Bugs and bacteria: a survey of water quality and ecosystem health in Rockbridge County

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<u>Abstract</u>

The Maury River and its tributaries comprise an understudied system within the Chesapeake Bay Watershed that is affected heavily by human disturbance. Water and sediment nitrate and *E. coli* concentrations were used in conjunction with the Virginia Save Our Streams benthic macroinvertebrate sampling method to assess the health of several waterways within Rockbridge County, Virginia, within which the Maury River is fully enclosed. Macroinvertebrate data did not correlate with nitrate or *E. coli* concentration but does indicate that the benthic macroinvertebrate community shows some resilience to disturbance. Nitrate and *E. coli* concentrations indicate that agricultural and recreational activities may be having an adverse effect on waterways.

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Introduction

The Chesapeake Bay Watershed is an incredibly important and vulnerable ecosystem, containing hundreds of waterways that drain five physiographic regions (Jantz et al. 2005). It is the nation's largest estuary and home to 348 species of finned fish and 173 species of shellfish (NOAA Fisheries), making the watershed a site of great economic and ecological importance. Urbanization and agriculture threaten the health of the various waterways within the watershed and by extension the animal and plant life that depend on its streams and rivers. Therefore, studies of the health of these waterways are of vital importance.

The Maury River is a part of the Chesapeake Bay Watershed and is contained entirely within Rockbridge County, VA (Virginia Department of Wildlife Resources). Agriculture is the main anthropogenic disturbance of the river and its tributaries, as livestock ranching is especially common in Rockbridge County (USDA 2020). Agricultural disturbances can lead to increased nitrogen in waterways, potentially leading to eutrophication (Romanelli et al. 2020). Other human disturbances to waterways in Rockbridge County may arise from recreational use, such as swimming or bathing. These disturbances raise concerns about the ecological health of these waterways, especially as little prior research has been done on the Maury River specifically. Given the Chesapeake Bay's economic and ecological importance, an understanding of the health of its waterways, including the Maury, is vital to its preservation.

The goal of this study is to examine the health of Rockbridge County's major waterway, the Maury River. We assessed the presence of *E. coli* and the concentration of nitrate in various waterways, both of which are generally accepted indicators of water quality (Arimoro et al., 2015, Jang et al., 2017). While there is some concern about environmental *E. coli* strains that may have adapted to life outside of the GI tract confounding the bacteria's usefulness as an indicator of water quality (Jang et al., 2017), it may remain useful for understanding relative amounts of contamination among sites and systems. Nitrate concentration is an especially important indicator because of the potential of eutrophication of waterways caused by agricultural runoff (Romanelli et al., 2020). We also used benthic macroinvertebrate diversity as a measure of ecosystem health, both as an important metric in its own right and because this diversity can be threatened by poor water quality as measured by nitrate concentration (Arimoro et al., 2015).

Site Descriptions

This survey focuses on five sites with varying degrees of known disturbance. The Chessie Nature Trail is a walking and biking trail which runs directly beside and parallel to the Maury River. The Chessie site is therefore susceptible to damage from human and pet waste, as well as to potential washout from nearby roadways. Ben Salem Wayside is a picnic ground and wading area where fishing and bathing regularly occur. The Woods Creek site lies along the Woods Creek Trail, another popular walking trail prone to disturbance by humans and their pets. Additionally, the Woods Creek sampling site has in the recent past been the focus of extensive macroinvertebrate sampling by Washington and Lee University's BIOL-113 laboratory course. This degree of interference has potential negative consequences for macroinvertebrate quantity and diversity. The two remaining sites are split into two sample locations each. The Jordan's Point upstream and downstream sites lie within Jordan's Point Park, the location of a large dam whose predecessors date to the the 19th century (*After Dam Removal, Archaeology at Jordan's Point, Maury River, Lexington*, n.d.). This dam was removed in 2019, leaving open the possibility of negative ecological effects downstream of the dam's former site. Thus, samples were taken both upstream and downstream of the former dam. The Hays Creek site lies within an extensive cattle pasture, with part of the waterway exclosed from cattle. Samples were taken in both this exclosed section and in the section the cattle could access.

Materials and Methods

Site Selection

Sites were selected based on knowledge of nearby potential anthropogenic stressors and contaminants and on proximity to Washington and Lee University.

Benthic Macroinvertebrate Sampling

Samples were collected in riffle regions of all sites and scored according to the Virginia Save Our Streams method. Three nets were taken at each site and all macroinvertebrates could be identified to the specificity of the scoring sheet for this method.

Collection and Analysis of Water and Sediment Samples

Water samples were collected by filling centrifuge tubes with surface water from each site. Sediment samples were obtained by collecting approximately 5 mL of sediment and allowing the centrifuge tube to fill with water while holding the centrifuge tube near the sediment collection site. Three water samples and three sediment samples were collected for each site. Samples were promptly transported from the sample site and refrigerated until they could be analyzed,

All analysis of samples took place within twenty-four hours of sample retrieval. The nitrate concentration in each sample was measured via the CHEMetrics Nitrate Vacu-vials® Kit according to the manufacturer's methods. *E. coli* and coliform counts were obtained via Micrology Laboratories' Coliscan® Easygel® procedure. 1 mL of each sample was mixed with Coliscan® medium and incubated in manufacturer-supplied treated petri dishes for 24 hours at 35℃. Coliform and *E. coli* colonies were manually differentiated and counted.

Statistical Analysis

Linear regression, univariate ANOVAs and post-hoc Tukey's honest significance test were performed using R version 4.1.2. Student's two-tailed unpaired t-tests assuming unequal variance were performed in Microsoft Excel. Significance was accepted at $p < 0.05$ for all data.

Results

Save Our Streams Multimetric Index Scores all ranged from 7-11, with Jordan's Point Upstream being the only site designated as having unacceptable ecological condition by the metric (Table 1). Two sites, Woods Creek and Chessie, scored at 8 and could not be determined to have either acceptable or unacceptable ecological conditions. The remaining sites scored at 11 and are thus designated as having acceptable ecological condition. Index score did not significantly correlate to mean water nitrate concentration ($b = 8.15$, $p = 0.310$), mean sediment nitrate concentration ($b = -2.77$, $p = 0.4344$), mean water *E. coli* colonies ($b = 0.00423$, $p = 0.7309$), or mean sediment *E. coli* colonies ($b = -0.009527$, $p = 0.7309$).

While no significant difference was found between sediment and water nitrate samples as a whole ($t = 1.297$, $p = 0.205$), significant site differences in nitrate concentrations were found for both water ($F = 28.05$, $p = 4.93 * 10^{-7}$) and sediment ($F = 11.4$, $p = 0.000107$) samples (Figure 1). Results of the Tukey's HSD post-hoc analysis are recorded in Table 2 and Table 3.

Summer water *E. coli* concentrations were significantly lower than summer sediment *E. coli* concentrations (t = 3.35441, p = 0.00333). Fall water *E. coli* concentrations were also significantly lower than fall sediment *E. coli* concentrations (t = 3.41856, p = 0.00352) (Figure 3). Summer samples had significantly greater concentrations of *E. coli* than fall samples (t = 2.824192, p = 0.007219) (Figure 2). Site-based differences were detected only for summer water samples ($F = 13.89$, $p = 3.5 * 10^{-5}$) (Figure 4). Results of the Tukey's HSD post-hoc analysis are recorded in Table 4.

Discussion and Conclusion

No correlation was found between Save Our Streams Multimetric Index Score and nitrate or *E. coli* concentration, indicating the possibility that index score is simply unrelated to these factors. Surprisingly, the Ben Salem and both the Hays Creek sampling sites scored 11 out of a max score of 12, indicating sound ecological condition despite the sites' clear known disturbances. This further implies that agricultural and recreational disturbance may not be severely negatively impacting benthic ecosystems. Still, the index score upstream of the former Jordan's Point Dam indicates unacceptable ecological condition, while the score downstream of the former dam indicates acceptable condition. In this most severe case of disturbance, there seems to be lasting ecological harm, indicating that there may be some level of disturbance which the benthic macroinvertebrate community cannot tolerate.

No significant difference was found between sediment and water nitrate concentration. Fewer sites significantly differed from other site in their sediment nitrate concentrations than in their water nitrate concentrations. In fact, all significant differences in sediment nitrate were between Woods Creek and all other sites, as the concentration of nitrate in sediment at Woods Creek was much higher than at any other sampling site. It is unclear why the Woods Creek value is so high, especially given that Woods Creek does not hold the highest concentration of nitrate in water.

The Hays Creek Exclosed and Hays Creek Unexclosed sampling sites were not found to have significantly different water nitrate concentrations, indicating dispersal of nitrate between the exclosed and unexclosed sections. Interestingly, Ben Salem does not show a significantly different mean water nitrate concentration than either Chessie or the Jordan's Point sites, despite the proximity of livestock to the Ben Salem sampling site. This may be explained by the section of the waterway being directly exposed to livestock being farther away from the Ben Salem site than the Hays Creek Unexclosed site, hence the dispersal of nitrate being detectable at Hays Creek but not at Ben Salem. Woods Creek's water nitrate concentration was found to be significantly higher than all sites except the Hays Creek sites, possibly due to nearby heavy trail use and university functions. The Hays Creek sites were found to have higher water nitrate concentrations than all other sites, with the exception of no significant difference being found between Woods Creek and Hays Creek Unexclosed. This indicates the impact of agricultural activity on Hays Creek and raises concerns about eutrophication.

Overall, *E. coli* had a much greater presence in sediment than in water. This corroborates earlier research indicating that *E. coli* is adapting to life outside of the gastrointestinal tract, and raises concerns about the long-term ramifications of fecal contamination of waterways. Significantly less *E. coli* was also detected in the fall than in the summer, possibly because *E. coli* cannot tolerate the cooler fall water temperatures (Ferrer et al., 2003). It may be that *E. coli*'s preference for warmer temperatures partially explains why site-based differences in concentration were only detected in summer water samples. It does not seem prudent, given the high degree of variance within the data, to draw any certain conclusions about the relative presence of *E. coli* within each site. Still, some general comparisons may be made. Hays Creek and Woods Creek emerge as the sites with the most colonies per 100 mL, with Hays Creek Unexclosed having significantly more colonies than every site other than Woods Creek and Hays Creek Exclosed. This likely reflects the very close proximity of cattle grazing and its negative effects on water quality. Significantly more *E. coli* colonies were detected at Woods Creek than at every other site excepting the Hays Creek sites, again potentially reflecting heavy trail use and the effects of university function.

It is clear that anthropogenic disturbance has adversely affected the Maury River and its tributaries as measured by the commonly accepted indicators of nitrate and *E. coli* concentration. There is some cause for concern about the potential dispersal of nitrate and other compounds from their immediate point of waterway entry to locations further away, as well as concern about the ability of *E. coli* to survive in stream sediments. While the apparent resilience of benthic macroinvertebrate communities to disturbance is reassuring, monitoring should continue to assure their safety as well as identify any causal links between specific disturbances and a decline in community diversity and health.

Figures

Table 1. Save Our Streams Multimetric Index Scores calculated from summer macroinvertebrate data.

Site	Multimetric Index Score
Woods Creek	8
Chessie	8
Hays Creek Unexclosed	11
Hays Creek Exclosed	11
Ben Salem	11
Jordan's Point US	7
Jordan's Point DS	11

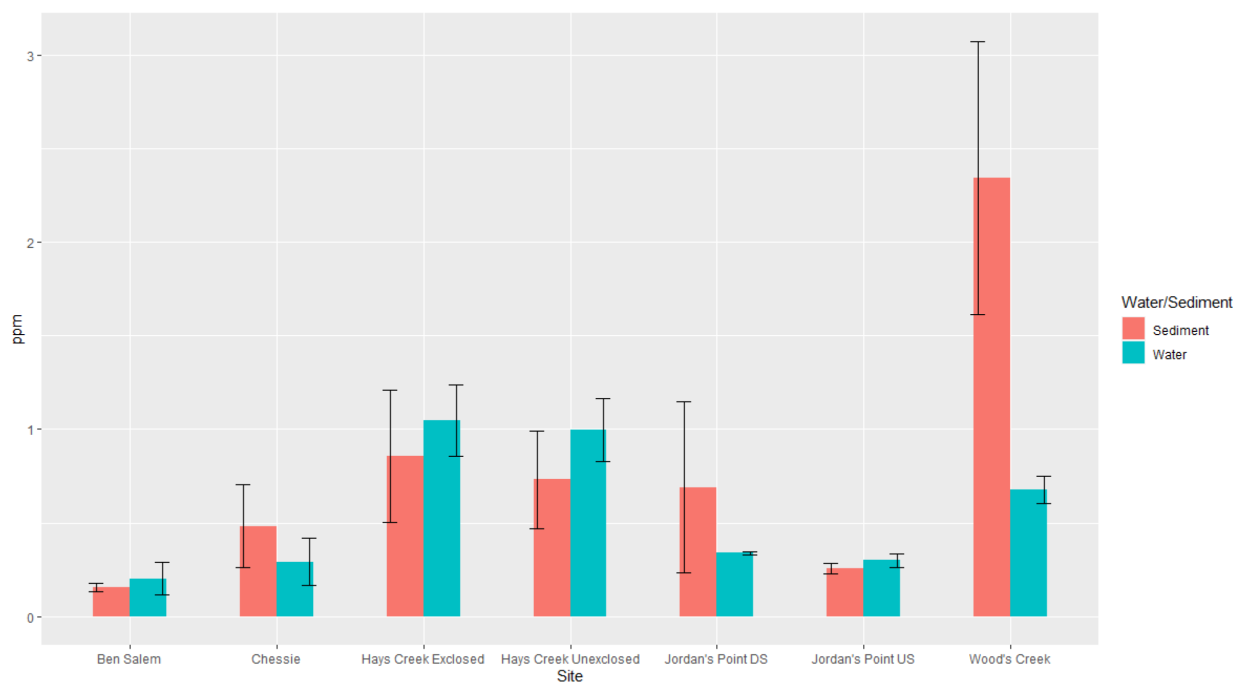

Figure 1. Mean nitrate concentration in ppm in sediment and water samples for each sample site.

Table 2. Results of post hoc Tukey's HSD tests on differences in sediment nitrate between sites.

Sites Compared	p
Chessie-Ben Salem	0.92923
Hays Creek Exclosed-Ben Salem	0.315854
Hays Creek Unexclosed-Ben Salem	0.524206
Jordan's Point DS-Ben Salem	0.602733
Jordan's Point US-Ben Salem	0.999875
Woods Creek-Ben Salem	8.12E-05*
Hays Creek Exclosed-Chessie	0.875059
Hays Creek Unexclosed - Chessie	0.979413
Jordan's Point DS-Chessie	0.991832
Jordan's Point US-Chessie	0.9871
Woods Creek-Chessie	0.000446*
Hays Creek Unexclosed-Hays Creek Exclosed	0.999514
Jordan's Point DS-Hays Creek Exclosed	0.997563
Jordan's Point US-Hays Creek Exclosed	0.476232
Woods Creek-Hays Creek Exclosed	0.0037*
Jordan's Point DS-Hays Creek Unexclosed	0.999999
Jordan's Point US-Hays Creek Unexclosed	0.710021
Woods Creek-Hays Creek Unexclosed	0.001799*
Jordan's Point US-Jordan's Point DS	0.783187
Woods Creek-Jordan's Point DS	0.001419*
Woods Creek-Jordan's Point US	0.000134*

Table 3. Results of post hoc Tukey's HSD tests on differences in water nitrate between sites.

Sites Compared	p
Chessie-Ben Salem	0.9578993
Hays Creek Exclosed-Ben Salem	0.0000064*
Hays Creek Unexclosed-Ben Salem	0.0000135*
Jordan's Point DS-Ben Salem	0.7781931
Jordan's Point US-Ben Salem	0.9416379
Woods Creek-Ben Salem	0.0027631*
Hays Creek Exclosed-Chessie	0.0000238*
Hays Creek Unexclosed - Chessie	0.0000531*
Jordan's Point DS-Chessie	0.9986931
Jordan's Point US-Chessie	1
Woods Creek-Chessie	0.0152764*
Hays Creek Unexclosed-Hays Creek Exclosed	0.9975136
Jordan's Point DS-Hays Creek Exclosed	0.0000485*
Jordan's Point US-Hays Creek Exclosed	0.0000264*
Woods Creek-Hays Creek Exclosed	0.0212801*
Jordan's Point DS-Hays Creek Unexclosed	0.0001115*
Jordan's Point US-Hays Creek Unexclosed	0.0000591*
Woods Creek-Hays Creek Unexclosed	0.0566232
Jordan's Point US-Jordan's Point DS	0.9994656
Woods Creek-Jordan's Point DS	0.0367833*
Woods Creek-Jordan's Point US	0.0173846*

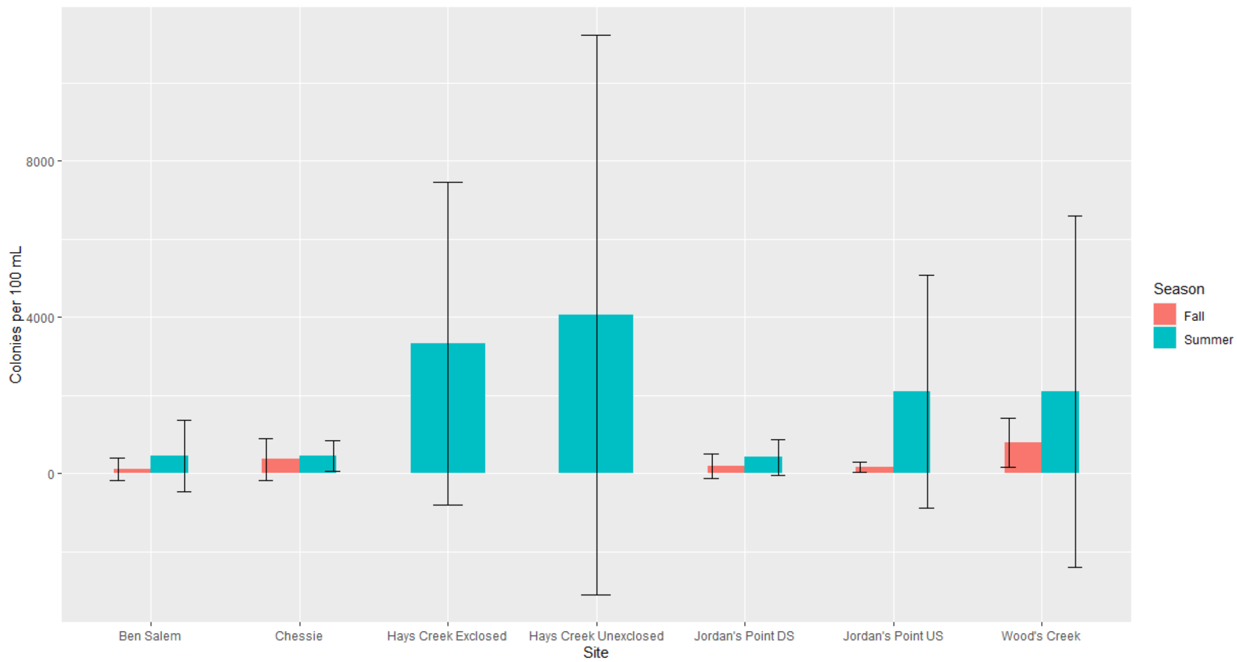

Figure 2. Mean *E. coli* colonies per milliliter at each site in summer and fall.

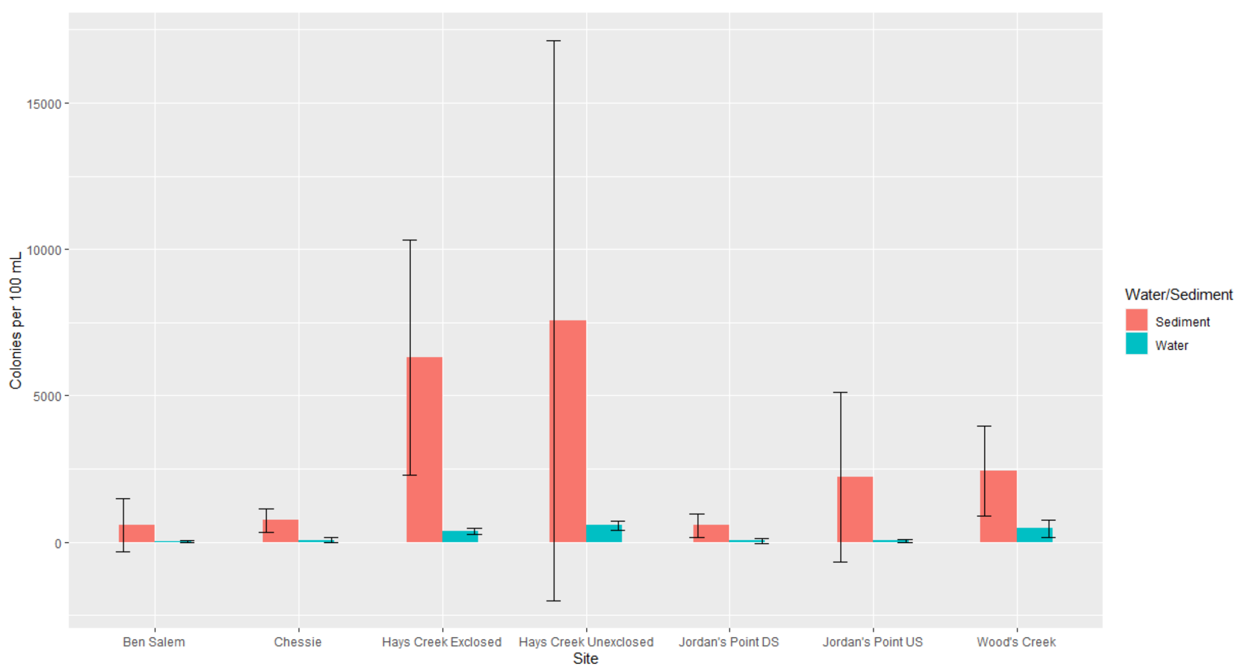

Figure 3. Mean *E. coli* colonies per 100 mL in sediment and water samples at each site.

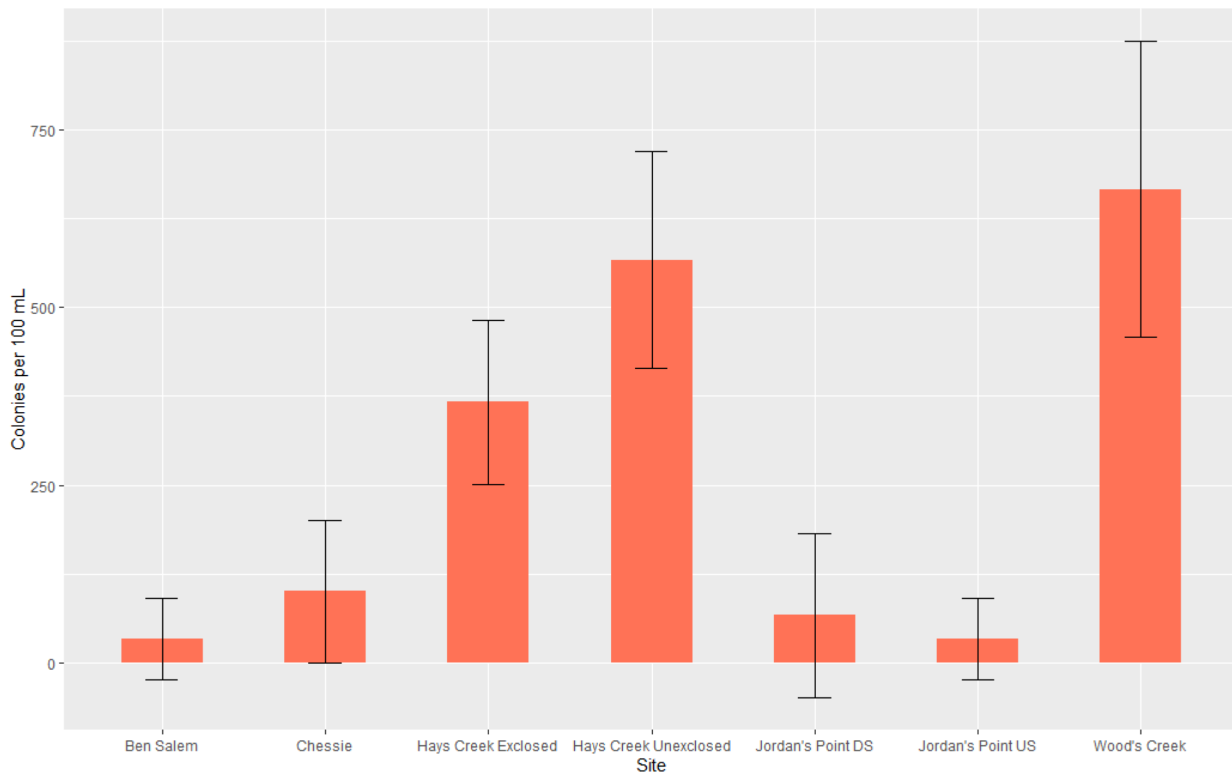

Figure 4. Mean *E. coli* colonies in summer water samples from each site.

Table 4. Results of post hoc Tukey's HSD tests on differences in summer water *E. coli* between sites.

Sites Compared	p
Chessie-Ben Salem	0.993414
Hays Creek Exclosed-Ben Salem	0.066099
Hays Creek Unexclosed-Ben Salem	0.001957*
Jordan's Point DS-Ben Salem	0.999864
Jordan's Point US-Ben Salem	1
Woods Creek-Ben Salem	0.000368*
Hays Creek Exclosed-Chessie	0.196122
Hays Creek Unexclosed - Chessie	0.006272*
Jordan's Point DS-Chessie	0.999864
Jordan's Point US-Chessie	0.993414
Woods Creek-Chessie	0.001109*
Hays Creek Unexclosed-Hays Creek Exclosed	0.480486
Jordan's Point DS-Hays Creek Exclosed	0.115686
Jordan's Point US-Hays Creek Exclosed	0.066099
Woods Creek-Hays Creek Exclosed	0.115686
Jordan's Point DS-Hays Creek Unexclosed	0.003489*
Jordan's Point US-Hays Creek Unexclosed	0.001957*
Wood's Creek-Hays Creek Unexclosed	0.951142
Jordan's Point US-Jordan's Point DS	0.999864
Woods Creek-Jordan's Point DS	0.000635*
Woods Creek-Jordan's Point US	0.000368*

References

After Dam Removal, Archaeology at Jordan's Point, Maury River, Lexington. (n.d.). Retrieved May 16, 2022, from https://www.dhr.virginia.gov/articles-blogs-contributions-in-newsletters/after-dam-removal-archaeology-at-jordans-point-maury-river-lexington/

Arimoro, F. O., Odume, O. N., Uhunoma, S. I., & Edegbene, A. O. (2015). Anthropogenic impact on water chemistry and benthic macroinvertebrate associated changes in a southern Nigeria stream. *Environmental Monitoring and Assessment*, *187*(2), 14. https://doi.org/10.1007/s10661-014-4251-2

Benthic Macroinvertebrates. (2019, August 28). *Virginia Save Our Streams*. https://vasos.org/benthic-macroinvertebrates/

Chesapeake Bay | NOAA Fisheries (National). (2022, April 4). NOAA. https://www.fisheries.noaa.gov/topic/chesapeake-bay

Ferrer, M., Chernikova, T. N., Yakimov, M. M., Golyshin, P. N., & Timmis, K. N. (2003). Chaperonins govern growth of Escherichia coli at low temperatures. *Nature Biotechnology*, *21*(11), 1267–1267. https://doi.org/10.1038/nbt1103-1266b

Jang, J., Hur, H.-G., Sadowsky, M. j., Byappanahalli, M. n., Yan, T., & Ishii, S. (2017). Environmental Escherichia coli: Ecology and public health implications—a review. *Journal of Applied Microbiology*, *123*(3), 570–581. https://doi.org/10.1111/jam.13468

Jantz, P., Goetz, S., & Jantz, C. (2005). Urbanization and the Loss of Resource Lands in the Chesapeake Bay Watershed. *Environmental Management*, *36*(6), 808–825. https://doi.org/10.1007/s00267-004-0315-3

Moore, T. (2014). *Nitrate-nitrogen effects on benthic invertebrate communities in streams of the Canterbury Plains*. https://doi.org/10.26021/8927

Nitrate Vacu-vials Instrumental Test Kit (K-6913) | CHEMetrics. (n.d.). *CHEMetrics, Inc.* Retrieved April 29, 2022, from https://www.chemetrics.com/product/nitrate-test-kit-vacu-vials-instrumental-kit-k-6913/

Romanelli, A., Soto, D. X., Matiatos, I., Martínez, D. E., & Esquius, S. (2020). A biological and nitrate isotopic assessment framework to understand eutrophication in aquatic ecosystems. *Science of The Total Environment*, *715*, 136909. https://doi.org/10.1016/j.scitotenv.2020.136909

US EPA, O. (2013, March 27). *Estimated Nitrate Concentrations in Groundwater Used for Drinking* [Data and Tools]. https://www.epa.gov/nutrient-policy-data/estimated-nitrate-concentrations-groundwater-used-drinking

USDA. (2020). *USDA - National Agricultural Statistics Service—Virginia—Annual Statistical Bulletin*. https://www.nass.usda.gov/Statistics_by_State/Virginia/Publications/Annual_Statistical_Bulletin/index.php

Virginia Department of Wildlife Resources. (n.d.). *Maury River*. Retrieved April 29, 2022, from https://dwr.virginia.gov/waterbody/maury-river/