reason we believe the Colonial portion of the stream has the highest pollutant measures and that this will move down stream slowly until all of the sediment released from Power Dam is flushed through the system which may take decades. The idea that legacy sediments are very slow moving and often remain in the river system for extended periods of time following dam removal is supported in the literature (Livers and Snyder, 2025).

Thus, the existence of sediment throughout the beds of rivers in these basins may be the most pervasive problem for water quality. This along with stream banks erosion due to inadequate stabilization with excessive flow of debris worsening the problem by creating log jams forcing water flow diversion and even greater rates of erosion. This belief does not agree with the prevailing idea that agricultural land management is the key to lowering *E. coli* to improve water quality (Soupir et al., 2006; Derlet et al., 2012; Hansen et al., 2020) where many studies found bacteria concentrations are highest in runoff samples from farmland from manure in streams and excessive cattle density. Conversely, we see *E. coli* concentrations at such high levels as a surrogate indicator of pollution rather than an indicator of health concerns.

The strongest evidence to support the idea that *E. coli* contamination is driven by sediment rather than other factors can be found in our disturbance experiments. We are able to measure considerable *E. coli* in the water when we disturbed the sediment and in particular at Chestnut and Colonial Turnpike sites closest to the power dam removal (Figure 6). Those same experiments did not produce increases in human, ruminant or bovine markers (Figure 7). This produced the conclusion that *E. coli* is prevalent throughout river sediment, is likely increasing in abundance in the sediment as no measurable genetic marker identified a source and this increase “masks” any ability to measure true environmental health contamination.

This does not suggest agricultural pollution is not a problem in the watershed just that *E. coli* as a health risk indicator in the Pigg River is a better indicator of pollution. *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 a significant 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.

Confounding this entire study is the removal of Power Dam in 2017 without control for sedimentation. Built in 1915, the release of a 102-year legacy of sedimentation uncontrolled to fill coating the entire Pigg River system with *E. coli* laden sediment masks our ability to discern potential health risks in the watershed. Not only will it likely take decades for this sediment to flush through the system (Pizzuto, 2023), it provides a perfect incubator for *E. coli* growth and ample nutrient supply for this problem to continue. These time lags and legacy sediments must be considered in the forefront for the management of this resource. A body of literature now exists (reviewed by Vero et al., 2018) 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, policymakers 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).

Conclusion

The damage to the Pigg River may have already occurred and what this study documented is part of a recovery process. All of the work on the TMDL along with best management practices (BMPs) will likely not be realized until this problem equilibrates. A sudden release of accumulated sediment behind a dam therefore compounds the problem and can be catastrophic to a river (Stanley and Doyle, 2003; Livers and Snyder, 2025) and is best understood as experimental and a gamble rather than sound environmental planning (Wohl et al., 2015). These sediments mask the otherwise necessary understanding of the health risk and even further complicated regulatory efforts to improve water quality such as the TMDL process. It is incumbent on us to manage these systems allowing them to recover first and most importantly equilibrate with the watershed then apply management techniques. Globally, streams and rivers need time to equilibrate, flush and mend before we see significant outcomes from our land management and treatment technologies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

TS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

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