SPRING 2023 WATER QUALITY MONITORING IN HUNTING CREEK

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LAND ACKNOWLEDGEMENT

In November 2023, ACLT's Board of Directors approved a new Land Acknowledgement statement that recognizes the contributions of those peoples who lived upon, cared for, were nurtured by, and worked the land in the Parkers Creek Preserve before us. By extension, we embrace this important formal recognition of those who inhabited the Hunting Creek watershed before us and include ACLT's Land Acknowledgement statement here in our report.

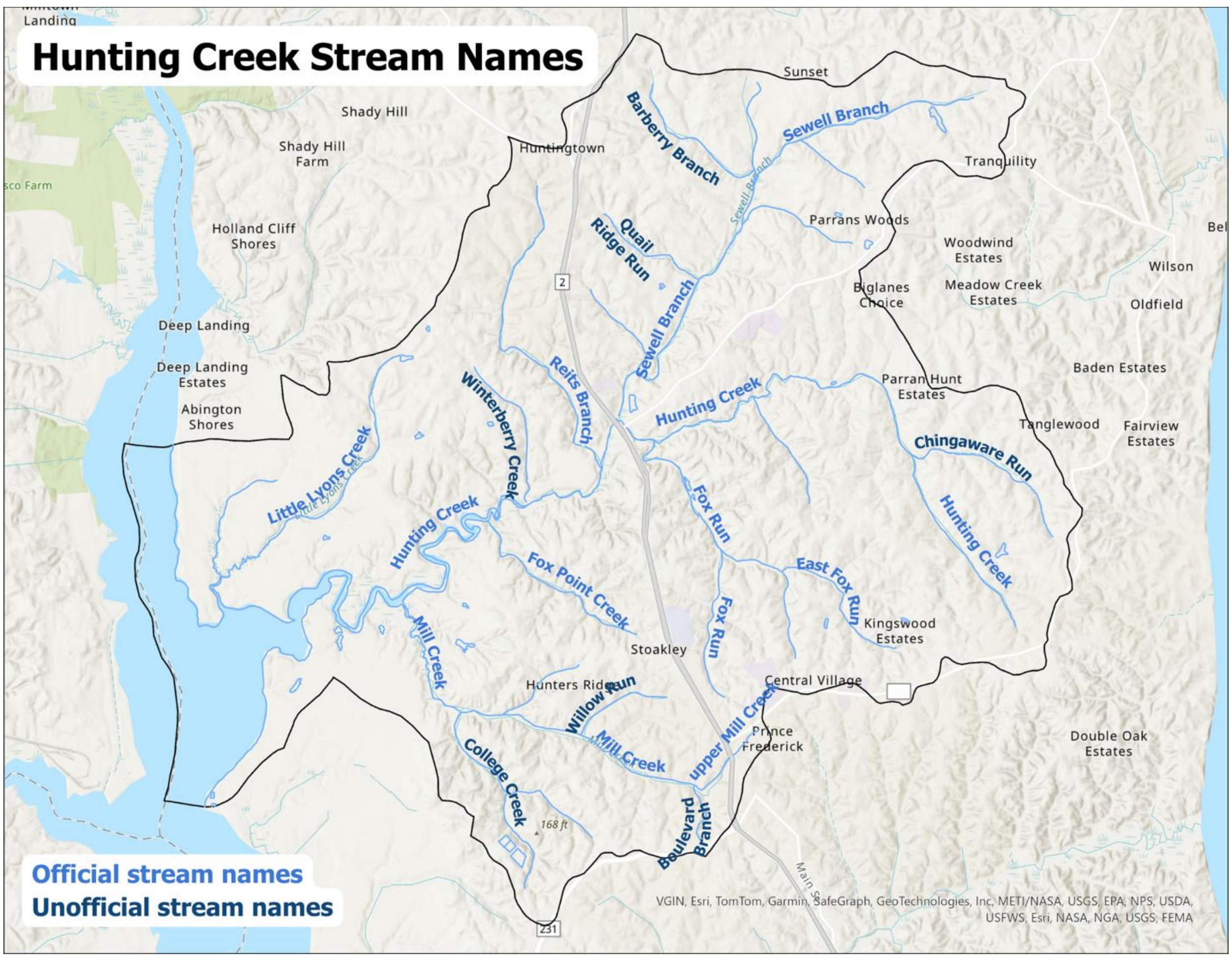
"ACLT acknowledges that the land it stewards, including Parkers Creek and Governors Run watersheds, is the ancestral home of the Peoples of the Piscataway Confederacy and other Indigenous People. Further, we recognize the people of African descent, enslaved and free, who once worked on or owned the farms, the land of which is now stewarded by ACLT. We recognize the contributions made by these displaced and enslaved peoples in the face of injustice and inequity and acknowledge our obligations to maintain the land and educate the public in such a way that their spirit and heritage are not forgotten.

Accordingly, ACLT pledges to continue its fundamental mission of connecting all people to the land by providing equitable and inclusive access to the lands we steward, through ongoing research and education about the land's inhabitants and their history, and through outreach to all, particularly those most affected by societal injustice. As a leader of land conservation and preservation we strive by example to foster a feeling of belonging to this region and at ACLT for all its diverse communities with the hope of a more equitable and sustainable future in Nature for all."

1 SUMMARY

The Friends of Hunting Creek (FOHC) completed our third Water Quality Blitz on April 1, 2023. We collected water samples in 11 catchments across almost the entire watershed. To assist our ongoing efforts to get "up close and personal" with the 50+ miles of streams that drain the 19,127-acre Hunting Creek watershed of Calvert County, Maryland, we have unofficially named several previously unnamed tributaries (see Figure 1.1). In addition to measuring nitrogen, phosphorus, and turbidity at 21 sites, we also sampled benthic macroinvertebrates (macros) and collected environmental DNA (eDNA) samples in four streams.

Figure 1.1 Map with both official (light blue) and unofficial (dark blue) names of Hunting Creek streams.



The FOHC are proud to have compiled three years of water quality data. We are committed to continuing and, if possible, expanding our monitoring activities, both spatially and temporally. Our goal is to see our young *database* develop over the next

7-8 years into a mature *time series* that will provide an opportunity to detect trends. Until then, what can we now say about the ecological health of streams in the Hunting Creek watershed?

For starters, baseflow concentrations of two nutrients (nitrogen and phosphorus) were relatively low in 2021, 2022, and 2023. NO23 levels were in the *Fair* condition range (0.7<x>or=2.1 mg/L) in only three streams: Little Lyons Creek (in 2021, 2022, 2023), Fox Point Creek (in 2021, 2022), and *Quail Ridge Run* (in 2022, 2023). We call these locations "*warm*" spots for baseflow nitrogen levels that are not alarming but deserve watching.

What we know about these three streams is that the Little Lyons Creek catchment has the highest percentage of agricultural land (26.8%) in the watershed. Fox Point Creek drains the northwest portion of the Prince Frederick Town Center, and its catchment is 26% developed and 8.6% impervious. *Quail Ridge Run* flows through the Marley Run community and its catchment is 37.6% developed and 8.9% impervious.

To date, the only stream in the Hunting Creek watershed with a PO4 concentration slightly elevated and in the *Fair* range (0.037<x>or=0.111 mg/L) was *College Creek.* Its catchment is 25.9% developed and 8% impervious.

In contrast to what we've observed with nutrients, baseflow turbidites, measured only in 2023, were elevated and in the *Fair* range (4.0<x>or=20.0 NTU) at all but one (Fox Run) of the 21 sampled streams. Turbidity refers to the cloudiness of a water and it is measured in NTUs, or nephelometric turbidity units. With only one year of baseflow measurements, we can't yet conclude that somewhat elevated turbidity is typical of streams in our watershed.

The steep topography in most areas of Calvert County, coupled with an abundance of highly erodible soils, is the perfect combination of conditions rendering less than crystal clear streams at baseflow and highly turbid streams during/after intense rainfall events that cause stormwater runoff and soil erosion. FOHC volunteers documented elevated turbidities ranging up to almost 90 NTU in Fox Run, Sewell Branch, Hunting Creek, *Willow Run,* upper Mill Creek, and *Boulevard Branch* in 2023 after 3.05" and 3.31" rain events.

Sampling macros is proven to be a useful tool for supplementing water chemistry monitoring to yield a more complete assessment of ecological health. Although limited to only four streams in the Hunting Creek watershed so far, Benthic Indices of Biotic Integrity (BIBI) scores, calculated from macros data collected in 2023, suggested a range of ecological health conditions from *Very Poor* in upper Mill Creek to *Good* in Fox Run. BIBI scores (2023) were *Fair* in *Chingaware Run* and *Poor* in *Willow Run.*

Our measurements of nitrogen, phosphorus, and turbidity do not reflect these differences in stream conditions suggested by the macros data. Rather, observed differences among the four streams appear to be more closely associated with differences in key physical habitat parameters. The habitat parameters most influential on BIBI scores were Epifaunal Substrate/Available Cover, Sediment Deposition, and Bank Stability. Habitat degradation appears to be most severe in upper Mill Creek, a stream that drains the southwest portion of the Prince Frederick Town Center. The section of this stream where our macros sampling site (HC20) is located has a deeply incised channel that is disconnected from its floodplain, is suffering from Urban Stream Syndrome, and would be a candidate for restoration.

The FOHC's eDNA Pilot Study conducted in 2023 in the four streams where macros were collected achieved two of our three objectives. We successfully collected sufficient volumes of stream water and did not contaminate the eDNA samples. The DNA analyses successfully identified many fish species with a high level of accuracy revealed differences in fish community composition among the four Pilot Study streams. eDNA revealed that Fox Run and *Chingaware Run* had the most fish species (16-18 and 12 or 13, respectively). The robust fish diversity observed in Fox Run in 2023 sets a record for Calvert County streams. The macros Benthic Index of Biotic Integricy (BIBI) score for Fox Run in 2023 was a very respectable 4.1, on a 1.0 to 5.0 scale, and in the *Good* range, indicating that this stream has a robust biological community and could be a viable candidate for Calvert County's first High Quality Tier II Waters Designation ("Maryland's High Quality Waters (Tier II)"). In contrast to these two Pilot Study streams, eDNA found far fewer fish species living in *Willow Run* and upper Mill Creek in 2023 (only 5 and 6 or 7, respectively). These two streams drain the west and southwest portions of the Prince Frederick Town Center and had low BIBI scores.

Our 2023 Pilot Study results tell us that eDNA sampling is an effective and feasible fish sampling method for use by volunteer-based watershed associations like the FOHC. We now have information from four streams in the Hunting Creek watershed that will allow us to include fish photos in our exhibits at public education/outreach events.

Unfortunately, the eDNA Pilot Study did not yield results useful to the FOHC for stream macros. In all four streams sampled by eDNA and D-net, DNA analysis revealed fewer macros families and genera than did the D-net collections. Plausible reasons for these differences include a need for more effective primers, incomplete DNA sequence reference libraries, and relatively low quantities of DNA shed by macros into the environment.

After three years of Blitz-related water chemistry measurements, two years of macros collections, and one year of eDNA sampling, FOHC volunteers are getting better acquainted with the 50+ miles of stream that drain the 19,127-acre Hunting Creek watershed. Our efforts have revealed a few “warm” spots for nitrogen levels to be watched and two streams that appear to be adversely impacted by past and current development in the Prince Frederick Town Center. But, in general, the picture of ecological health being revealed in our watershed is mostly positive and encouraging.

How this current picture might change for the worse in the future is uncertain. But, here’s one reason to be concerned. The recently completed 2023 Report Card for the Parkers Creek watershed contains a disturbing graphic showing that between 2013 and 2017, a total of 291 acres of natural vegetation (forests and wetlands) was lost to development in the Hunting Creek watershed. This acreage represents 1.52% of the entire watershed. For comparison, net loss of natural vegetation in the Hunting Creek watershed during those four years ranked fourth among Calvert County’s watersheds, behind North Battle Creek (3.12%), Mill Creek (1.76%), and Fishing Creek (1.64%). To round out the top five for this troubling statistic is the Parkers Creek watershed, at 1.08%. Between 2013 and 2017, the Hunting Creek watershed also suffered a net loss of 82 acres of agricultural land to development, another statistic that is not good news (“Parkers Creek Watershed Report Card-2023 Update” 2024, p.12).

Steadfast vigilance, a commitment to long-term water monitoring, inspiring/informing watershed residents, and data-based advocacy for stream health will continue to be major responsibilities for the Friends of Hunting Creek into the foreseeable future.

2 WHAT DID WE ACCOMPLISH?

Formed in 2020, the Friends of Hunting Creek (FOHC) adopted the Mission "…*to promote the ecological health and resiliency of the watershed's 50 miles of streams and landscape so that landowners, citizens, government agencies, and elected officials together take an active role in protecting and sustaining the natural and cultural resources."* During the past four years, a small group of committed watershed residents, supported by American Chestnut Land Trust (ACLT) staff, has worked diligently to inspire, inform, and investigate. We investigate to "*expand the scientific understanding of our land and water resources"* by monitoring water quality and assessing stream health throughout the Hunting Creek watershed.

2.1 2021

The FOHC launched our first water monitoring initiative by participating in the Water Quality Blitz, led by the American Chestnut Land Trust (ACLT), on April 3, 2021. We sampled 10 non-tidal stream sites. Details can be found in our 2021 report (Klauda and Estes 2021).

2.2 2022

The FOHC's second Water Quality Blitz was conducted on April 2, 2022. Water samples were collected at the 10 stream sites sampled in 2021 and at seven new sites added to expand our coverage of the watershed. In addition to collecting water samples at 17 sites, we also sampled aquatic benthic macroinvertebrates (macros) and scored 10 physical habitat parameters at two sites, to enhance our assessments of stream health. Details can be found in our 2022 report (Klauda et al. 2023).

2.3 2023

The FOHC's third Water Quality Blitz was conducted on April 1, 2023. Water samples were collected at 21 sites and analyzed for nitrogen (as NO23), phosphorus (as PO4), and turbidity. The growth i the number of sites is a result of significant efforts to physically travel the watershed by vehicle and on foot to add to our coverage and to increase the resolution of the gathered data. The catchment information of all our sites can be found in Appendix F Catchments of Test Sites. Macros sampling was expanded from the two sites sampled in 2022 (HC18 and HC19) to include two new sites: HC6 and HC20. What macros are and why they are excellent indicators of stream health is discussed on pgs. 4-5 of the FOHC's Spring 2022 Water Quality Monitoring report (Klauda et al. 2023).

A new biological sampling tool was evaluated by the FOHC in 2023. An environmental DNA (eDNA) Pilot Study was conducted at the four stream sites sampled for macros to determine (a) if our volunteers could collect uncontaminated eDNA samples, (b) if eDNA sampling is as effective as a D-net for describing macros diversity, and (c) what eDNA can also tell us about fish diversity in the Hunting Creek watershed.

Environmental DNA is the genetic material shed by macros, fish, and other aquatic animals and plants that live in streams, rivers, ponds, lakes, wetlands, vernal pools, estuaries, and oceans. By carefully collecting water samples that contain mucus, skin, scales, other tissues, urine, and yes, even “poop”, scientists can extract and process eDNA to learn who lives in the sampled environments.

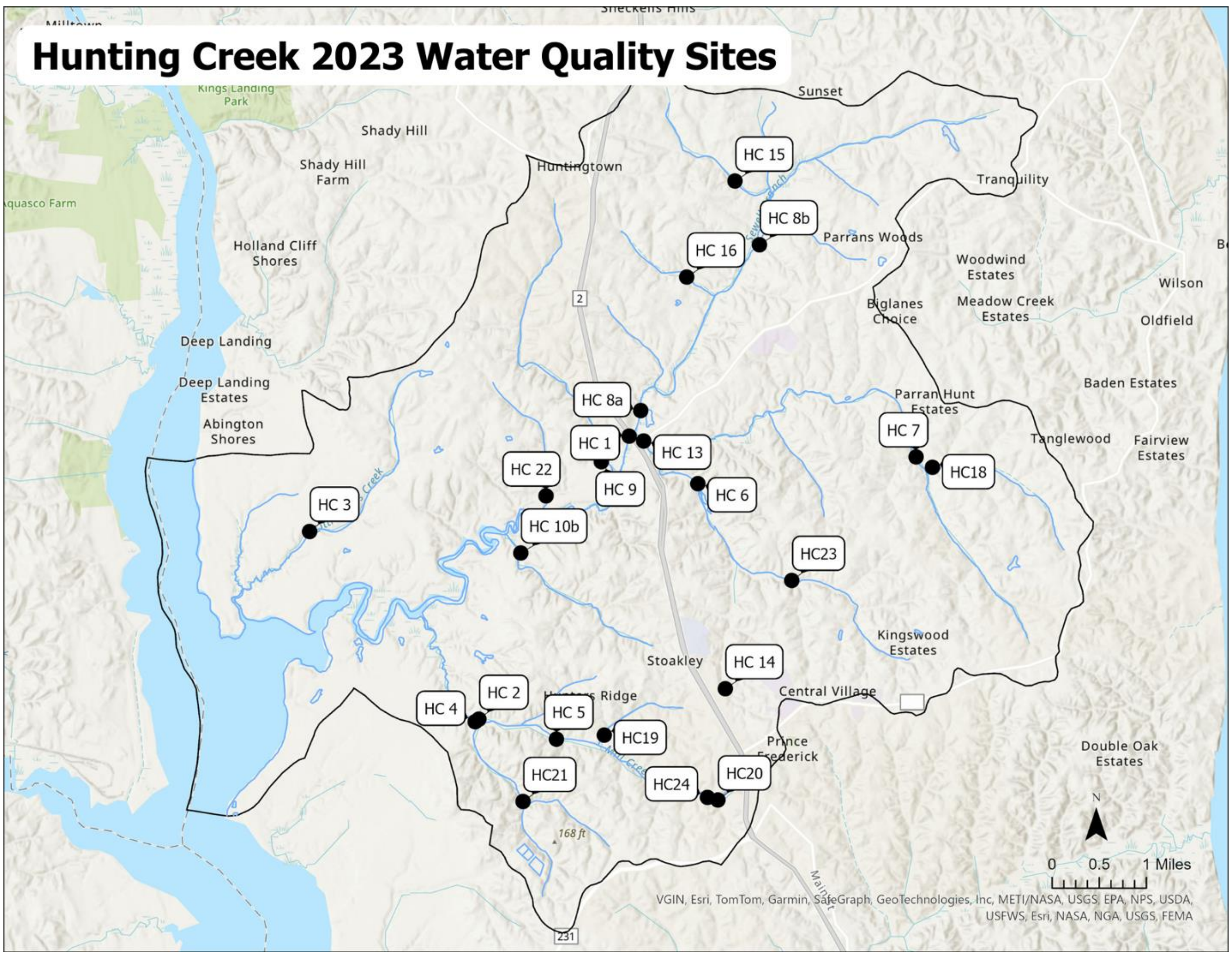
There is a growing consensus that eDNA analysis may be a complimentary and perhaps an alternative sampling approach to describe the diversity of aquatic communities and locate non-native and rare/threatened/endangered species. eDNA sampling has the potential to be easier, quicker, and cheaper than traditional sampling methods. eDNA sampling is revolutionizing biological monitoring by enabling non-disruptive, efficient, and less-costly surveys of diverse taxa in a range of aquatic ecosystems. There is also evidence to support using eDNA concentrations in water samples as an ancillary tool for estimating fish relative abundance (Rourke et al. 2022).

3 HOW DID WE DO IT?

3.1 Water Chemistry Methodology

Two grab water samples were collected at each of the 21 stream sites by FOHC volunteers in the morning of April 1, 2023 (Figure 3.1). Water samples were transported to ACLT headquarters in insulated coolers with ice. Those samples destined for measurements of NO23 and PO4 at the Nutrient Analytical Services Laboratory, Chesapeake Biological Laboratory, Solomons MD, were filtered at ACT within 2-4 hours after collection. The filtrates were frozen and transported to CBL. The other water samples were analyzed for turbidity by FOHC volunteers at ACLT using an Apera TN400 Meter.

Figure 3.1



3.1.1 Rationale for Blitz Sampling Dates

A major goal of the Water Quality Blitz is to characterize concentrations of inorganic nitrogen (measured as nitrite NO2 + nitrate NO3) in near-surface groundwater. Scientists at the University of Maryland's Center for Environmental Science, Appalachian Laboratory, in Frostburg MD, found after making many measurements of dissolved inorganic nitrogen throughout all months of the year that a good approximation of average annual concentrations in near-surface groundwater can be obtained by collecting a single stream water sample, if two conditions are met. First, the water samples should be collected in early spring, before leaf-out occurs. In Calvert County, early to mid-April typically precedes leaf-out. Once trees have fully leafed-out and transpiration increases, nitrogen concentrations in surface waters are depressed. Second, the stream water samples should be collected under dry (baseflow) conditions (Eshelman et al. 2009). The rule of thumb followed by ACLT is no rainfall and associated surface run-off for three days prior to sample collection. Typically, surface run-off causes decreased inorganic nitrogen concentrations in stream water and would thus hamper our goal to obtain average annual concentrations. Dissolved inorganic nitrogen (i.e., NO2 and NO3) are the two forms of bioavailable nitrogen in near-surface groundwater. Other forms of nitrogen (e.g., ammonium, dissolved organic nitrogen, particulate organic nitrogen) are generally at low concentrations in groundwater and, except for ammonium, are not directly available to support plant growth.

3.2 Aquatic Biology and Physical Habitat Methodology

3.2.1 Benthic Macroinvertebrates (Macros) Methodology

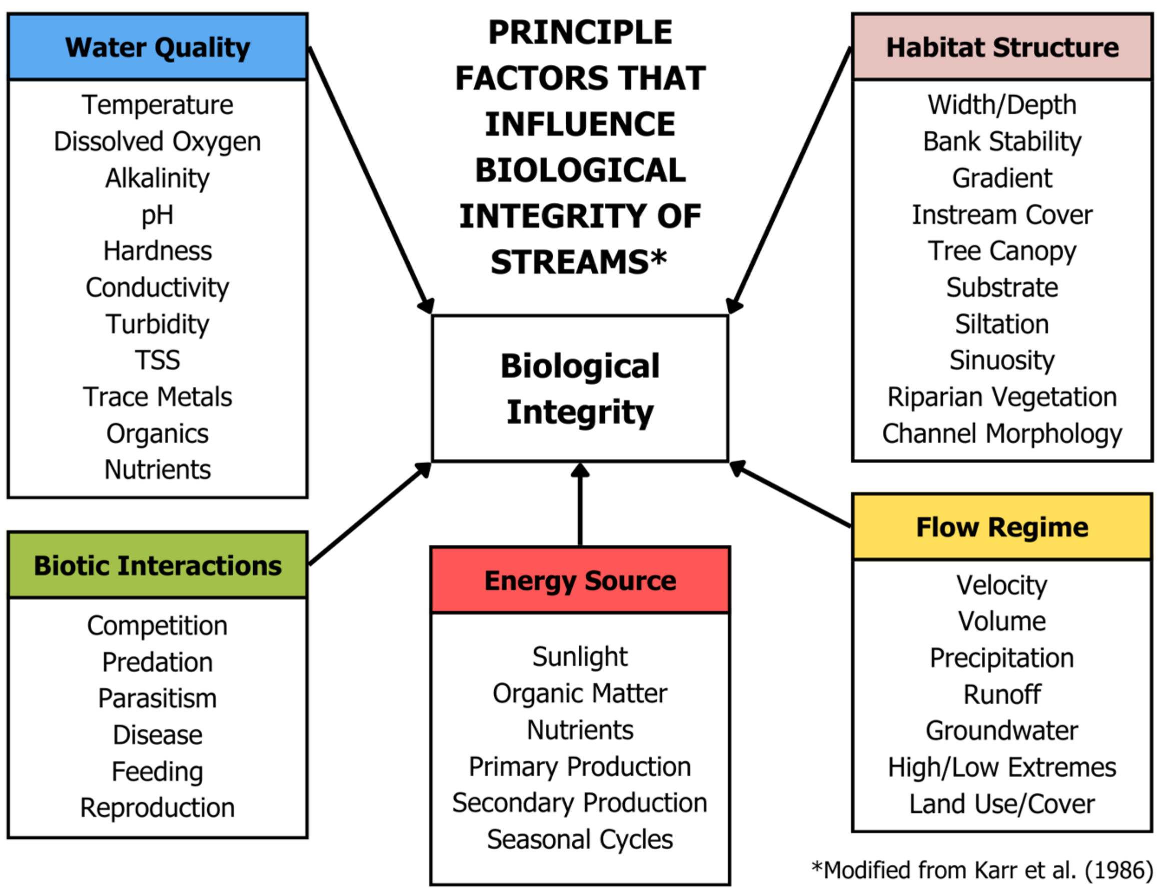
Using a D-net (equipped with a frame 12 in. wide by 10 in. high and a 540-micron mesh net) and following protocols used by the Maryland Department of Natural Resources (MD/DNR) for their Maryland Biological Stream Survey, or MBSS (see MBSS sampling manual here), we sampled macros in a 75-m (246 ft) long segment at each of four sites: HC6 (Fox Run), HC18 (*Chingaware Run),* HC19 (*Willow Run*), and HC20 (upper Mill Creek). See site locations on Figure 3.1. Sampling occurred on April 15, 2023, at HC18 and HC19, and on April 16, 2023, at HC6 and HC20. As stated above, macros were also sampled at HC18 and HC19 in 2022.

After collecting macros in the D-net, the contents were washed and strained through a sieve bucket with a 540-micron mesh bottom and then the captured organisms were transferred to sample jars containing 95% ethanol for preservation. The preserved samples were transported to Dr. John Cooper at Cooper Environmental Research, Constantia, NY for processing and identification of all macros to the family and genus levels. Dr. Cooper also calculated Benthic Index of Biotic Integrity (BIBI) scores for each of the four sampled stream sites using MBSS methods (Southerland et al., 2005).

The BIBI is a multi-metric measure of biological integrity than can be used to assess the condition (health) of a given stream site based on the kinds and numbers of macros taxa (families and genera) that were collected there. BIBI scores range from 1.0 (worst) to 5.0 (best), dependent upon how far a given stream site's condition deviates from minimally-disturbed reference streams in the appropriate physio-graphic region. The Hunting Creek watershed is in the Coastal Plain. BIBI scores fall into four stream health assessment categories: 1.0-1.9 = very poor, 2.0-2.9 = poor, 3.0-3.9 = fair, and 4.0-5.0 = good.

BIBI scores calculated from macros data are useful for distinguishing degraded from healthy streams. The integrity of macros communities (and other aquatic biota) is influenced by many factors (Figure 3.2). Therefore, identifying the specific stressors that are impacting macros in degraded streams is challenging.

Figure 3.2



3.2.2 Physical Habitat Methodology

To assess the condition of one group of factors that influence the integrity of the macros communities, we evaluated ten physical habitat parameters at each macros site on the same day. Each physical habitat parameter was scored from 0 points (poor) to 20 points (optimal). For a description of the ten parameters and scoring criteria, see the Habitat Assessment Field Data Sheet in Appendix D. In addition to scoring the ten habitat parameters, we also measured wetted stream width and thalweg depth (deepest point) at each of four cross-stream transects located at the 0m, 25m, 50m, and 75m locations along each 75m-long stream segment. Maximum stream depth within the entire segment was also measured.

3.2.3 eDNA Pilot Study Methodology

Water samples were collected on April15, 2023, at HC18 and HC19 and on April 16, 2023, at HC6 and HC20 using a Smith-Root eDNA Citizen Scientist sampling pump and their Self-Preserving Filter Packs with 5-micron mesh filters. Two water samples (about 2-L each) were collected along the same 75-m long stream segments also sampled for macros with a D-net. The two eDNA water samples were collected at the 25m and the 50m transects. A total of two distilled water field blanks were also collected with the same pump and filters, then analyzed to check for sampling related contamination. At each of the four stream sites, the eDNA samples were collected first, followed immediately by the collection of macros samples with the D-net.

The ten filter packs (two per site X four sites + two field blanks) were mailed to Jonah Ventures, a commercial genetics lab in Boulder, CO, for metabarcoding analysis. In the lab, the DNA in each sample was extracted, amplified, sequenced, and then the found sequences were compared to known DNA sequences in the Jonah Ventures reference library to determine which macros genera and fish species were present at each of our four stream sites. Jonah Ventures used the MiFish-u primer to identify fish DNA sequences and the ArthCOi primer to identify macros DNA sequences in our samples. A primer is a short section of synthesized DNA. Its purpose is to bind complementary DNA segments during the PCR (polymerase chain reaction) step in the lab analysis, when millions of copies of DNA are made from a few original pieces in the sample being analyzed.

To reduce the number of base pair mismatches per DNA sequence, the spreadsheets we received from Jonah Ventures were filtered to remove lines of detected sequences with a <97% match to a known macros genus or fish species in Jonah Venture's DNA reference library. In addition, Rob Aguilar, a Research Technician at the Smithsonian Environmental Research Center in Edgewater, MD, kindly BLASTED our fish spreadsheet using SERC's private reference sequence library (CBBI: Chesapeake Bay

Barcode Initiative) and increased % match values for many lines of detected sequences. BLAST refers to a Basic Local Alignment Search Tool and is a technique used to match a particular DNA sequence with sequences in a reference library.

For those readers who are interested in more details about what eDNA is, how it behaves in a stream, definitions of DNA sequencing key words, metabarcoding details, and other related topics, we suggest you go to the Jonah Ventures website (https://jonahventures.com/).

4 WHAT HAVE WE LEARNED?

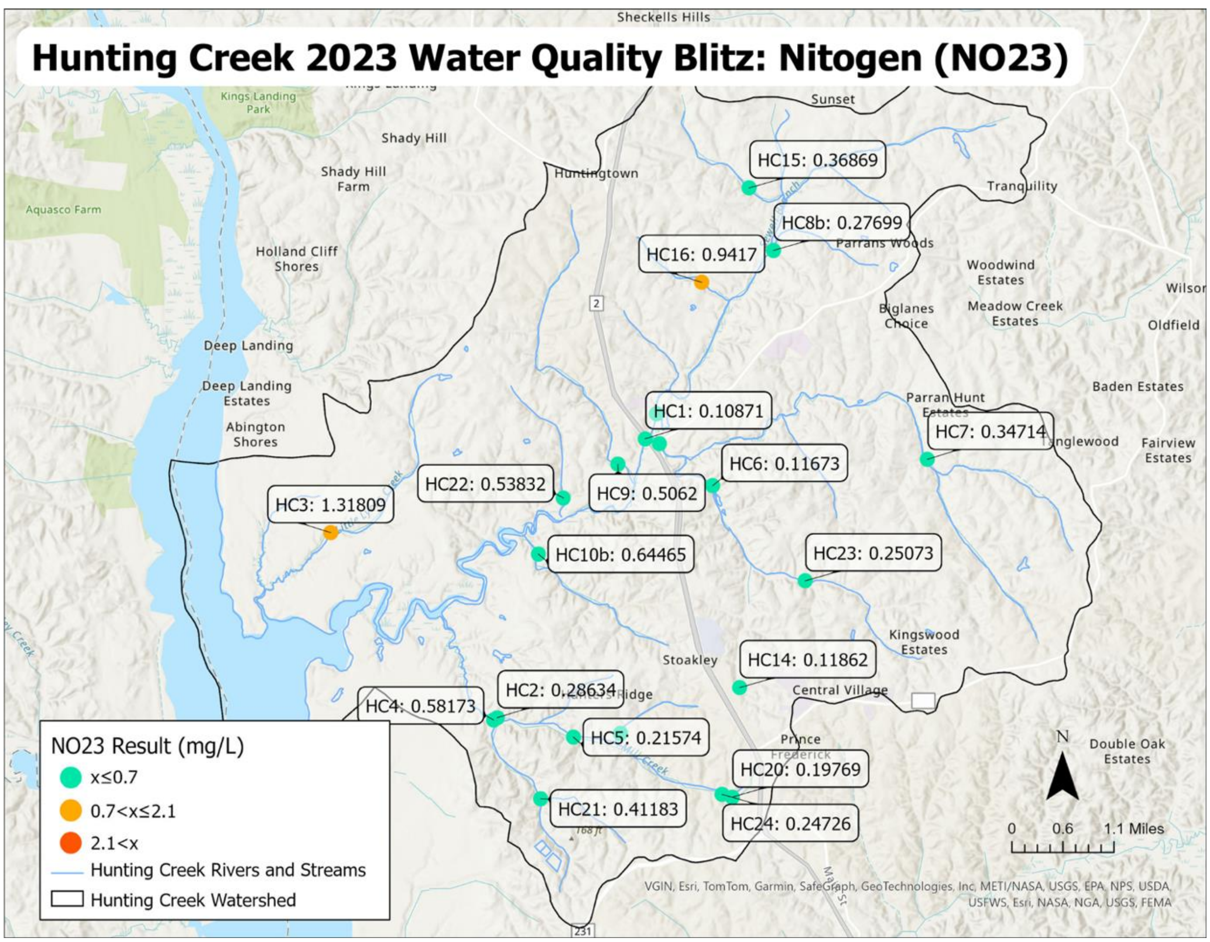
4.1 Water Chemistry

The 21 Blitz sites samples were measured for nitrogen (NO23 = Nitrite and nitrate) and phosphorus (PO4) in 2023. See Appendix 1 for supplemental information on NO23. A discussion of the results is below.

4.1.1 2023 NO23 Results

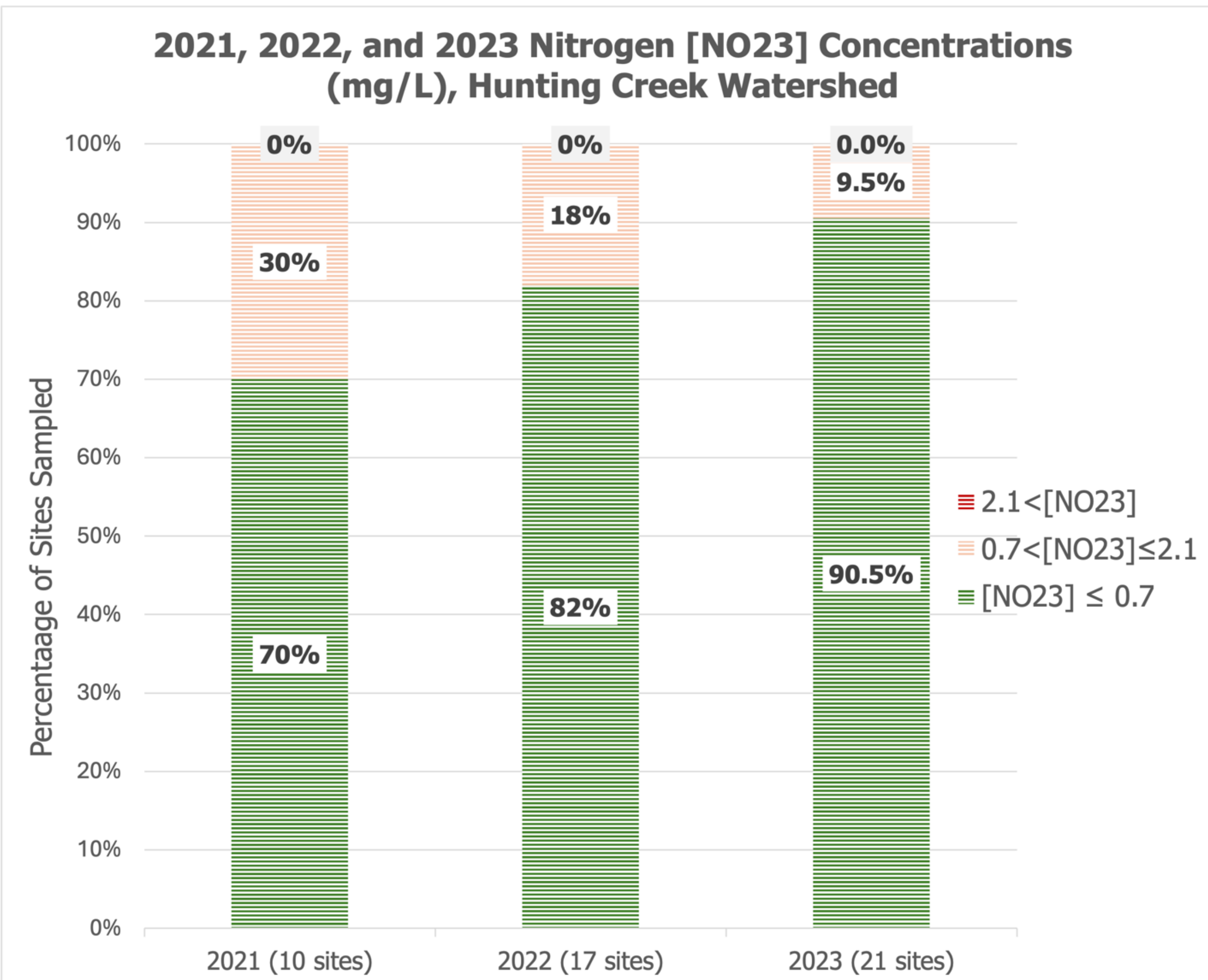
Nitrogen (NO23) concentrations were not significantly different between 2022 and 2023. As shown in Figure 3.3, only two sites, HC3 and HC16, exhibited elevated NO23 concentrations in 2023, falling within the *Fair* range, between 0.7 and 2.1 mg/L. HC3 and HC16 also had elevated NO23 concentrations in the *Fair* range in 2022. HC10b, despite having elevated NO32 concentrations in 2022, had a *Good* NO23 concentrations in 2023. Overall, NO23 levels have been relatively low in the Hunting Creek watershed.

Figure 4.1



The American Chestnut Land Trust uses 0.7 and 2.1 mg/L as the upper thresholds of the *Good* and *Fair* NO23 categories, respectively. These categories have changed since 2021, which had 0.7 and 1.05 mg/L as the upper thresholds for the *Good* and *Fair* categories, respectively. Overall, 90.5% of the sites tested in 2023 had *Good* NO23 concentrations, while the remaining 9.5% of sites had *Fair* NO23 concentrations. Compared across the three years of FOHC participation in the Water Quality Blitz, 2023 had the highest percentage of *Good* sites, as shown in figure 3.4. It is noteworthy, however, that the number of sites has increased from 10 sites in 2021 to 17 sites in 2022 to 21 sites in 2023. Although the number of sites tested has increased, the number of sites exhibiting elevated NO23 concentrations has shown little change.

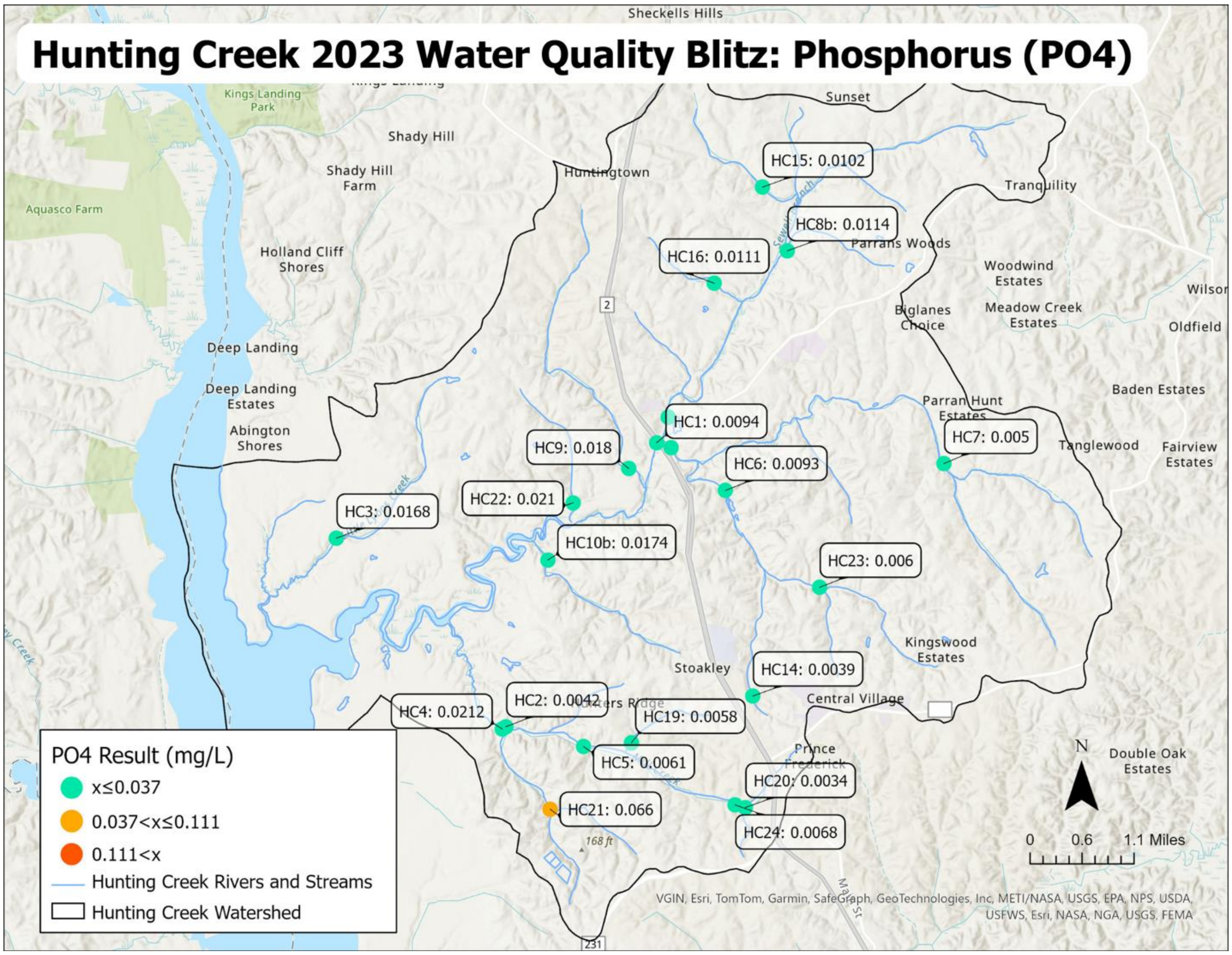
Figure 4.2



4.1.2 2023 PO4 Results

Phosphorus (PO4) levels in the Hunting Creek watershed were overall *Good* in 2023. As depicted in Figure 3.4, only one out of 21 sites exhibited elevated PO4 concentrations. Site HC21 had phosphorus levels within the *Fair* threshold, between 0.037 and 0.111 mg/L.

Figure 4.3

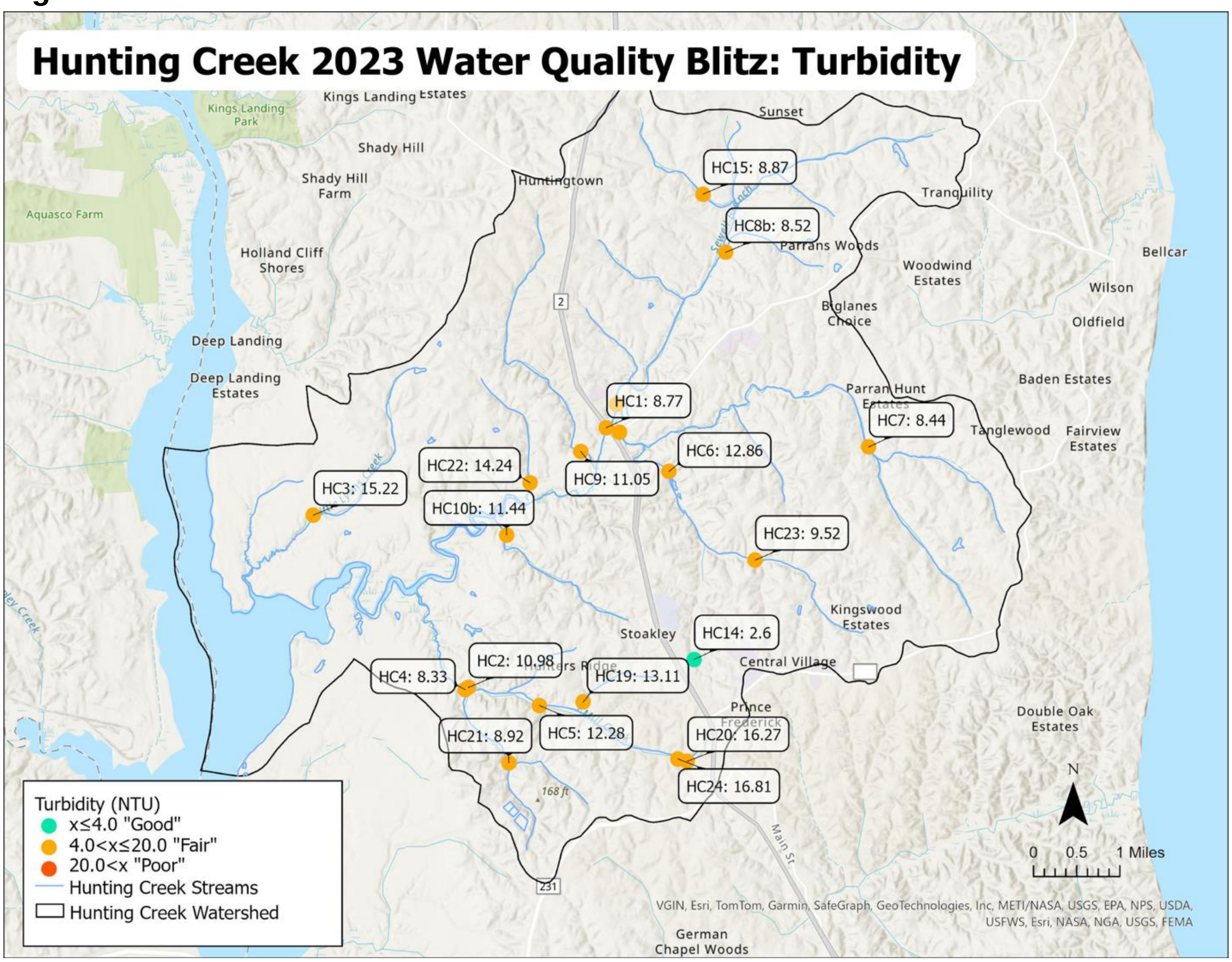


4.1.3 Turbidity

Turbidity measurements were made on water samples collected on April 1, 2023, during baseflow conditions, at the 21 Blitz sites across the Hunting Creek watershed. Turbidities varied from a low of 2.6 Nephelometric turbidity units or NTUs (*Good* condition) at HC14 to a high of 22.1 NTUs (*Poor* condition) at HC16, with turbidities at the other 20 sites in the *Fair* condition range (Figure 4.4). The full set of turbidity data is available in Appendix B. The mean (average) and median turbidities across all 21 sites were 11.05 NTUs and 10.25 NTUs, respectively.

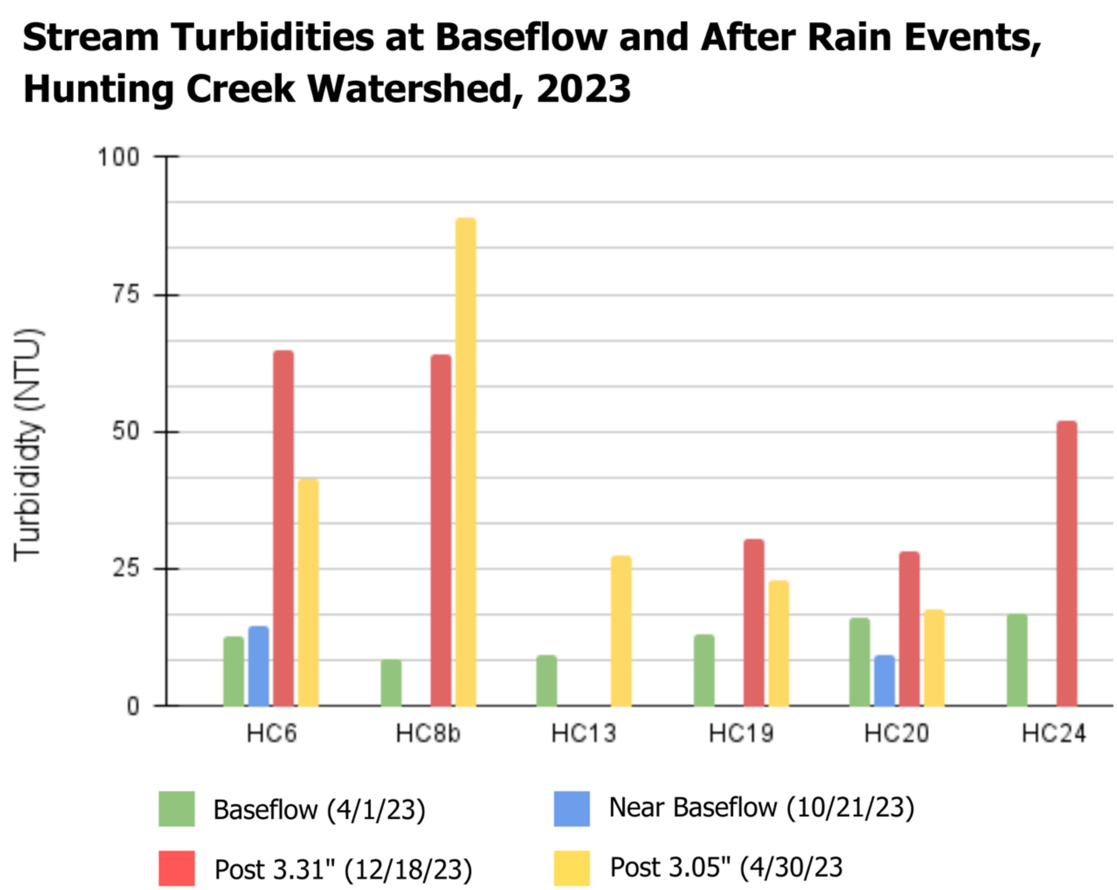
Turbidity is a measure of the cloudiness or haziness of a water sample caused by suspended particles that are usually not visible to the naked eye. Elevated turbidites that persist in streams for more than a few hours can harm macros, fish, and other aquatic organisms via mechanical damage to gills, smothering eggs and developing. larvae, making food gathering more difficult, altering dissolved oxygen levels, and temperature-related impacts. Most macros and fish function optimally at turbidity levels below 10 NTUs. Chronic, several-day exposures to turbidity levels above 20-25 NTUs are generally problematic for macros, fish, and aquatic plants. High turbidity levels, short-term and continuous, indicate that sediments and probably nutrients, organic contaminants, heavy metals, and other pollutants are being transported downstream. The turbidity levels shown in Figure 4.4 were measured in water samples collected during baseflow conditions when turbidities should be at their lowest levels and what the aquatic organisms living in those streams regularly experience and are adapted to. As mentioned above, baseflow conditions are defined as "no measurable rainfall amount during 48-72 hours preceding water sample collection."

Figure 4.4



FOHC volunteers also measured turbidity at six sites in the Hunting Creek watershed immediately after above average rainfall events in 2023: 3.05 in. on 4/1/23 and 3.31 in. on 12/18/23. As shown in Figure 4.5, stream turbidity increased by a factor of only 1 at HC20, compared to baseflow levels, but by a factor of 10.5 (to 88.9 NTUs) at HC8b. Turbidity levels in the 50 to almost 90 NTUs range that persist for more than a day or two would almost certainly be detrimental to aquatic organisms exposed to these conditions.

Figure 4.5



4.2 Macros

Genus-level BIBI scores calculated from D-net sample collections for each of the four stream sites sampled in 2023 ranged from a low of 1.6 (very poor) at HC20 to a high of 4.1 (good) at HC6 (Figure 4.6). BIBI scores in 2023 increased at one of the two sites where macros were also sampled by FOHC volunteers in 2022. The BIBI score at HC18 increased from 2.71 (poor) in 2022 to 3.6 (fair) in 2023. However, at HC19, the BIBI score in 2023 was unchanged from 2022 (2.7, poor).

Figure 4.6

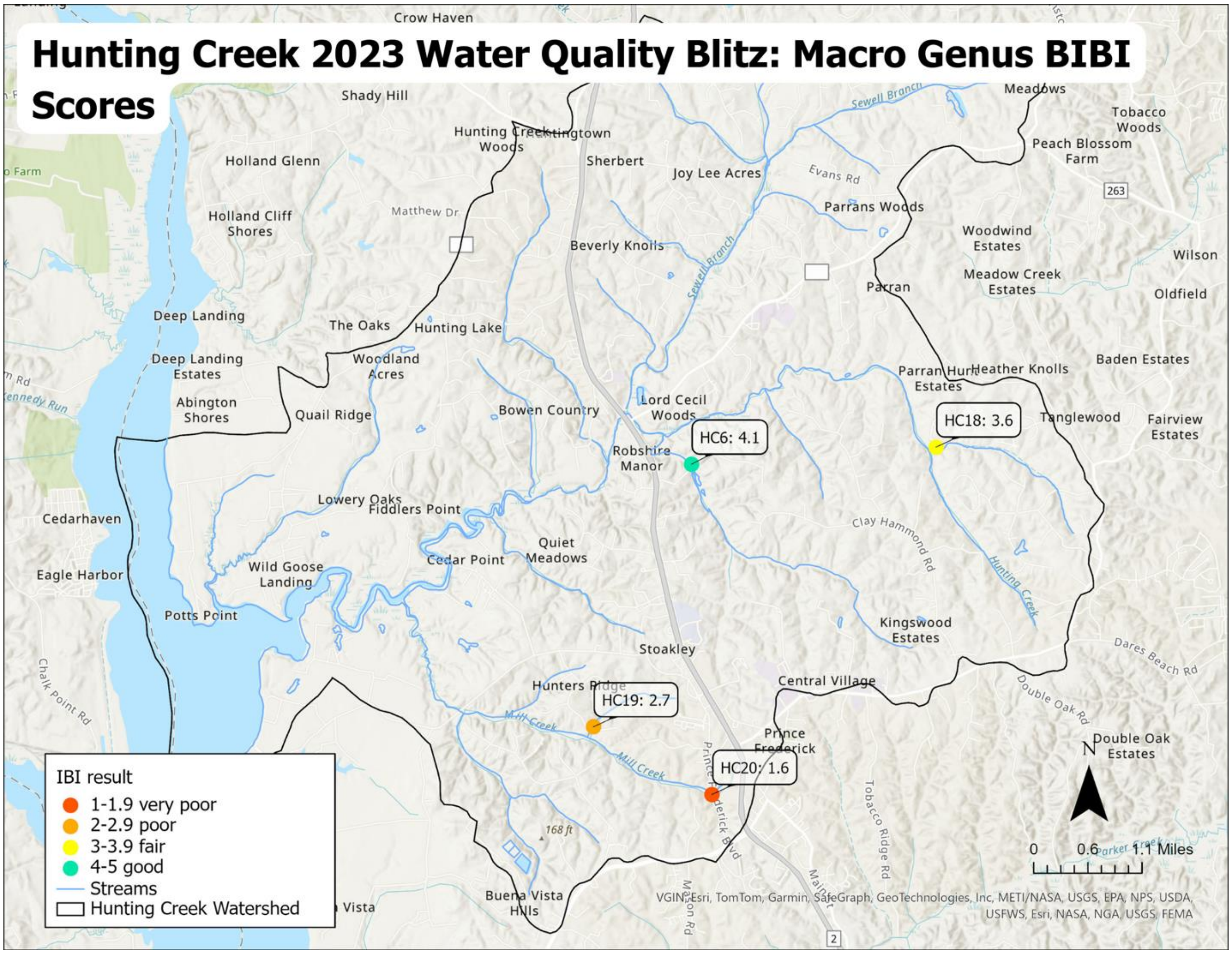


Table 4.1 presents an array of data that allows some speculation about why the BIBI scores indicate that ecological health differed among the four stream sites. For starters, HC6 and HC18, the two sites with the highest BIBI scores, had more kinds (taxa) of macros (43 and 48) than did the two sites with the lowest BIBI scores, HC19 and HC20 (30 and 13). Generally, more taxa (i.e., higher biodiversity) reflect better stream condition.

Overall, the macros collected at HC6 and HC18 included more taxa considered to be sensitive to water pollution and habitat disturbance compared to the macros collected at HC19 and HC20. Most macros genera within the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) are intolerant taxa and often decline in abundance and diversity following environmental degradation. Their presence in a stream is an indication of a healthy and high-quality resource. Based on wetted width measurements, HC6 and HC18 are somewhat wider streams than HC19 and HC20. Larger streams should offer more habitat for macros. HC20 not only had the lowest BIBI score of the four sites (1.6), but also the lowest total physical habitat score (118 out of 200 points possible), reflecting upper Mill Creek's generally degraded status.

Table 4.1 Macros metric vales, benthic index of biotic integrity (BIBI) scores, and physical habitat data at four stream sites, Hunting Creek watershed, 2023.

Genus Level Data	**HC6**	**HC18**	**HC19**	**HC20**
No. of Taxa	43	48	30	13
No. of EPT* Taxa	8	7	3	1
No. of Ephemeroptera Taxa	2	2	1	0
Percent Intolerant Taxa	13.2	7.8	8.7	0.7
Percent Ephemeroptera Taxa	37.7	1.4	0.3	0
No. of Scraper Taxa	0	0	0	0
Percent Climbers	42.4	26.6	42.1	10.6
BIBI Score	4.1 Good	3.6 Fair	2.7 Poor	1.6 Very Poor
Total Physical Habitat Score Maximum=200	141 (71%)	156 (78%)	162 (81%)	118 (59%)
Average Wetted Width (m)	2.9	2.4	2.1	1.5
Maximum Depth (cm)	NM	46	46	NM

- E = Ephemeroptera (mayflies), P = Plecoptera (stoneflies), T = Trichoptera (caddisflies), NM = not measured

With only two years of macros data at HC18, we can't conclude that the increase in BIBI scores from 2022 to 2023, moving from the poor to fair condition category, is a positive trend. But we are encouraged by this improving direction of change. The number of macros taxa at HC18 almost doubled from 26 in 2022 to 48 in 2023, another notable change in the right direction. At HC19, the number of macros taxa also increased from 19 in 2022 to 30 in 2023, even though the BIBI score did not change.

For those readers who want more details on the macros taxa that were collected at the four stream sites by FOHC volunteers in 2023 and identified to family and genus, please refer to Tables C-1 through C-4 in Appendix C. Available Tolerance Values that range from 1 (least tolerant) to 10 (most tolerant) are also included for the macros genera found at each site. Taxa considered to be intolerant of urbanization (development) have Tolerance Values of 0 to 3.

4.2.1 More Details on Physical Habitat Conditions at HC20

As mentioned above, the macros site that had the lowest BIBI score in 2023 (HC20) also had the lowest total Physical Habitat score (118 out of 200 possible points) of all four sampled sites (Table 4.2). In addition to having a narrower average wetted width, HC20 scored in the lowest (poor) condition category for channel flow status and in the marginal condition category for pool substrate characterization, pool variability, sediment deposition, and bank stability. HC20 also scored in the suboptimal category for epifaunal substrate/available cover, a key habitat requirement for macros. In contrast, HC20 scored in the optimal (best) condition category for three physical habitat parameters: channel alteration, vegetative protection, and riparian vegetative zone width.

Table 4.2 Physical habitat parameter scores and condition categories at four stream sites sampled for macros, Hunting Creek watershed, 2023.

Habitat Parameters	HC6	HC18	HC19	HC20
Epifaunal Substrate/ Available Cover	14 suboptimal	11 suboptimal	10 marginal	11 suboptimal
Pool Substrate Characterization	8 marginal	9 marginal	13 suboptimal	8 marginal
Pool variability	18 optimal	10 marginal	16 optimal	7 marginal
Sediment Deposition	7 marginal	11 suboptimal	10 marginal	9 marginal
Channel Flow Status	11 suboptimal	20 optimal	18 optimal	5 poor
Channel Alteration	20 optimal	20 optimal	20 optimal	19 optimal
Channel Sinuosity	11 suboptimal	17 optimal	15 suboptimal	11 suboptimal
Bank Stability	12 suboptimal	20 optimal	20 optimal	8 marginal
Vegetative Protection	20 optimal	20 optimal	20 optimal	20 optimal

Riparian Vegetative Zone Width	20 optimal	18 optimal	20 optimal	20 optimal
Total Score (200 Possible Points)	141 71%	156 78%	162 81%	118 59%

- For more information about how these ten physical habitat metrics are described and scored, see Appendix D.

These habitat scores for HC20 suggest that stream channel degradation, likely resulting from stormwater runoff in high imperviousness areas upstream, is a major factor contributing to very poor BIBI scores. Because the stream channel there is deeply incised (see photos of HC20 in Appendix A), storm-associated high flow events cannot easily overflow into the adjacent, well-vegetated flood plain and dissipate current velocity. By comparison, the sampled stream segments at HC6, HC18, and HC19 do not show evidence of channel incision (see photos of these sites in Appendix A).

Upper Mill Creek is clearly suffering from Urban Stream Syndrome (Walsh et al. 2005). But we believe there is a positive course of action. MD/DNR staff and Joe Berg, a stream restoration expert, visited upper Mill Creek in 2023 to assess the stream's condition. They informed the FOHC that a low-tech restoration project designed to reconnect the stream channel at and downstream from HC20 to its floodplain could halt stream channel incision, reduce the downstream transport of sediment and associated pollutants, create more instream aquatic habitat during baseflow conditions, increase macros and fish diversity and abundance, and restore several acres of adjacent non-tidal wetlands.

This improvement would not only benefit upper Mill Creek, but also Hunting Creek, the Patuxent River, and the Chesapeake Bay. Finding funding sources to support a restoration of upper Mill Creek, hopefully in coordination with Calvert County's Department of Public Works, is a key objective for the FOHC.

4.3 eDNA Pilot Study

Although limited to four stream sites in the Hunting Creek watershed, the eDNA Pilot Study conducted by FOHC volunteers in 2023 proved to be a valuable learning experience. We gained several insights that will help us understand and talk about what lives in our streams and how to monitor them.

What else did we learn?

1. Jonah Ventures did not find any DNA, human or otherwise, in our two field blank samples. So, we learned that we collected uncontaminated water samples for eDNA analyses from our four target streams.

4.3.1 A Little More on the Macros

2. Jonah Ventures found macros DNA in water samples collected at the four stream sites (see Tables 1-4 in Appendix E), but the number of macros taxa (families and genera) revealed by eDNA analysis was lower than the taxa numbers collected by FOHC on the same day at the same four sites with a D-net (Figure 4.7). The largest differences in macros diversity revealed by the two sampling methods occurred at HC6, HC18, and HC19. Among the four Pilot Study sites, there were more macros taxa unique to each sampling method than similar (Figure 4.8). Only Six families and two genera of macros were found in both D-net and eDNA samples (Table 4.3), so not much taxa overlap.

Figure 4.7

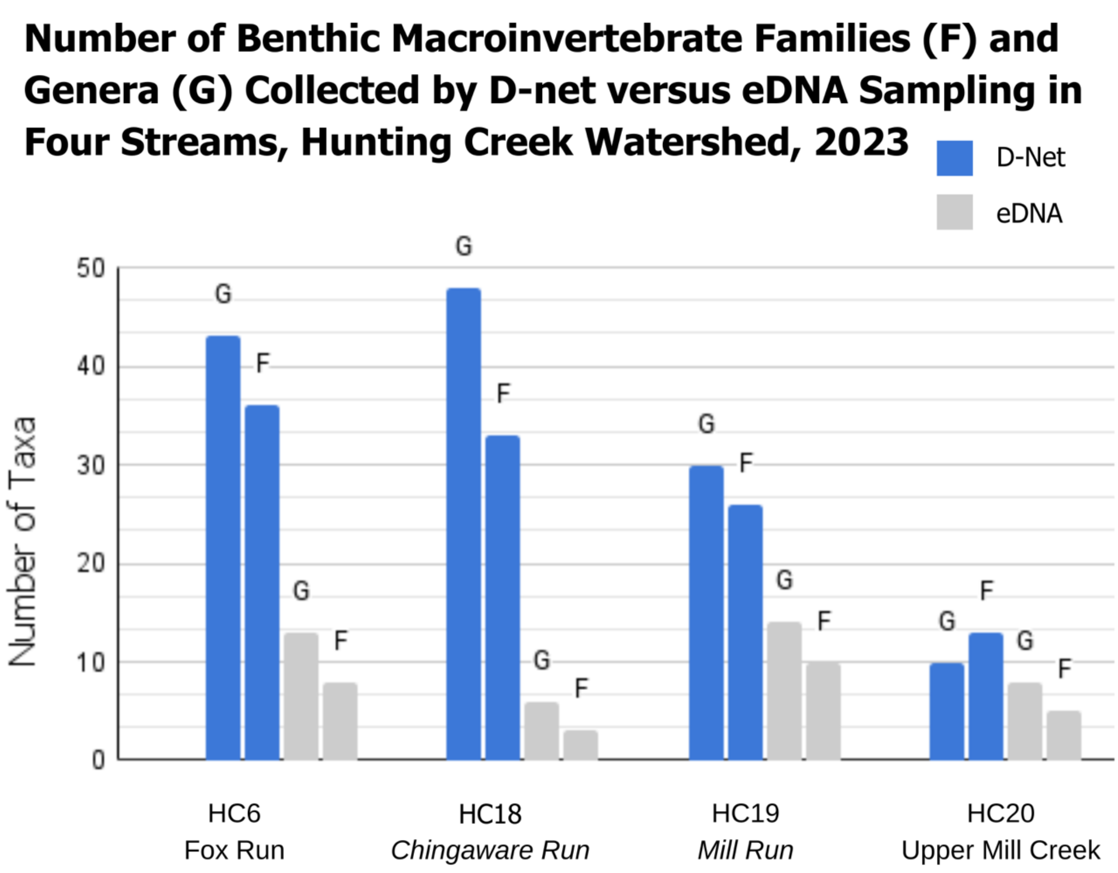


Figure 4.8

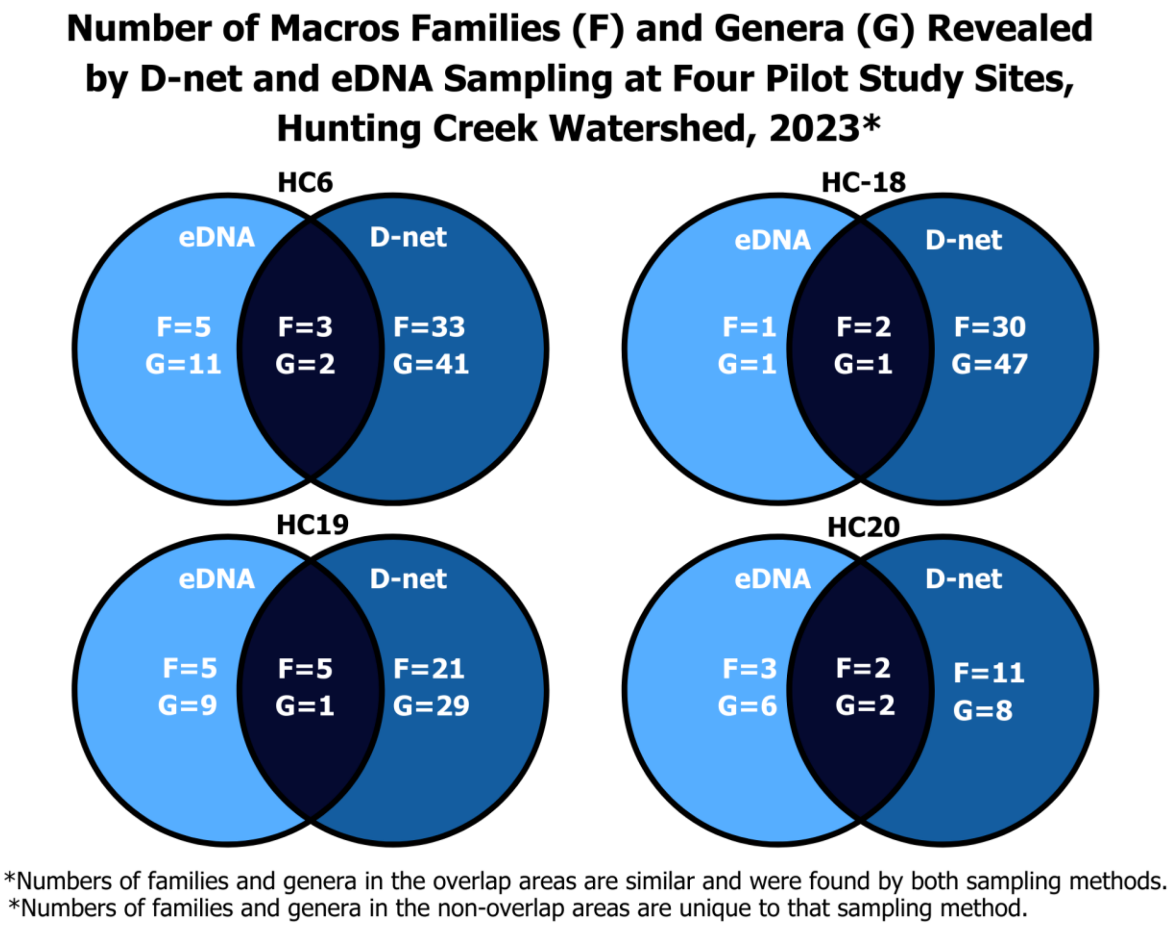


Table 4.3 Benthic macroinvertebrate families and genera collected with a D-net and revealed by eDNA sampling in four streams, Hunting Creek watershed, 2023.

Site ID	Family	Genus
HC6	Physidae Chironomidae Simulidae	Physa/Physella ---- Simulium
HC18	Physidae Chironomidae	Physa ----
HC19	Naididae Lymnaeidae Physidae Limnephilidae Chironomidae	---- ---- Physa/Physella ---- ----

HC20	Physidae Chironomidae	Physa/Physella

There are several plausible explanations for why the D-net collected more macros taxa that eDNA sampling, but determining which factors are most plausible goes beyond our areas of expertise and the scope of this report. We conducted the eDNA Pilot Study in 2023 and sent water samples to Jonah Ventures knowing that existing reference libraries for freshwater stream macros are incomplete and still being developed/updated. It's also likely that macros don't shed large quantities of DNA. So, these differences, although somewhat disappointing, were neither surprising nor unexpected.

Nevertheless, with regards to sampling macros to assess stream health, our Pilot Study yielded answers to these important questions:

(a) *Can eDNA sampling/analysis find macros in Hunting Creek watershed streams?* Yes, but as discussed above, the macros taxa richness revealed by DNA analysis was lower than what D-net samples collected.

(b) *Is eDNA sampling easier and quicker, on site, than using a D-net and MBSS protocols for collecting macros?* Yes. To collect two water samples per stream site for eDNA analysis takes about 10 minutes/per sample or 20 minutes/site, and the procedure can be done by one person. However, for safety reasons, FOHC always stives to use at least two-person sampling teams. To collect a D-net sample, pour it through the sieve bucket, carefully sort/wash off/discard sticks/large leaves, transfer the sieve bucket contents to one or more sample jars, and then preserve the macros samples takes two people at least 1-1/2 to 2 hours per site.

(c) *Is eDNA sample analysis cheaper than processing (sorting/Identifying) macros taxa collected in D-net samples?* No, but there is not a huge difference. The per site cost for eDNA analysis (not including sample shipping) to identify macros families and genera is about $290 (by Jonah Ventures), compared to a per site cost of about $175-$225 (depending upon the company/individual selected to process the samples). However, because of their larger volume and weight, ethanol-preserved D-net samples are more expensive to ship.

(d) *Can eDNA sampling/analysis be used as a replacement for D-net sampling and MBSS protocols to collect macros, calculate BIBI scores, and assess the health of non-tidal streams in the Hunting Creek watershed?* No, not currently. If the available primers and DNA reference libraries for freshwater macros improve significantly, eDNA sampling may be able to at least complement and could perhaps eventually replace conventional D-net sampling.

4.3.2 *Let's Also Talk About Fish*

Jonah Ventures also analyzed eDNA samples we collected at the four Pilot Study sites in April 2023 for fish DNA. These results are more satisfying and encouraging than the macros results (Table 4.4). All fish species revealed by eDNA analysis are native to some portion of Maryland. None are rare, threatened, endangered, or Species of Greatest Conservation Need (SGCN) in Maryland. Fish species found in the eDNA samples were "all the usual suspects" for Coastal Plain Maryland streams. And, perhaps of most importance for our Pilot Study, eDNA analysis did not find any "odd ball" fish species that should not be living in the Hunting Creek watershed.

Table 4.4 Fish species revealed by eDNA pilot study in four streams, Hunting Creek watershed 2023*

HC 6 **Fox Run**	**HC18** ***Chingaware Run***	**HC19** ***Willow Run***	**HC20** **upper Mill Creek**
American Eel Blacknose Dace Bluegill Brown Bullhead Creek Chubsucker-2 Chain Pickerel or Redfin Pickerel Eastern Mosquitofish Eastern Mudminnow-4 Golden Shiner-3 Green Sunfish Largemouth Bass Redbreast Sunfish or Pumpkinseed Satinfin Shiner Spottail Shiner Tessellated Darter-1 Yellow Bullhead	American Eel-3 Blacknose Dace Bluegill Creek Chubsucker-2 Eastern Mosquitofish Eastern Muddminnow-1 Green Sunfish Largemouth Bass Redbreast Sunfish or Pumpkinseed Redear Sunfish Tessellated Darter-4 Yellow Bullhead	Blacknose Dace-1 Creek Chubsucker-4 Eastern Mosquitofish-2 Golden Shiner Green Sunfish-3	Blacknose Dace-1 American Eel-3 Eastern Mosquitofish-4 Eastern Mudminnow Green Sunfish-2 Redbreast Sunfish or Pumpkinseed

*Numbers are the relative abundance ranks of the four most common species in each stream

HC6 had the most fish species: 18 if you count all the possibilities, or 16 if you count only one species in each of the two pairs of species that DNA analysis could not distinguish. Furthermore, if one accepts the assumption that the sum of the number of DNA sequence reads for each line of base pair sequences for a given fish species in the Jonah Ventures spreadsheet approximates the relative abundance of that species, then the four most common fishes found at HC6 were Tesslated Darter (#1), Creek Chubsucker (#2), Golden Shiner (#3), and Eastern Mudminnow (#4). These four species comprised 82.5% of the fish community at HC6.

In addition to this site's robust fish diversity, eDNA analysis also revealed two species categorized by MD/DNR as intolerant/pollution sensitive fishes found only in good quality streams: Satinfin Shiner and Spottail Shiner. Spottail Shiner is a lithophilic spawner, meaning they require clean sand, gravel, and cobble substrates for spawning sites, where their eggs can develop in cracks and crevices. If sediment deposition buries the developing eggs, spawning success is greatly diminished. Redfin pickerel, also possibly found at HC6, is somewhat sensitive to pollution and habitat degradation and therefore found only in fair to good quality streams. The other fish species found at HC6 by eDNA analysis are pollution tolerant and can be found in any quality stream.

eDNA analysis also revealed that site HC18 has a relatively robust fish community of 12 or 13 species, including many of the same species found at HC6 plus Green Sunfish, Largemouth Bass, Redear Sunfish, and Yellow Bullhead. All fish species found at HC18 are native to some portion of Maryland. None are rare, threatened, endangered, or SGCN in Maryland However, the International Union for Conservation of Nature (IUCN) added American Eel to its RED LIST (species at very high risk of extinction) in 2014. The U.S. Fish and Wildlife Service does not think American Eel warrants being listed under the Federal Endangered Species Act at this time. Nevertheless, the Atlantic States Marine Fisheries Commission is working to reduce mortality and increase conservation of American Eel due to its currently depleted status. All fish species found at HC18 by eDNA analysis are pollution tolerant and can be found in any quality stream. The four most common fishes (Eastern Mudminnow, Creek Chubsucker, American Eel, Tessellated Darter) comprised 82.9% of the fish community at HC18.

In contrast to sites HC6 and HC18, fish diversity, as revealed by eDNA analysis, was much lower at HC19 and HC20, with only 5 and 6 or 7 species found, respectively. Blacknose Dace, a pollution tolerant species and probably the most widely distributed stream fish in Maryland, was most common at both HC19 and HC20. These two streams drain the western portion of the Prince Frederick Town Center, where extensive deforestation, excavating, grading, soil erosion, and often inadequate stormwater management has occurred in the past and is continuing.

In 2017, the upper Mill Creek catchment was 14.6 % impervious, above the 10% imperviousness threshold where stream habitats often show signs of stress and degradation. HC20 is in upper Mill Creek, a stream with a deeply incised channel that has been and still is being impacted by stormwater runoff from developed areas upstream with high imperviousness. So, it is not surprising to us that HC20 and HC19 had, by far, the lowest fish species diversity of the four Pilot Study sites. Other factors may be contributing to the low biodiversity, but it's likely that development is playing a major role.

4.3.3 How Does Fish Diversity at HC6 and HC18 Compare to Diversity in Other Calvert County Streams?

MD/DNR's MBSS sampled several County streams with electrofishing gear between 1997 and 2017. None of these streams had as many fish species as our eDNA Pilot Study found in Fox Run (HC6) in 2023 (16-18). A close second was a site in Lyons Creek, sampled by the MBSS in 1997, that had 15 fish species, including five that our 2023 Pilot Study did not find in the Hunting Creek watershed: Fallfish, Least Brook Lamprey, Rosyside Dace, Tadpole Madtom, and Yellow Perch. The Fish Index of Biotic Integrity (FIBI) score at the Lyons Creek site is 4.7 (good), on a scale from 1.0 to 5.0. A second site in Lyons Creek, also sampled by the MBSS in 1997, had 14 fish species and a FIBI score of 5.0 (good). Lyons Creek drains portions of northern Calvert County and southern Anne Arundel County before confluencing with the Patuxent River.
Two other Calvert County streams sampled by the MBSS had relatively high fish diversity (10 or more species): Hall Creek (2004), with 13 species and a FIBI score of 4.3 (good); and Tucker Creek (1997), with 10 species and a FIBI score of 3.0 (fair).

High fish diversity is associated with higher FIBI scores in these Calvert County streams sampled by the MBSS. This relationship suggests to us that two of our four eDNA Pilot Study streams in the Hunting Creek watershed, Fox Run and *Chingaware Run* (HC6 and HC18), would probably have FIBI scores in the high 3's (fair) and maybe into the 4.0 to 5.0 (good) range, if FIBIs could be calculated from eDNA sample analysis results. Currently, these calculations are not possible. Two of the six metrics used by MD/DNR to calculate fish IBIs in Coastal Plain streams include measures of absolute abundance, specifically Abundance Per Square Meter and Percent Abundance of Dominant Taxa (Southerland et al. 2005). It's not yet certain that DNA analysis can provide estimates of fish species abundance with known and acceptable levels of accuracy.

4.3.4 What About Other Stream Fish Data for the Hunting Creek Watershed?

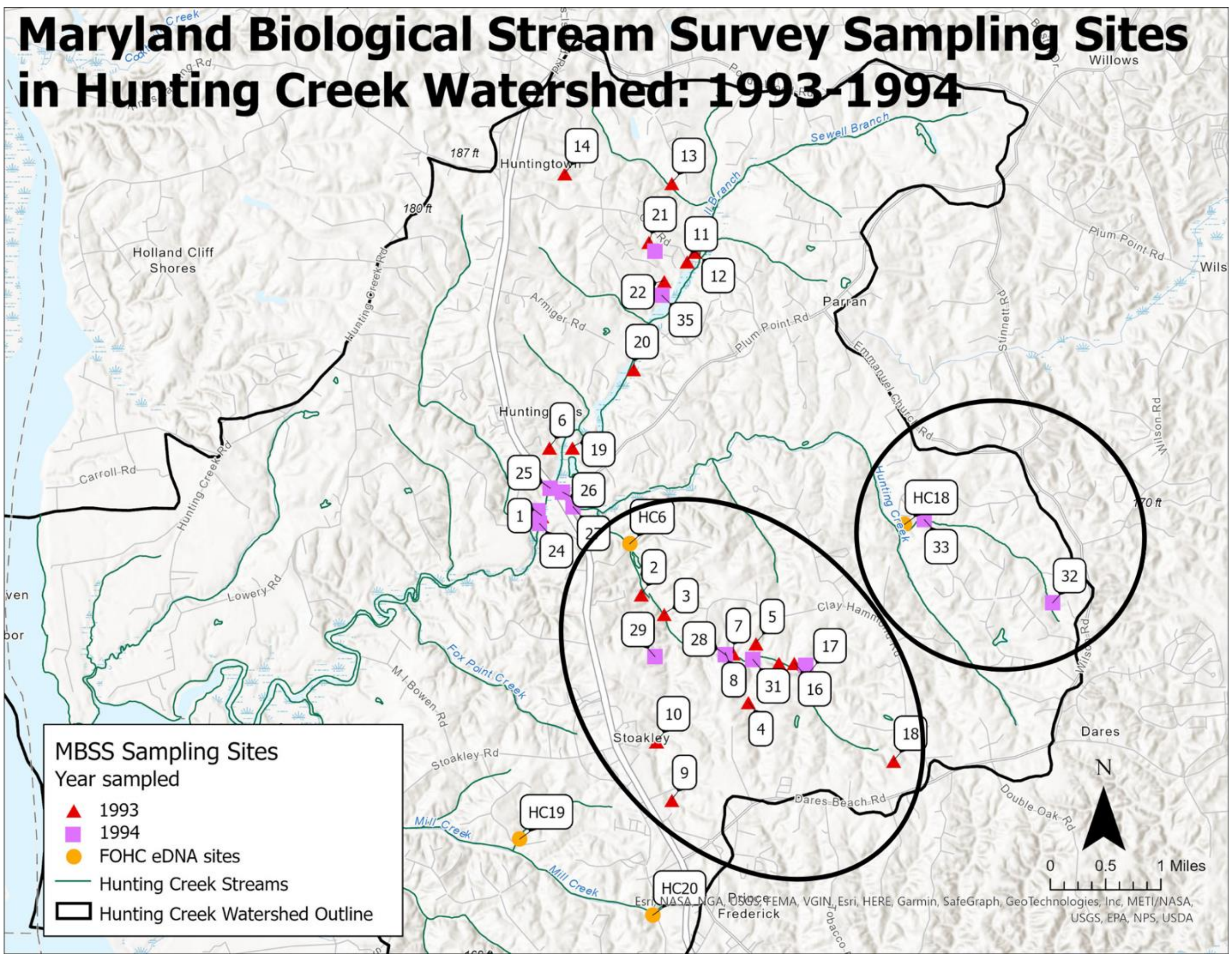
Our 2023 eDNA Pilot Study found as many or more fish species in two Hunting Creek watershed streams (Fox Run and *Chingaware Run*) than the MBSS found in other

Calvert County streams. But are there any MBSS fish data collected in the Hunting Creek watershed? If so, can a comparison with these data sets tell us anything more about the value of eDNA sampling for describing fish diversity in our watershed?

Most recently, in 2004, the MBSS sampled one unnamed tributary to Sewell Branch (a stream the FOHC has "unofficially" named *Barberry Branch*) in the northeast portion of the Hunting Creek watershed. Only three fish species were found: Blacknose Dace, Eastern Mudminnow, and Tessellated Darter.   No MBSS sampling has occurred in the Hunting Creek watershed since then. So, there are no recent stream fish data for comparison.

However, during MD/DNR's 1993 and 1994 MBSS Pilot Studies, they sampled 31 sites throughout the Hunting Creek watershed (Figure 4.9). Fortunately, 13 sites with fish data were in Fox Run and East Fox Run, upstream from our site HC6, and two sites were located in *Chingaware Run*, upstream from our site 8b.

Figure 4.9



The MBSS Pilot Study and our eDNA Pilot Study were conducted 30 years apart, so these data sets don't offer the ideal comparison to address the question: "Did eDNA sampling reveal more or few fish species than conventional electrofishing gear?" But since we don't have any other options, we took the plunge.

The short answer to the question is that more species were found using eDNA. eDNA samples collected in 2023 found 8 or 9 more fish species in *Chingaware Run* (Figure 4.10) and 5 more species in Fox Run (Figure 4.11) than did the MBSS in 1993-94 using electrofishing. eDNA sampling found the same fish species that were collected by electrofishing (see areas where the circles overlap in Figures 9 and 10), with one exception. The MBSS collected Chain Pickerel in *Chingaware Run*, a species not revealed by eDNA sampling at HC18 in 2023.

Because of the 30-year time span between the MBSS sampling and our eDNA Pilot Study, many factors, in addition to sampling method, could explain the observed differences in fish species diversity revealed by electrofishing and eDNA sampling. Attempting to elucidate these other factors goes beyond the scope of our Pilot Study and this report and is probably not feasible with any reasonable degree of certainty.

Figure 4.10

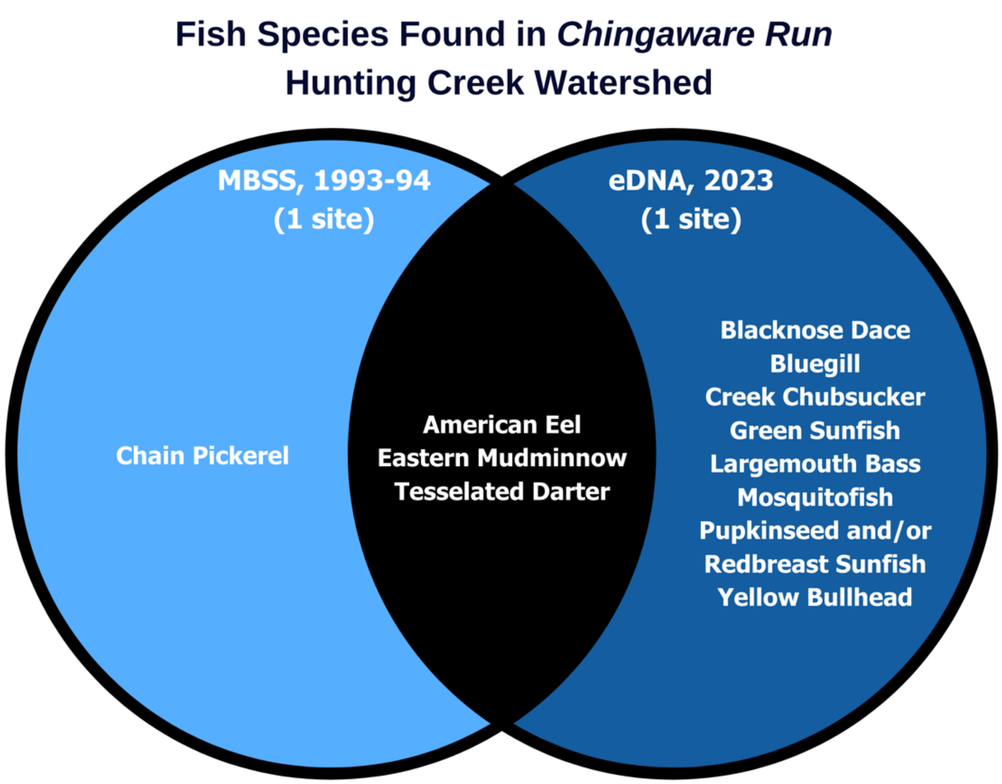
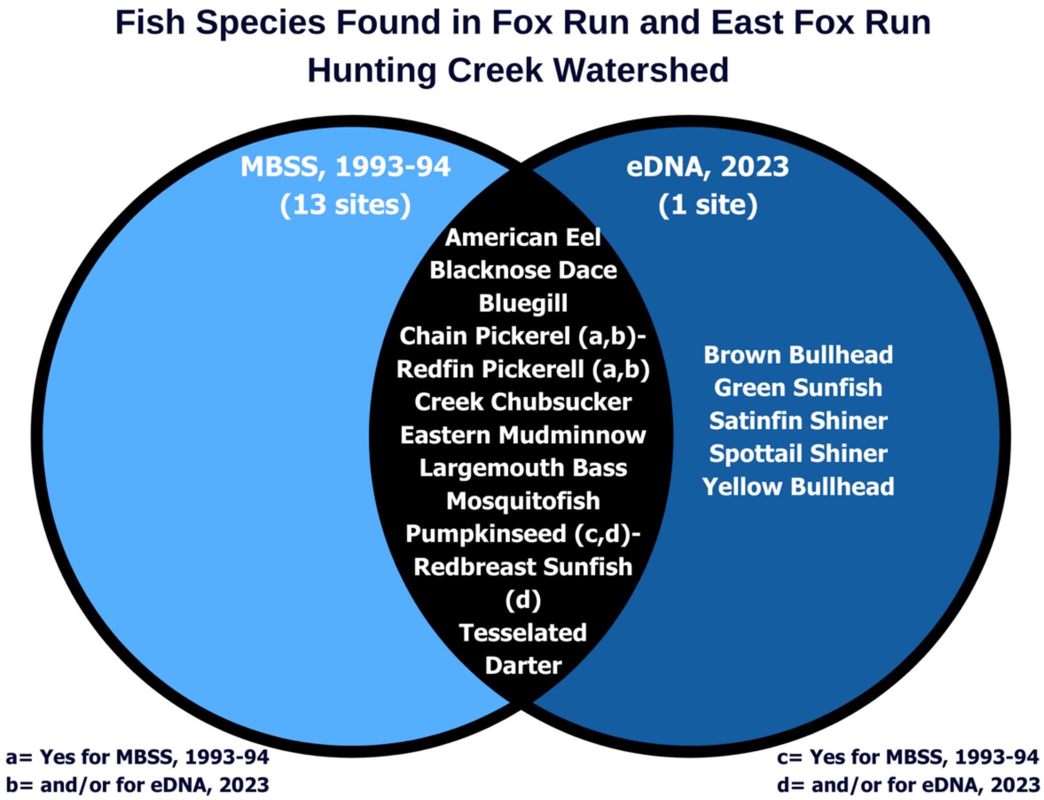


Figure 4.11



Based on what we've learned, eDNA sampling appears to be an acceptable and doable sampling method for a volunteer-based, watershed association (like the FOHC) to use to find out what fish species live in their streams. The FOHC has neither the budget to purchase a backpack electrofisher, blocking seines, dip nets, and other fish sampling equipment like the MBSS uses (a rough cost estimate of $25,000), nor volunteer numbers to staff a four-person sampling team with sufficient expertise to accurately identify all fish species collected in the field.

Even if eDNA sampling alone cannot yet yield FIBI scores that, in combination with BIBI scores, can help us assess stream health, just knowing what fish species live in our watershed is valuable information. FOHC members participate in exhibits at various public events (e.g. Patuxent River Appreciation Days), to *inspire* and *inform* people who stop by to talk with us about the Hunting Creek watershed. In addition to scenic photos of the tidal creek, we show them maps and graphs of water quality monitoring results. Now, knowing how many and which fish species live in at least four of our streams, we can add some nice fish photos to our exhibit and, hopefully, encourage visitors to linger longer.

5 LOOKING AHEAD

In three years, 2021-2023, the FOHC more than doubled the number of non-tidal stream sites sampled during the annual Water Quality Blitzes from 10 to 21. In addition to measuring nitrogen, phosphorous, and turbidity, we added macros sampling at two sites in 2022 and at four sites in 2023, to help us assess ecological integrity. To describe fish diversity in our watershed, the FOHC conducted an eDNA Pilot Study at four sites in 2023. To garner more insights into seasonal variability in water quality, we launched a Quarterly Water Monitoring Program in October 2023. Nitrogen, ammonium, total suspended solids, turbidity, conductivity, dissolved oxygen, and temperature are being measured at six of the 21 Blitz sites. The findings from the Quarterly will be presented in a separate report in late 2024.

So, what else should the FOHC strive to accomplish in 2024 and beyond? If we had more active volunteers and a significant budget, we could accomplish a lot. But reality says otherwise.

Here's an ambitious list of objectives ranging from most feasible/higher priority to less feasible/lower but still important priority.

1. Continue to participate in ACLT's annual Water Quality Blitz and continue our Quarterly Water Monitoring Program. Add measurements of pH to the Quarterly Program using the recently acquired pH meter.

   Keeping our current monitoring activities going into the foreseeable future is important. Detecting changes in water quality that are occurring/may occur in the Hunting Creek watershed is complicated by annual fluctuations in the weather and by climate change. To account for this background variability and increase our abilities to detect changes in nitrogen, for example, requires at least 10 years of monitoring data.

   Another reason for why the FOHC should continue our water monitoring programs is because the US EPA's Chesapeake Bay Program may be shifting some of its focus to smaller watersheds, to better understand the effectiveness of best management practices (BMPs) on water quality (Blankenship 2024). The Hunting Creek watershed encompasses about 30 square miles, perhaps in the size range of interest to the Bay Program. This shift in focus may also include a community science aspect and efforts to involve watershed groups, like the FOHC, and their citizen science monitoring programs.

2. Continue to use our water quality monitoring results as one source of support for advocacy positions taken by the FOHC that are focused on protecting the environment.

3. Continue to share our water quality monitoring data with Calvert County agencies and elected officials.

4. Explore ways to increase the FOHC's active core membership and connect with watershed residents who are willing to help fund our water monitoring activities.

5. Consider collecting macros and eDNA samples (for fish and mussel diversity) at several new stream sites in the Hunting Creek watershed where we have not collected macros or eDNA samples before. Candidate sites are HC3 (Little Lyons Creek), HC8a (Sewell Branch), HC9 (Reits Branch), and HC10b (Fox Point Creek).

6. Consider adding a new Blitz site in upper Sewell Branch, upstream from its confluence with *Barberry Branch*, in the far northeast corner of the watershed.

7. Apply for a Watershed Assistance Grant to fund the development of a Watershed Assessment and Action Plan for the Hunting Creek watershed. A primary objective of the plan will be to examine all available water monitoring data to locate high quality streams that deserve extra protection and degraded streams that should be restored.

8. Consider conducting stream corridor and habitat assessments along high priority tributaries in the Hunting Creek watersheds, beginning with the segment of upper Mill Creek that flows through County-owned property. MD/DNR has a protocol ("Coastal Stream Corridor and Habitat Assessment"). and field data sheet ("Physical Assessment: Coastal Stream Corridor and Habitat Assessment") for walking along stream segments, observing stream habitat in and adjacent to the channel, and scoring/recording the condition (quality) of 10 habitat characteristics from Poor (1 point) to Marginal (2 points) to Fair (3 points) to Good (4 points).

9. Consider implementing a plan to continuously monitor changes in stream depth in upper Mill Creek, during rainfall/runoff events, to describe the characteristics of the stream's hydrograph. Because Mill Creek drains an urban area and the segment flowing through County-owned property has a deeply incised channel, it likely has a flashy hydrograph during rain events that should be documented.

10. Consider mapping the locations and describing the sizes/other characteristics of small ponds found throughout the Hunting Creek watershed. This topic is discussed in more detail on pp. 16-17 of our Spring 2022 report (Klauda et al. 2023).

11. Consider measuring stream discharge at several stream sites in the watershed, coincidentally with either spring Blitz or Quarterly sampling, if the appropriate equipment is available to the FOHC, so we can estimate nitrogen loads being transported downstream, in addition to measuring concentrations.

6 ACKNOWLEDGEMENTS

The coauthors of this report extend our appreciation to several property owners in the Hunting Creek watershed who gave us permission to access our sampling sites in 2023: Patty Long-Bradley and Bruce Bradley (HC6), Kyle Pellegrino (HC7), Calverton School and Hawit family (HC8a), Wahl family (HC10b), Craig and Judith Weilmuenster (HC16), Danielle Reader-Jolley and Twin Ponds HOA (HC15), Michael O'Donnell (HC18), Michael Clark Jr. (HC19), John and Lynda Williams (HC12), Charlz and Anna Cortex (HC21), Susan Noble (HC22), Karyn Molines (Calvert County, HC23), and the Calvert County Board of Commissioners (HC20 and 24).

We would not have been able collect water, macros, and eDNA samples in spring 2023 without the help of these dedicated volunteers: Frank McPhillips, Erin McPhillips, Kyle Greene, Kathryn Klauda, Evan Klauda, Michele Lyons, Becky Hunter, Ray Malmrose, Danielle Reader-Jolley, Alyssa Matanin, and students from the Calverton School.

We also thank Walter Boynton, Greg Bowen, and the staff at the American Chestnut Land Trust for leading the charge on the annual Water Quality Blitz event that started in the Parkers Creek watershed in 2017 and has expanded to include the Hunting Creek, St. Leonard Creek, Mill Creek (near Solomons), and Fishing Creek watersheds in Calvert County, plus several watersheds throughout southern Maryland.

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8 APPENDICES

A 2023 Sampling Sites

B Water Chemistry Data

C Benthic Macroinvertebrates

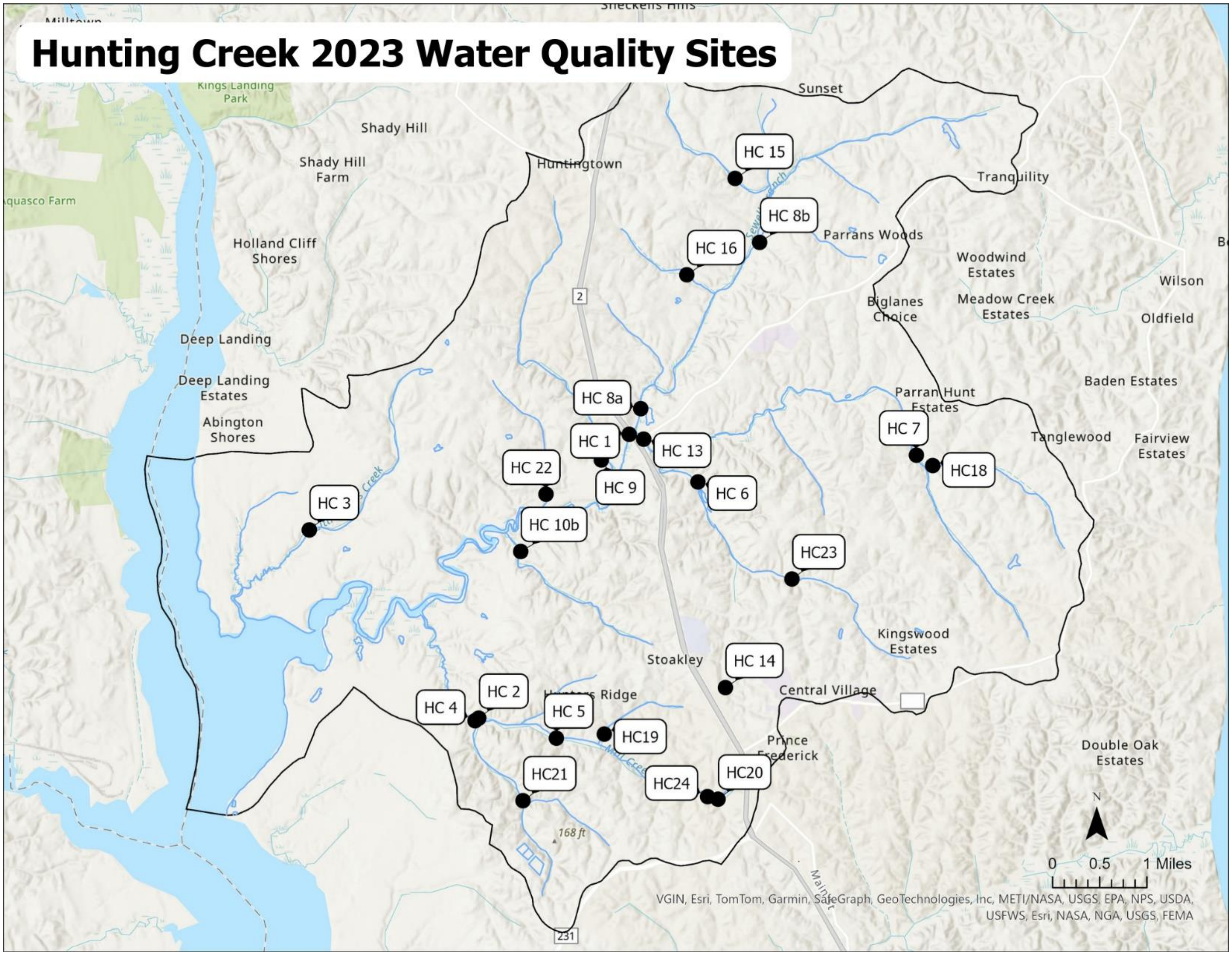
D Physical Habitat Assessment Field Data Sheet—Low Gradient Streams

E eDNA Pilot Study

F Catchments of Test Sites

Appendix A. 2023 Sampling Sites

Figure A1 Locations of Sites Per Table A2-1 Coordinates
The outline is the Hunting Creek watershed



*Site HC18 used for eDNA and macroinvertebrate samples only

Table A1 Site Coordinates and Stream Names

Site ID	Latitude	Longitude	Stream name
HC1	38.584843	-76.607017	Hunting Creek, Rt 2/4 Bridge
HC2	38.550865	-76.630076	Mill Creek, Stoakley Rd
HC3	38.573407	-76.656031	Little Lyons Creek, Hunting Creek Rd
HC4	38.550589	-76.630649	*College Creek**, Stoakley Rd
HC5	38.548495	-76.618217	Mill Creek, Hunters Ridge
HC6	38.579128	-76.596507	Fox Run, Hunting Farms Ln
HC7	38.582335	-76.563076	Hunting Creek, Queensberry
HC8a	38.587877	-76.605287	Sewell Branch, near Calverton school
HC8b	38.607776	-76.587049	Sewell Branch, Cox Rd.
HC9	38.581740	-76.611284	Reits Branch, Walton Rd
HC10b	38.570806	-76.623676	Fox Point Creek, upstream of Hunting Creek
HC13	38.584250	-76.604804	Hunting Creek, Plum Pt. Rd.
HC14	38.554528	-76.592331	Fox Run, Fox Run Blvd.
HC15	38.615396	-76.590811	*Barberry Branch*, Ponds Woods
HC16	38.603900	-76.598206	*Quail Ridge Run*, Marley Run
HC18	38.581099	-76.560572	*Chingaware Run*, Queensberry (Macroinvertebrates only)
HC19	38.548962	-76.610882	*Willow Run*, Hunters Ridge
HC20	38.541126	-76.594114	upper Mill Creek, Prince Frederick Blvd
HC21	38.541040	-76.62333	*College Creek*, College Station,
HC22	38.577751	-76.61970	*Winterberry Creek*, Hunting Fields Manor
HC23	38.56750	-76.58208	East Fox Run, Hughes Tree Farm
HC24	38.541467	-76.595070	*Boulevard Branch* (Tributary to upper Mill Creek)

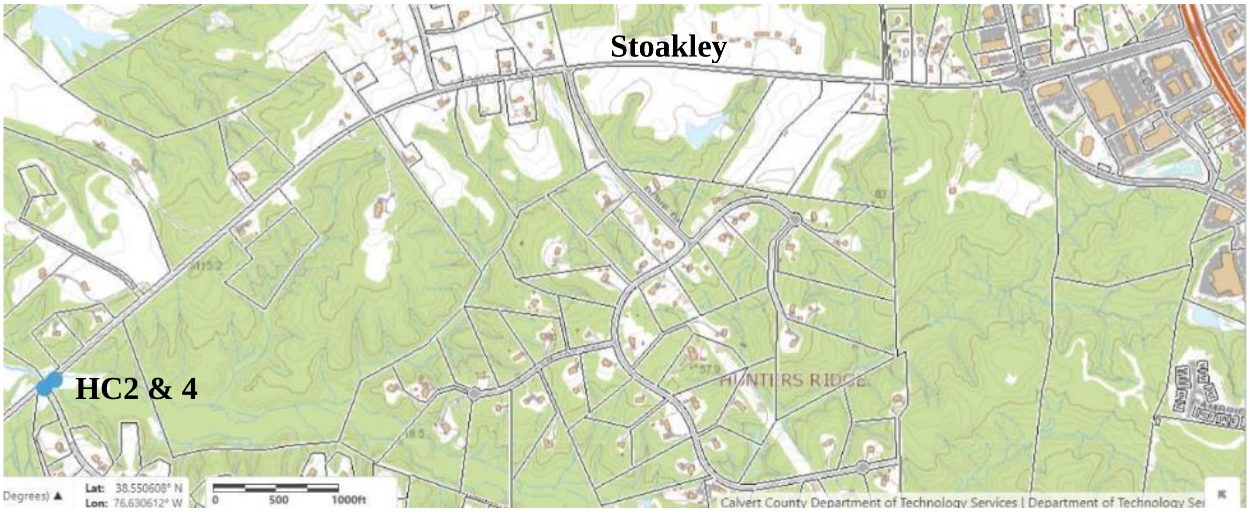
Coordinates are generally within 50’ of sample site, Features such as which side of a bridge or relationship to incoming tributary are reflected in coordinates detail maps in Appendix A
* *Unofficial FOHC names signified by italics*

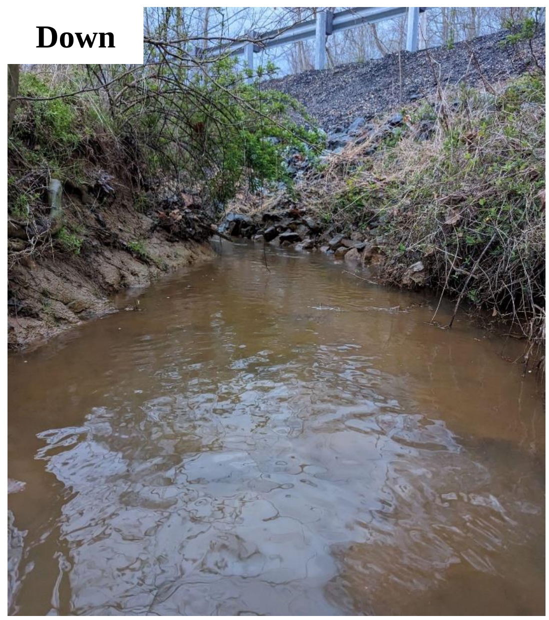
HC1
Hunting Creek at Route 2/4 bridge, 38.584843 -76.607017


Up

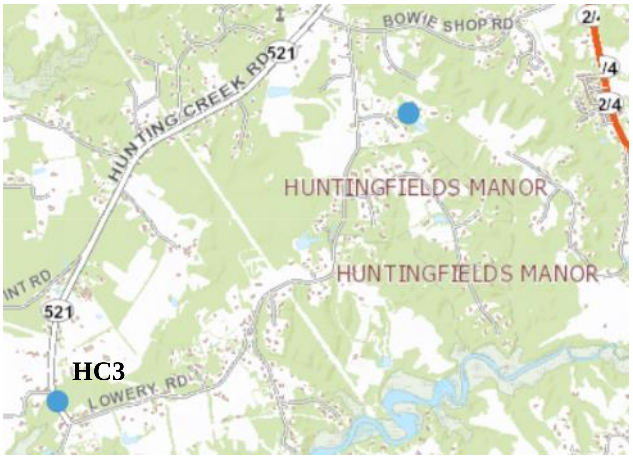

Down

HC2
Mill Creek at Stoakley Rd. bridge, 38.550865, -76.630076

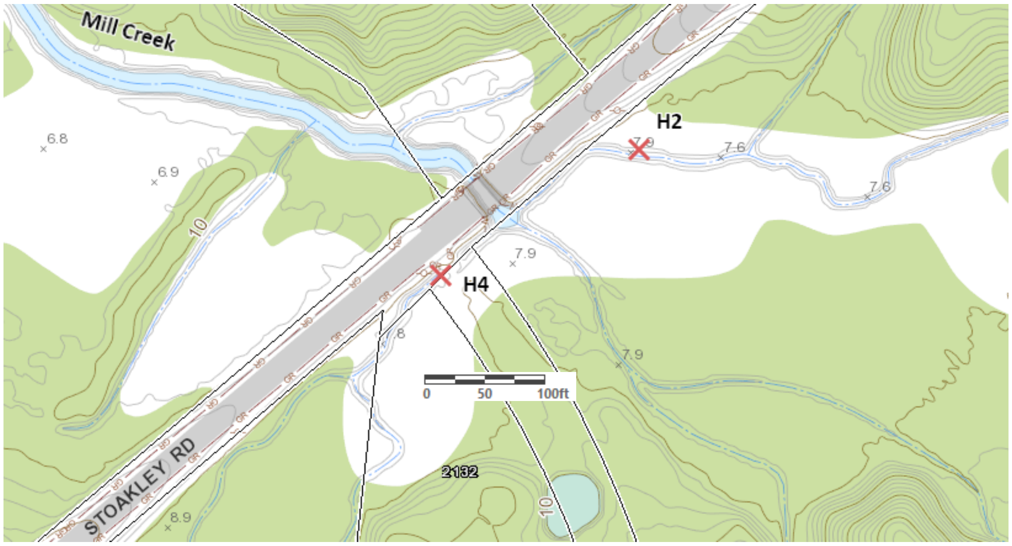




HC3
Little Lyons Creek at Hunting Creek Rd. bridge, 38.573407, -76.656031



HC4
***College Creek,* Unnamed Tributary (UT) to Mill Creek, just upstream from Stoakley Rd. bridge, 38.550589, -76.630649**

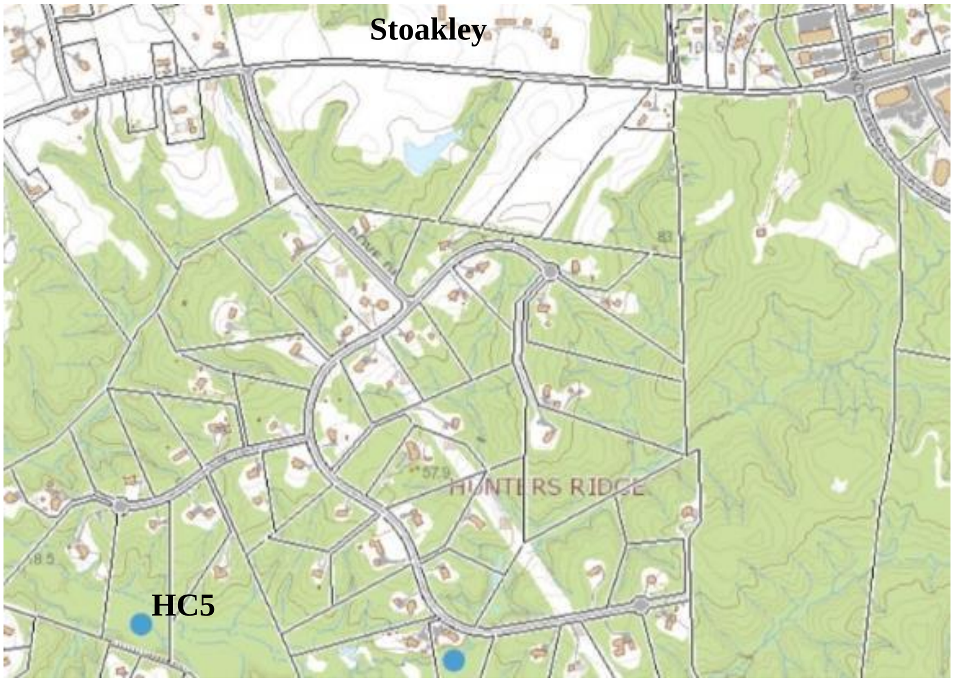


See HC2 for general map

Up

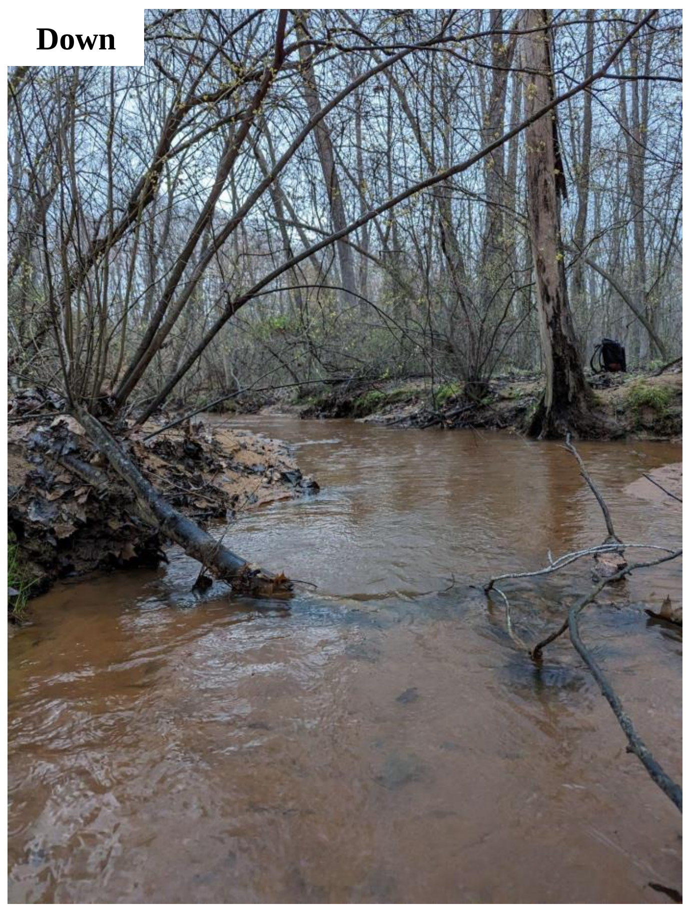

Down

HC5
Mill Creek, Behind 1440 Foxtail Lane, Hunters Ridge, 38.548495, -76.618217



Up

Down



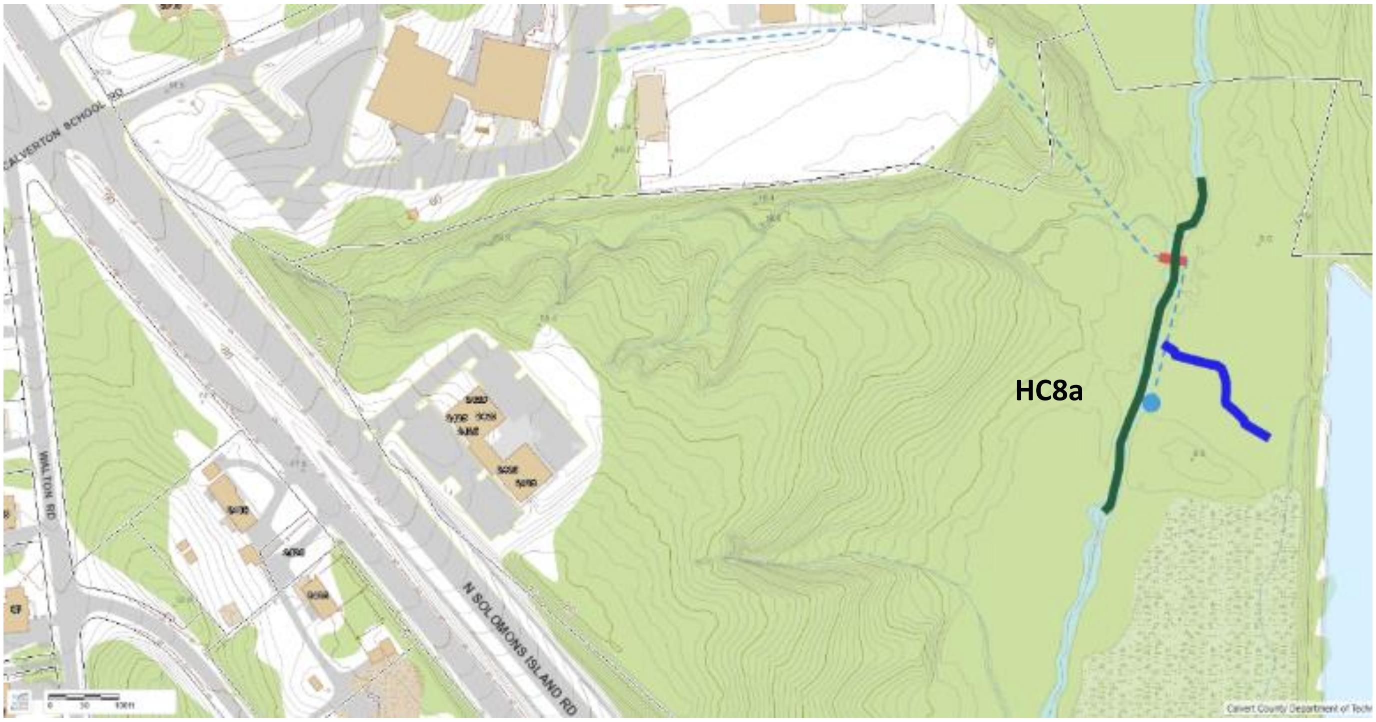
HC6
Fox Run at Hunting Farms Lane bridge, 38.579128, -76.596507

HC7
upper Hunting Creek west of Queensberry; 38.582335 -76.563076

Up

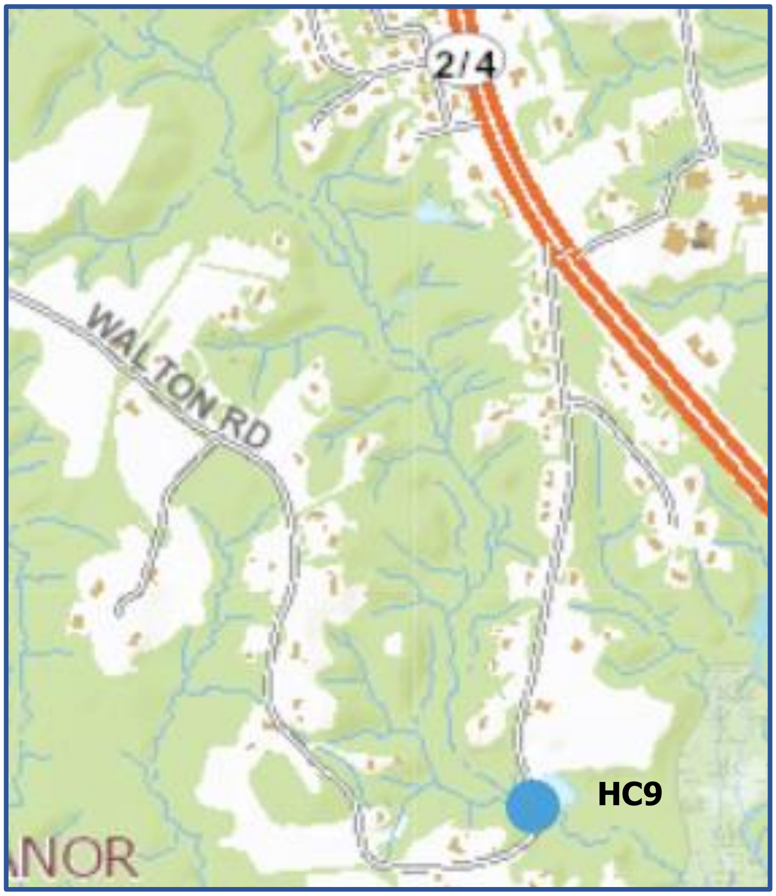
Down

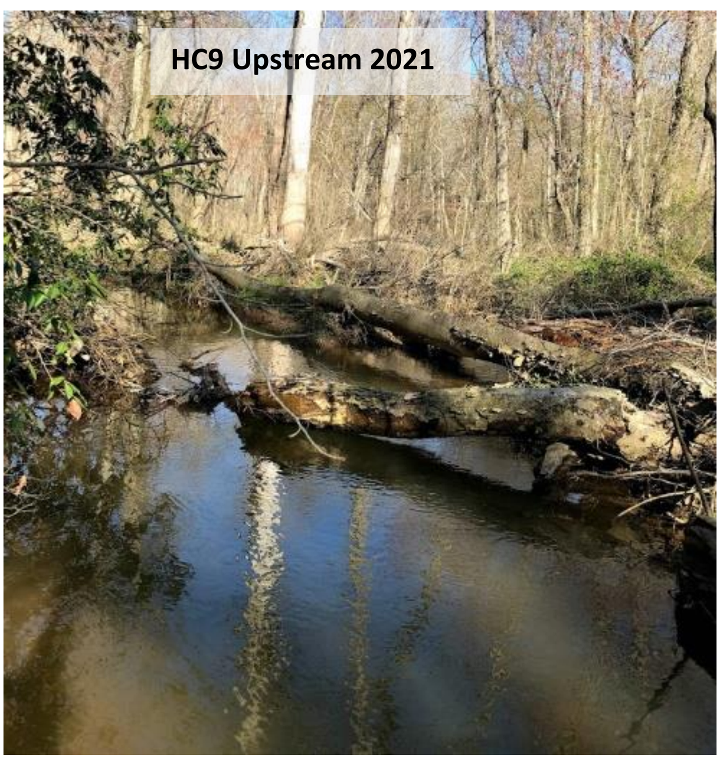
HC8a
Sewell Branch, Upstream of confluence w/Hunting Creek, 38.587877, -76.605287

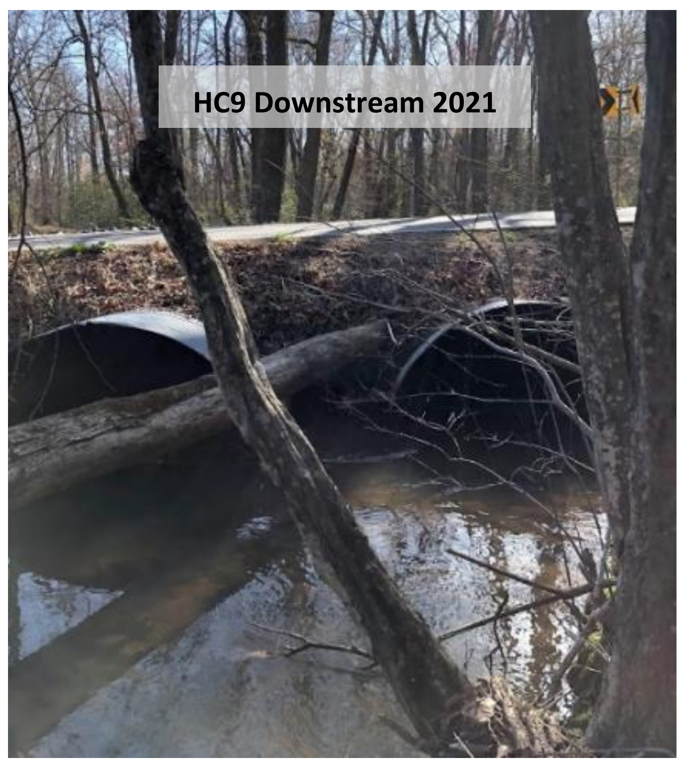


HC8b
Sewell Branch at Cox Rd. bridge, 38.607776, -76.587049

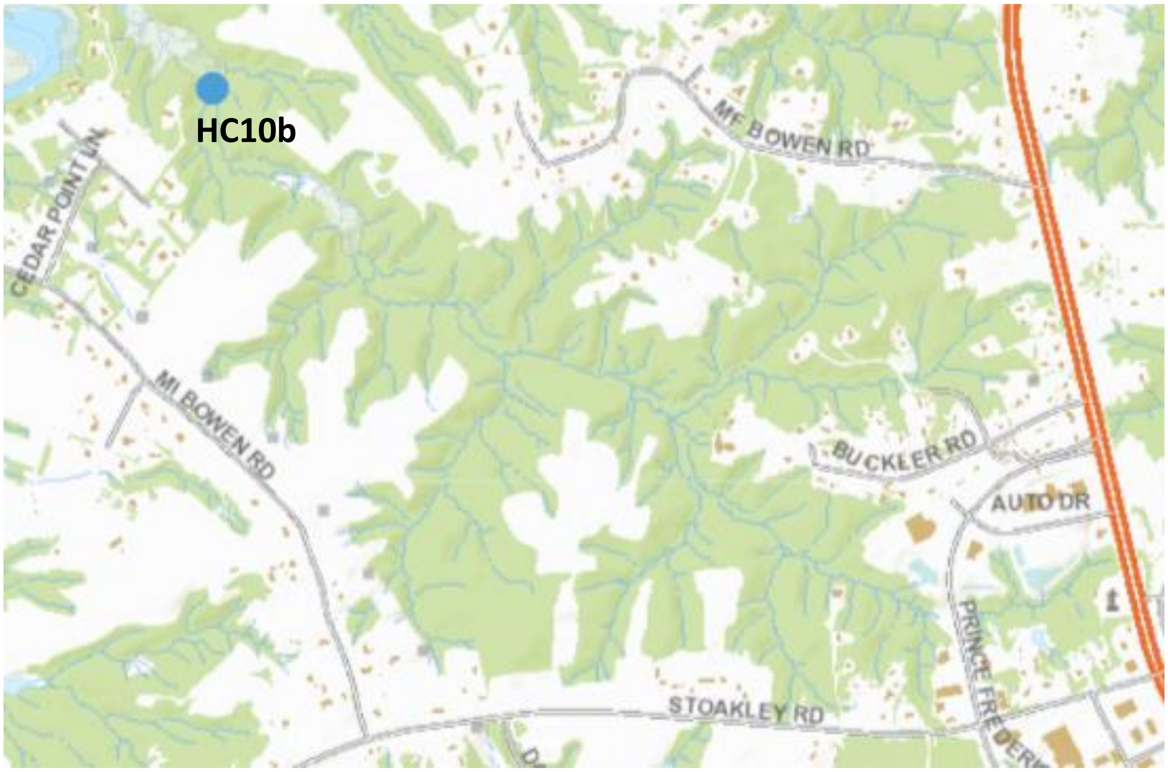
HC9
Reits Branch at Walton Rd. bridge, 38.581740, -76.611284



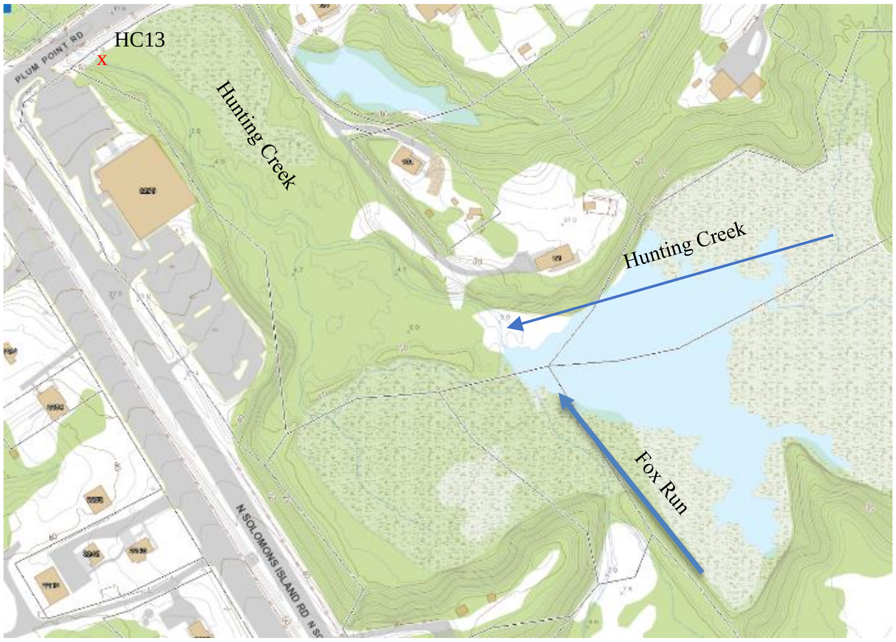




HC10b
Fox Point Creek, Upstream from confluence w/Hunting Creek,
38.570806 -76.623676

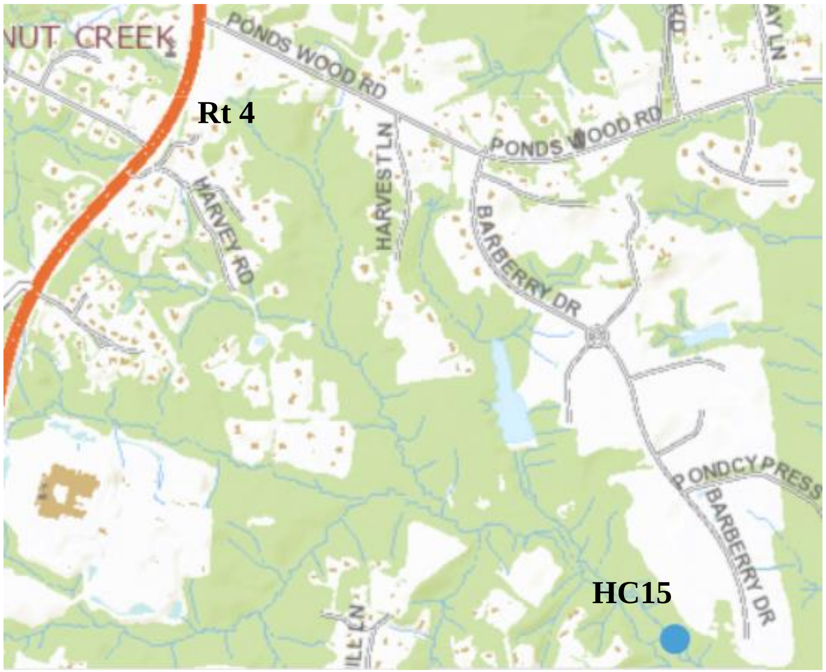


HC13
Hunting Creek, Just upstream from Plum Pt. Rd. bridge, 38.584250, -76.604804



HC14
Fox Run, access via Fox Run Blvd 38.554528 -76.592331

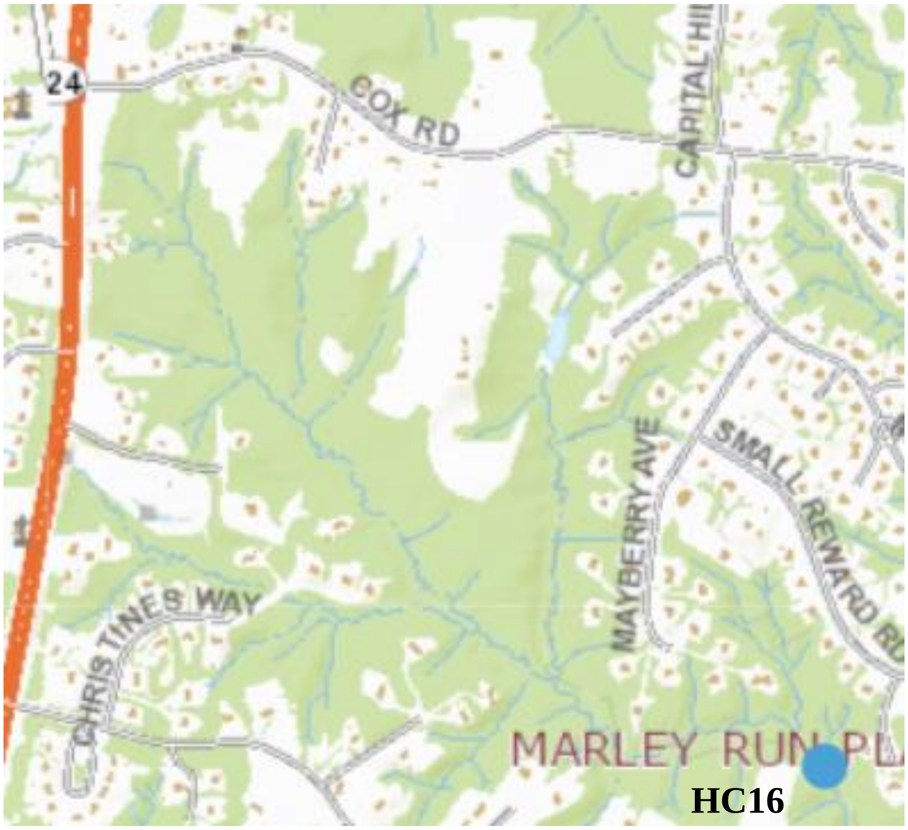
HC15
***Barberry Branch*, UT to Sewell Branch, Ponds Woods, 38.615396, -76.590811**



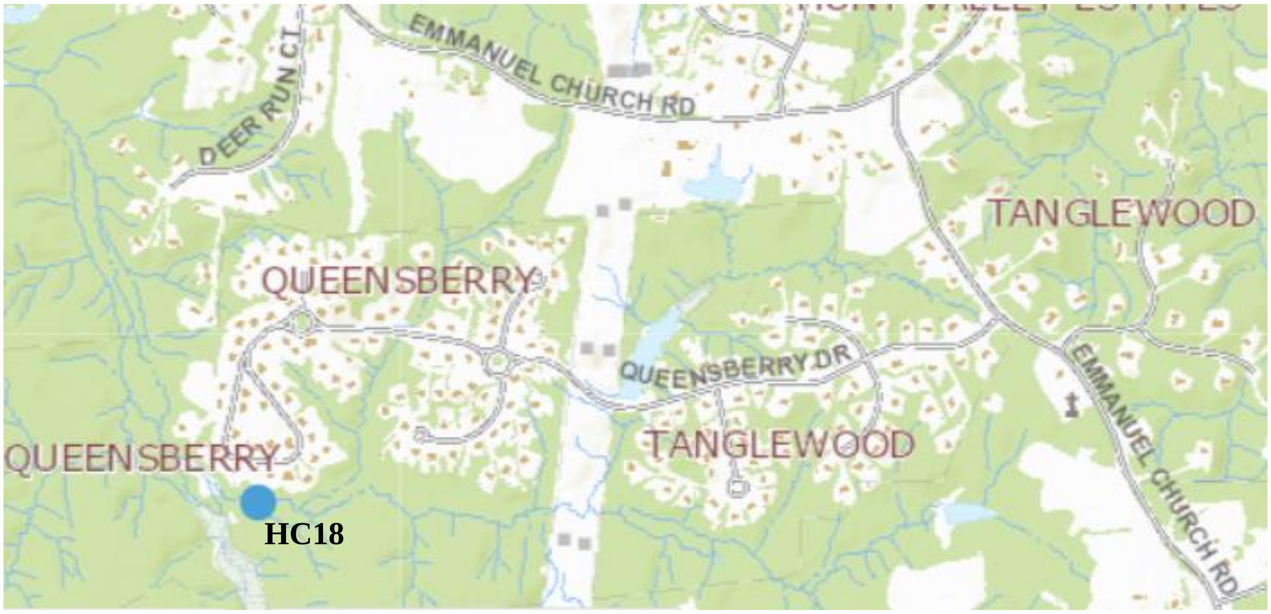
Up

Down

HC16
***Quail Ridge Run*, UT to Sewell Branch, Marley Run, Quail Ridge Way, 38.603900, -76.598206**



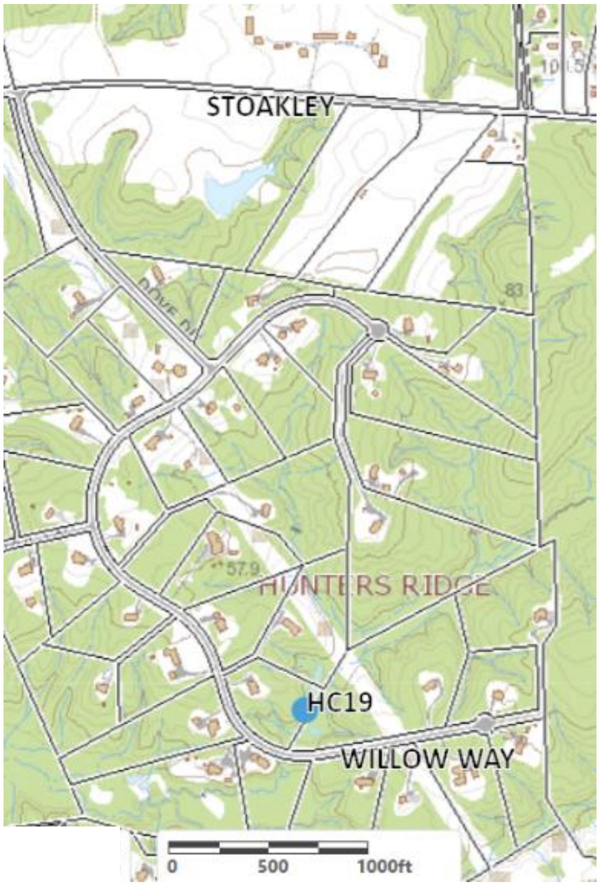
HC18 Macroinvertebrates and eDNA only
UT *Chingaware Run*, Queensberry, 38.581099, -76.560572 (Sample taken over a 75-m long segment of stream, coordinates roughly in middle of span)



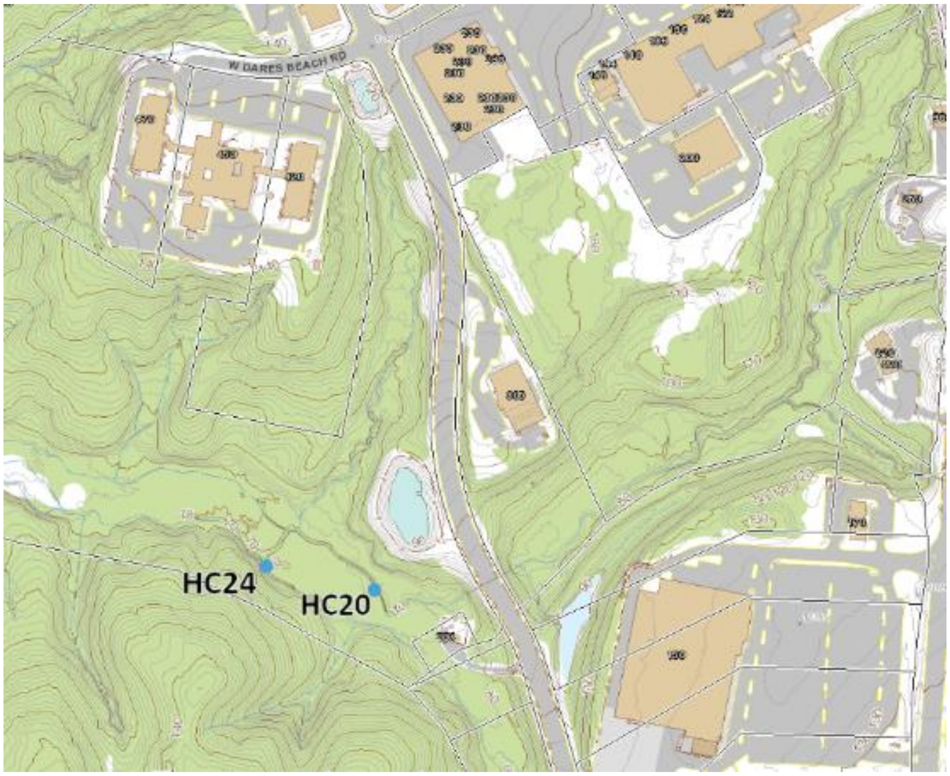
Picture was taken approximately midway along the 75m run.

HC19

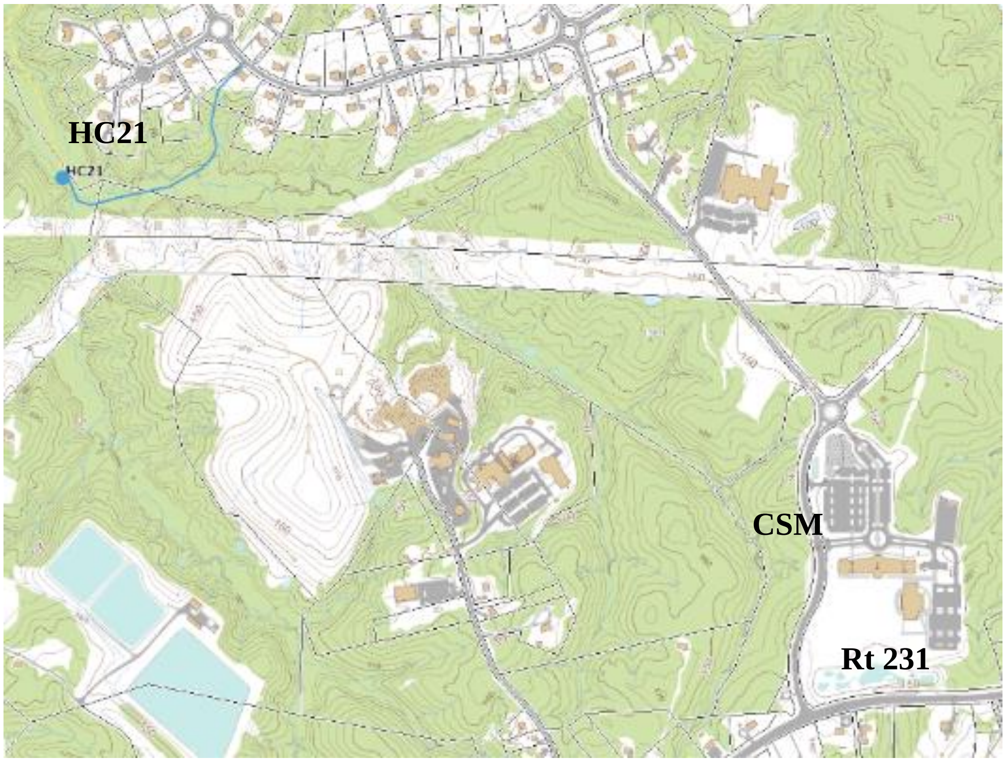
***Willow Run*, UT to Willow Way, Hunters Ridge, 38.548962, -76.610882 (Takes the place of HC11 for future sampling)**



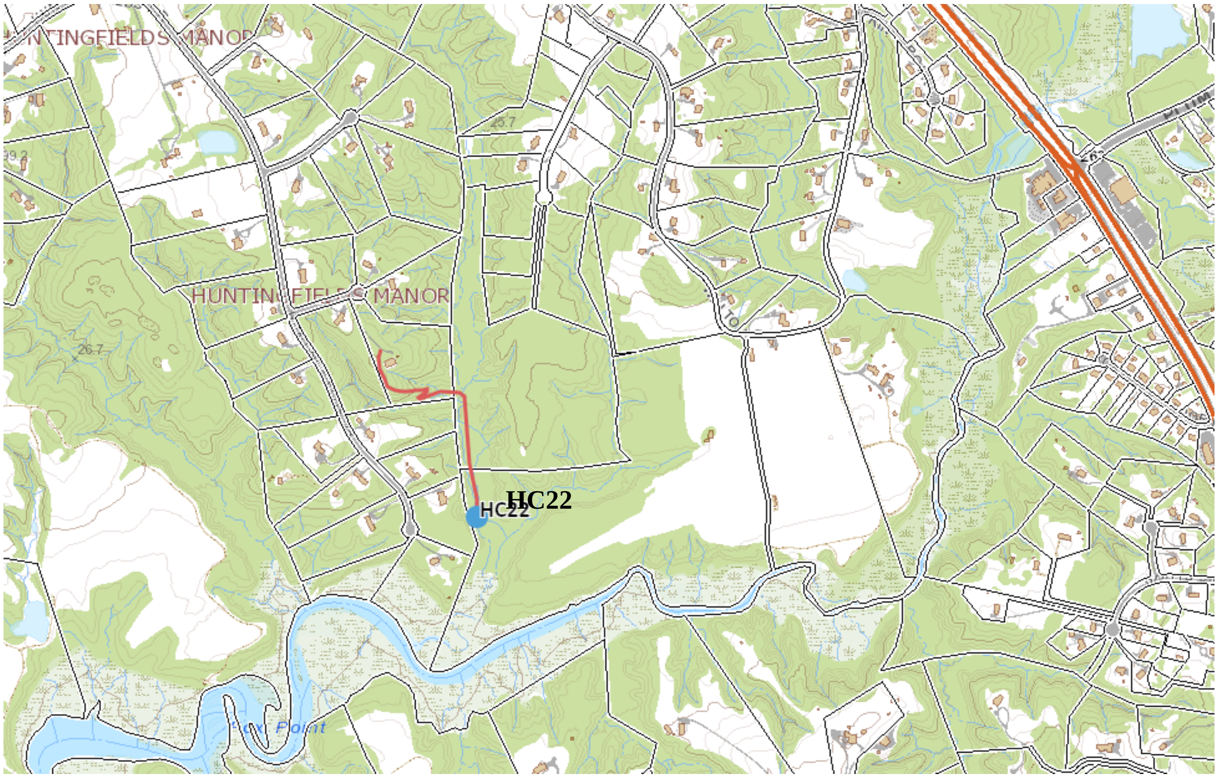
HC20
upper Mill Creek, 38.541126 -76.594114



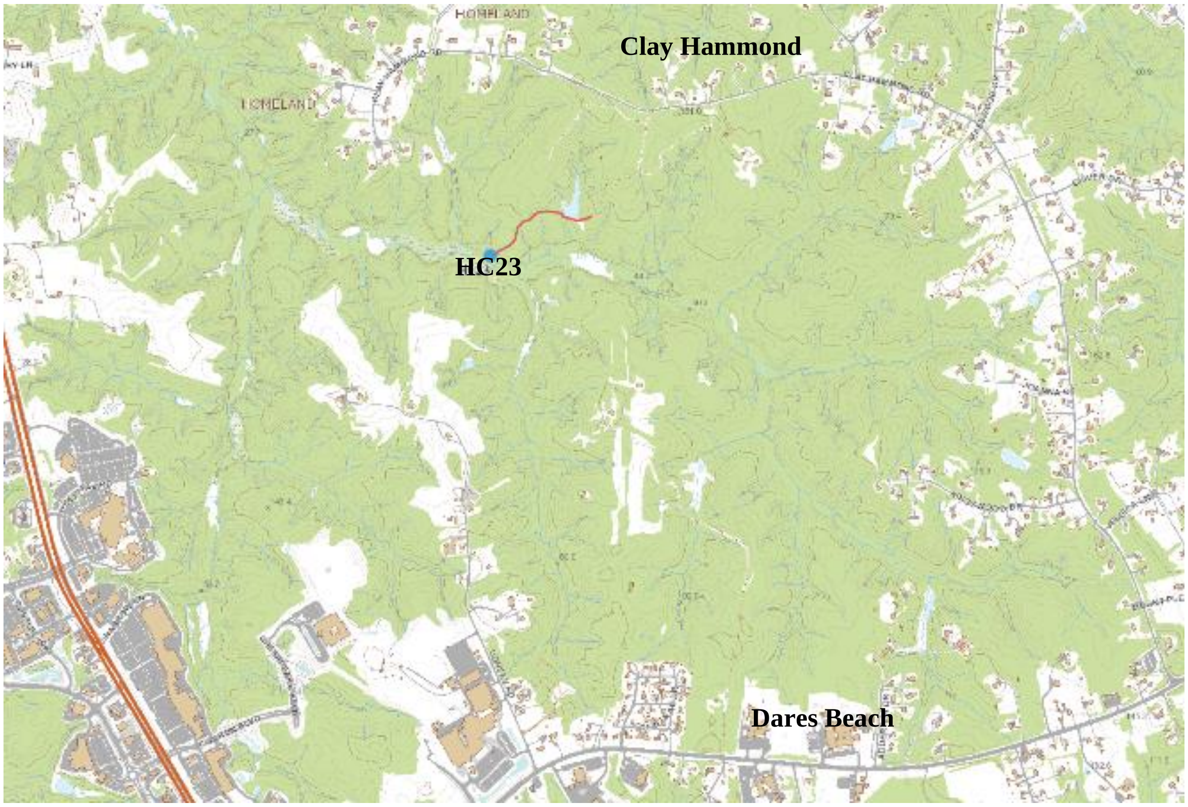
HC21
***College Creek*, UT, College Station subdivision (38.541040, -76.62333)**

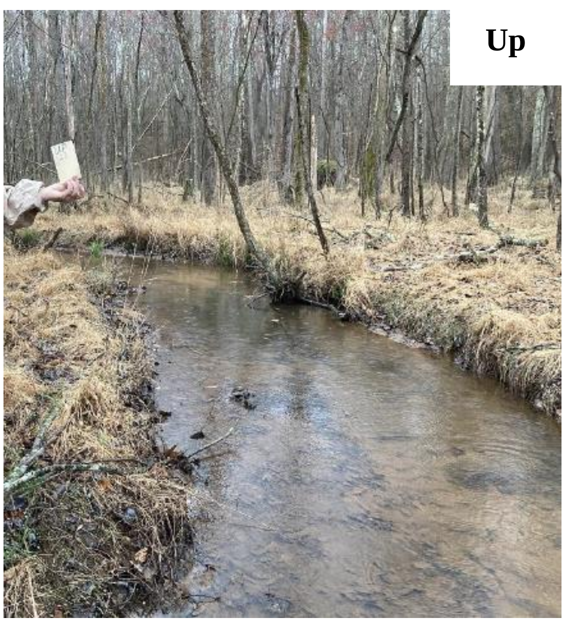


HC22
***Winterberry Creek,* UT, 38.57751, -76.61970 (avg of picture coordinates)**



HC23
East Fox Run, Hughes Tree Farm, 38.56750, -76.58208 (avg of pic coordinates)





HC24
***Boulevard Branch*, UT, 38.541467, -76.595070**

Appendix B. Water Chemistry Data

Site ID	2021	2022		2023		
	NO23 (mg/L)	NO23 (mg/L)	PO4 (mg/L)	NO23 (mg/L)	PO4 (mg/L)	Turbidity (NTU)
HC1	0.444	0.161	0.0074	0.1087	0.0094	8.77
HC2	0.465	0.473	0.0063	0.2863	0.0042	10.98
HC3	1.660	1.88	0.0092	1.3180	0.0168	15.22
HC4	0.542	0.609	0.0096	0.5817	0.0212	8.33
HC5	0.419	0.287	0.0036	0.2157	0.0061	12.28
HC6	0.335	0.201	0.0064	0.1167	0.0093	12.86
HC7	0.534	0.441	0.0037	0.3471	0.005	8.44
HC8a	0.538	0.264	0.0053	0.392	0.0077	4.64
HC8b	NS	0.349	0.0062	0.2769	0.0114	8.52
HC9	0.712	0.153	0.0066	0.506	0.018	11.05
HC10b	1.093*	1.14	0.0095	0.6446	0.0174	11.44
HC11		0.156	0.0034	NS	NS	NS
HC13		0.586	0.0085	0.0782	0.009	9.17
HC14		0.175	0.0034	0.1186	0.0039	2.6
HC15		0.38	0.0058	0.3686	0.0102	8.87
HC16		0.938	0.0095	0.9417	0.0111	22.1
HC17		0.311	0.0034	**NS**	**NS**	**NS**
HC19		**c**	**c**	0.0387	0.0058	13.11
HC20				0.1976	0.0034	16.27
HC21				0.4118	0.066	8.92
HC22				0.5383	0.021	14.24
HC23				0.2507	0.006	9.52
HC24				0.2472	0.0068	16.81

*HC10, Downstream of HC10b

Notes

a) PO4 was not measured in 2021 **b)** NS = Not sampled **c)** HC18 macroinvertebrates and eDNA sampling only

Appendix C. Benthic Macroinvertebrates

Tables C-1 through C-4 include benthic macroinvertebrate (macros) families and genera (with available Tolerance Values) collected by the FOHC with a D-net at four streams sites in the Hunting Creek watershed in 2023. Tolerance Values can range from 1.0 (least tolerant/most sensitive to pollution) to 10.0 (most tolerant/least sensitive). Taxa considered to be intolerant of urbanization (development) have Tolerance Values of 0 to 3. Macros taxa found in the D-net samples at a given site that are not included in the MBSS calculation of BIBI scores are listed below the table for that site. *

Table C-1. Benthic macroinvertebrates collected at HC6 by D-net sampling (2023)

Family	Genus	Tolerance Value
Naididae	Nais (aquatic worm)	9.1
Ancylidae	Ferrissia (freshwater limpet)	6.7
Lithoglyphidae	Gillia altilis (buffalo pebble snail)	----
Lymnaeidae	Fossaria (pond snail)	7.9
Lymnaeidae	Stagnicola (freshwater snail)	7.8
Physidae	Physa (freshwater snail)	7
Pisidiidae	Pisidium (pill clam or pea clam)	5.7
Sphaeriidae	Sphaerium (fingernail clam)	5.7
Viviparidae	Bellamya chinensis (Chinese mystery snail)	----
Gammaridae	Gammarus (scud)	6.7
Asellidae	Caecidotea (waterslater)	2.6
Baetidae	Baetis (small minnow mayfly)	3.9
Heptageniidae	Stenonema (moth)	4.6
Aeshnidae	Boyeria (spotted darner)	6.3
Calopterygidae	Calopteryx (black-winged damselfly)	8.3
Gomphidae	Gomphus (club-tailed dragonfly)	2.2
Nemouridae	Amphinemura (spring stonefly)	3
Perlidae	Paragnetina (common stonefly)	2.2
Perlodidae	Isoperla (green-winged stonefly)	2.4
Notonectidae	Notonecta (common blackswimmer)	10
Goeridae	Goera (little grey sedge caddisfly)	3.4
Leptoceridae	Oecetis (long-horned caddisfly)	4.7
Phyrganeidae	Limnophilus (caddisfly)	----
Polycentropodidae	Polycentropus (tube maker caddisfly)	1.1
Curculionidae	Perenthis (snout beetle)	----
Dryopidae	Postelichus (long-toed water beetle)	----
Dytiscidae	Hydroporus (predaceous diving beetle)	5.4
Elmidae	Dubiraphia (riffle beetle)	5.7
Elmidae	Macronychus (riffle beetle)	6.8
Elmidae	Optioservus (riffle beetle)	5.4
Elmidae	Oulimnius (riffle beetle)	2.7
Elmidae	Stenelmis (riffle beetle)	7.1
Hydrophilidae	Sperchopsis (water scavenger beetle)	4.1
Ceratopogonidae	Bezzia (biting midge)	3.3
Chironomidae	Cardiocladius (non-biting midge)	10
Chironomidae	Chironomus (non0biting midge)	8
Chironomidae	Eukiefferiella (non-biting midge)	8
Chironomidae	Odontomesa (non-biting midge)	6.6

Chironomidae	Pentaneura (non-biting midge)	6.6	Plus:
Chironomidae	Rheotanytarsus (non-biting midge)	7.2	
Empididae	Hemerodromia (dancefly)	7.1	
Ephydridae	Ephydra (shorefly)	6	
Simuliidae	Simulium (blackfly)	5.7	
Tabanidae	Tabanus (horsefly)	2.8	
Tipulidae	Hextoma (cranefly)	1.5	

Cladocera—Chydoridae—Camptocerus (water flea)
Nematomorpha (horsehair worm)

Table C-2. Benthic macroinvertebrates collected at HC18 by D-net sampling (2023).

Family	Genus	Tolerance Value
Naididae	*Nais* (aquatic worm)	9.1
Naididae	Stylaria (aquatic worm)	8
Viviparidae	Campeloma (freshwater snail)	6
Lithoglyphidae	Gillia altilis (buffalo pebble snail)	----
Lymnaeidae	Fossaria (pond snail)	7.9
Physidae	Physa (freshwater snail)	7
Pisidiidae	Pisidium (pill clam or pea clam)	5.7
Sphaeriidae	Sphaerium (fingernail clam)	5.7
Planorbidae	Menetus (freshwater snail)	7.6
Gammaridae	Gammarus (scud)	6.7
Crangonyctidae	Crangonyx (scud)	6.7
Asellidae	Caecidotea (waterslater)	2.6
Baetidae	Baetis (mayfly)	3.9
Siphlonuridae	Siphlonurus (minnow mayfly)	7
Noctuidae	Simyra (owlet moth)	----
Aeshnidae	Boyeria (spotted darner)	6.3
Calopterygidae	Calopteryx (black-winged damselfly)	8.3
Libelludidae	No genus identified	----
Corduligastridae	No genus identified	----
Nemouridae	Amphinemura (spring stonefly)	3
Nemouridae	Ostrocerca (sprig stonefly)	1.7
Perlodidae	Isoperla (green-winged stonefly)	2.4
Naucoridae	No genus identified	----
Hydropsychidae	Hydropsyche (net-spinning caddisfly)	6.5
Limnephilidae	Limnephilus (northern caddisfly)	3.4
Dryopidae	Helichus (long-toed water beetle)	6.4
Dryopidae	Helichus (long-toed water beetle) adult	6.4
Dytiscidae	Hydroporus (predaceous diving beetle)	5.4
Elmidae	Dubiraphia (riffle beetle)	5.7
Elmidae	Macronychus (riffle beetle)	6.8
Elmidae	Optioservus (riffle beetle)	5.4
Elmidae	Stenelmis (riffle beetle)	7.1
Ceratopogonidae	Bezzia (biting midge)	3.3
Chironomidae	Alotanypus (non-biting midge)	6.6
Chironomidae	Cardiocladius (non-biting midge)	10
Chironomidae	Chaetocladius (non-biting midge)	8

Chironomidae	Constempellina (non-biting midge)	8
Chironomidae	Eukiefferiella (non-biting midge)	8
Chironomidae	Macropleopia (non-biting midge)	6.6
Chironomidae	Microtendipes (non-biting midge)	4.9
Chironomidae	Parachironomus (non-biting midge)	6.6
Chironomidae	Paradentipes (non-biting midge)	6.6
Chironomidae	Polypedilum (non-biting midge)	6.3
Chironomidae	Psectrocladius (non-biting midge)	6.6
Chironomidae	Rheotanytarsus (non-biting midge)	7.2
Chironomidae	Tanypus (non-biting midge)	6.6
Dixidae	Dixella (meniscus midge)	5.8
Ephydridae	Ephydra (shorefly)	6
Ephydridae	No genus identified	--
Simuliidae	Simulium (blackfly)	5.7
Tabanidae	Tabanus (horsefly)	2.8
Tipulidae	Dicranota (cranefly)	1.1
Tipulidae	Hextoma (cranefly)	1.5
Tipulidae	Tipula (cranefly)	6.7

Plus:

Celeoptera—Staphylinidae—Psephidonus (beetle), adult
Cyclopoida—Cyclopidae—Eucyclops (water flea)
Harpacticoida—Phyllognathopodidae—Phyllognathopus (copepod)
Planaria (flatworm) No family or genus identified

Table C-3. Benthic macroinvertebrates collected at HC19 by D-net sampling (2023)

Family	Genus	Tolerance Value
Naididae	Nais (aquatic worm)	9.1
Lymnaeidae	Fossaria (pond snail)	7.9
Planorbidae	Menetus (freshwater snail)	7.6
Physidae	Physa (freshwater snail)	7
Pisidiidae	Pisidium (pill clam or pea clam)	5.7
Sphaeriidae	Sphaerium (fingernail clam)	5.5
Crangonyctidae	Crangonyx (scud)	6.7
Gammaridae	Gammarus (scud)	6.7
Asellidae	Caecidoeta (waterslater)	2.6
Caenidae	Caenis (small square-gill mayfly)	----
Calopterygidae	Calopteryx (black-winged damselfly)	8.3
Coenagrionidae	Ischnura (forktail damselfly)	9
Gomphidae	Gomphus (club-tailed dragonfly)	2.2
Nemouridae	Amphinemura (spring stonefly)	3
Perlidae	Paragnetina (common stonefly)	2.2
Saldidae	Salda (shorebug)	6
Hydropsychidae	Hydropsche (net-spinning caddisfly)	6.5
Limnephilidae	Limnephilus (northern caddisfly)	3.4
Corixidae	Trichocorixa (water boatman)	5.6
Dryopidae	Helichus (long-toed beetle)	----
Dytiscidae	Hydroporus (predaceous diving beetle)	5.4
Elmidae	Dubiraphia (riffle beetle)	5.7
Hydrophilidae	Tropisternus (water scavenger beetle)	4.1

Chironomidae	Cardiocladius (non-biting midge)	10
Chironomidae	Chironomus (non-biting midge)	8
Chironomidae	Eukiefferiella (non-biting midge)	8
Chironomidae	Odontomesa (non-biting midge)	6.6
Chironomidae	Pentaneura (non-biting midge)	6.6
Chironomidae	Polypedilum (non-biting midge)	6.3
Chironomidae	Psectrotanypus (non-biting midge)	6.6
Chironomidae	Rheotanytarsus (non-biting midge)	7.2
Ephydridae	Hydrellia (shorefly)	----
Tabanidae	Tabanus (horsefly or deerfly)	2.8
Tipulidae	Tipula (cranefly)	6.7

Plus:
Hirudinea—Glossophonidae—*Glossophonia* (leech)
Porifera—Spongillidae—*Spongilla* (freshwater sponge)
Cladocera—Daphnidae—*Ceriodaphnia* (water flea)
Ostracoda—Cypridopsidae—*Potamocypris* (water flea)
Coleoptera—*Pterostichus* (ground beetle)
Coleoptera—*Scarabaeidae—Popillia japonica* (Japanese beetle)
Mymaridae (fairy wasp)
Nematomorpha (horsehair worm)

Table C-4. Benthic macroinvertebrates collected at HC20 by D-net sampling (2023)

Family	Genus	Tolerance Value
Naididae	Nais (aquatic worm)	9.1
Physidae	Physa (freshwater snail)	7
Gammaridae	Gammarus (scud)	6.7
Isotomidae	Isotomurus (springtail)	4.8
Limnephiliidae	Limnephilus (northern caddisfly)	3.4
Pyralidae	Crambus (grass-veneer moth)	1
Dytiscidae	Hydroporus (predaceous diving beetle)	5.4
Dytiscidae	Matus (predaceous diving beetle)	5.4
Chironomidae	Cardiocladius (non-biting midge)	10
Chironomidae	Chironomus (non-biting midge)	8
Chironomidae	Eukiefferiella (non-biting midge)	8
Chironomidae	Odontomesa (non-biting midge)	6.6
Chironomidae	Psectrotanypus (non-biting midge)	6.6
Chironomidae	Tanypus (non-biting midge)	6.6
Ephydridae	Hydrellia (shorefly)	----
Tipulidae	Hexatoma (cranefly)	?

Plus: Porifera—Spongillidae—*Spongilla* (freshwater sponge)
Cladocera—Cyclopoidae—*Eucyclops* (water flea)
Coleoptera—Staphylinidae—Stenus (rove beetle)
Nematomorpha (horsehair worm)
Aphid
Scholopocryptops (bark centipede)
Gyrinophilus porphyriticus (spring salamander)

Appendix D. Habitat Assessment Field Data Sheets Low Gradient Streams

In addition to measuring the concentrations of some major water chemistry parameters (e.g., nitrogen) and collecting macros/calculating BIBI scores, assessing the condition of the physical habitat is essential in evaluating the ecological integrity of streams in the Hunting Creek watershed. Assessing habitat conditions using the ten parameters listed on these field data sheets essentially describes their degradation due to human activities. The presence of altered habitat structure is often a major stressor in freshwater streams. (https://www3.epa.gov/region1/npdes/merrimackstation/pdfs/ar/AR-1164.pdf).

From an overall perspective, “habitat” includes the physical and chemical constituents in a stream, along with the biological interactions. The FOHC narrowed the definition of “habitat” for the Spring 2023 water monitoring effort to the instream and riparian (streamside) habitats that influence the structure and function of macros, fish, and other aquatic communities

The habitat assessments were conducted by FOHC volunteers, using a visually-based approach, on the same day that macros and eDNA samples were collected at the four stream sites. Ten habitat features (parameters) were rated (scored) throughout each 75-m long stream segment and recorded on the field data sheet for low-gradient streams included here in Appendix D.

Two in-stream habitat parameters of most importance to macros and fish are Epifaunal Substrate/Available Cover and Sediment Deposition. The Epifaunal Substrate/Available Cover parameter captures the relative amount and variety of natural structures present in each stream segment, such as gravel, cobble, fallen trees, submerged logs, branches, tree roots, undercut banks, and leaf packs that provided places for macros and fish to live. More secure places for macros and fish to live typically lead to more diverse taxa and higher abundances. Sediment Deposition assesses the amount of silt, sand, and fine gravel resulting from large-scale transport of sediment that has accumulated in pools and point bars. High levels of sediment deposition are symptoms of an unstable stream environment, usually influenced by human activities, that can have negative impacts on macros and fish communities.   Channel Flow Status, the degree to which the stream channel is filled with water, is another very important in-stream habitat parameter, especially for fish.

HABITAT ASSESSMENT FIELD DATA SHEET-LOW GRADIENT STREAMS (FRONT)

STREAM NAME	LOCATION
STATION # ________	STREAM CLASS
LAT ____________	RIVER BASIN
STORET #	AGENCY
INVESTIGATORS	
FORM COMPLETED BY	DATE ________ TIME / REASON FOR SURVEY

Parameters to be evaluated in sampling reach

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/ Available Cover	Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	30-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Pool Substrate Characterization	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Pool Variability	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than <20% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50-80% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

HABITAT ASSESSMENT FIELD DATA SHEET-LOW GRADIENT STREAMS (BACK)

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Channel Sinuosity	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.)	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank)	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank) Note: determine left or right side by facing downstream.	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ___ (LB)	Left Bank 10 9 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Total Score _______

Appendix E. eDNA Pilot Study

Tables E-1 through E-4 include benthic macroinvertebrate (macros) families and genera (with available Tolerance Values) revealed by lab analysis of eDNA samples collected by the FOHC at four streams sites in the Hunting Creek watershed in 2023. Tolerance Values can range from 1.0 (least tolerant/most sensitive to pollution) to 10.0 (most tolerant/least sensitive). Taxa considered to be intolerant of urbanization (development) have Tolerance Values of 0 to 3. Macros taxa revealed by eDNA sampling at a given site that are not included in the MBSS calculation of BIBI scores are listed below the table for that site.

Table E-1. Benthic macroinvertebrates found at HC6 by eDNA sampling (2023).

Family	Genus	Tolerance Value
Physidae	*Physella* (Tadpole Snail)	7
Cambaridae	*Faxonius limosus* (Spinycheek Crayfish)	2.8
Ephemeridae	*Hexagenia limbata* (Giant Burrowing Mayfly)	2.6
Taeniopterygidae	*Taeniopteryx* (Winter Stonefly)	4.8
Limnephilidae	*Ironoquia* (Northern Caddisfly)	4.9
Phyganeidae	*Ptilostomis* (Giant Casemaker)	4.3
Chironomidae	*Cladotanytarsus* (non-biting midge)	6.6
Chironomidae	*Circotopus* (non-biting midge)	9.6
Chironomidae	*Orthocladius* (non-biting midge)	9.2
Chironomidae	*Parametriocnemus* (non-biting midge)	4.6
Chironomidae	*Tanytarsus* (non-biting midge)	4.9
Chironomidae	*Thienemanniella* (non-biting midge)	5.1
Simuliidae	*Simulium* (Blackfly)	5.7

Plus: Batrahospermacae---*Vivescentia viride-americana* (Red Algae)

Table E-2. Benthic macroinvertebrates found at HC18 by eDNA sampling (2023).

Family	Genus	Tolerance Value
Megascolecidae	*Metaphire hilgendorfi* (jumping worm)	----
Physidae	*Physella* (Tadpole Snail)	7
Chironomidae	*Corynoneura* (non-biting midge)	4.1
Chironomidae	*Orthocladius* (non-biting midge)	9.2
Chironomidae	*Parametriocnemus* (non-biting midge)	4.6
Chironomidae	*Tanytarsus* (non-biting midge)	4.9

Plus: Hydridae---Hydra vulgaris (swiftwater hydra)
Bosminidae---*Bosmina* (water flea)

Table E-3. Benthic macroinvertebrates found at HC19 by eDNA sampling (2023).

Family	Genus	Tolerance Value

Naididae	*Aulodrilus pluriseta* (tubificid worm)	----
Naididae	*Chaetogaster diaphanous* (aquatic worm)	----
Lymnaeidae	No genus identified	----
Physidae	Physella (Tadpole Snail)	7
Cambaridae	Lacunicambarus diogenes (Devil Crayfish)	----
Limnephilidae	Ironoquia (Northern Caddisfly)	4.9
Phyrganeidae	Ptilostomis (Giant Casemaker)	4.3
Cecidomyiidae	No genus identified	----
Chironomidae	Corynoneura (non-biting midge)	4.1
Chironomidae	Microtendipes (non-biting midge)	4.9
Chironomidae	Orthocladius (non-biting midge)	9.2
Chironomidae	Procladius (non-biting midge)	1.2
Chironomidae	Rheocricotopus robacki (non-biting midge)	6.2
Chironomidae	Tanytarsus (non-biting midge)	4.9
Simuliidae	Simulium (blackfly)	5.7
Tupulidae	Tipula (cranefly)	1.5

Plus: Murrayidae—Dactylobiotus parathenogeneticus (waterbear)
Saprolegniaceae—Achyla (water mold)
Crypridida—Cypridopsis (seed shrimp)

Table E-4. Benthic macroinvertebrates found at HC20 by eDNA sampling (2023).

Family	Genus	Tolerance Value
Megascolecidaae	*Metaphire hilgendorfi* (jumping worm)	----
Physidae	*Physella* (Tadpole Snail)	7
Noctuidae	*Agrochola biocolorago* (Bicolored Sallow)	----
Cecidomyiidae	No genus identified	----
Chironomidae	*Chironomus* (non-biting midge)	8
Chironomidae	*Corynoneura* (non-biting midge)	4.1
Chironomidae	*Cricotopus* (non-biting midge)	9.6
Chironomidae	*Limnophyes* (non-biting midge)	8.6
Chironomidae	*Orthocladius* (non-biting midge)	9.2

Plus: Hydridae---*Hydra vulgaris (*swift water hydra)
Synchaetidae---*Synchaeta tremula* (rotifer)
Vacuolariaceae---*Gonyostomum semen* (nuisance freshwater algae)

Appendix F. Test Site Catchments

Introduction

The following Figure F1 and Table F1 are provided as orientation to the test sites from which samples were gathered during the 2023 Blitz and to provide background for some of the representative land cover data in the Hunting Creek watershed. Appendix A contains a map of the watershed and additional maps with higher resolution locations for each test site. (Note: This appendix will not appear in subsequent Blitz reports but will exist as a stand-alone document)

Figure F1. Locations of Sites Per Table A2-1 Coordinates

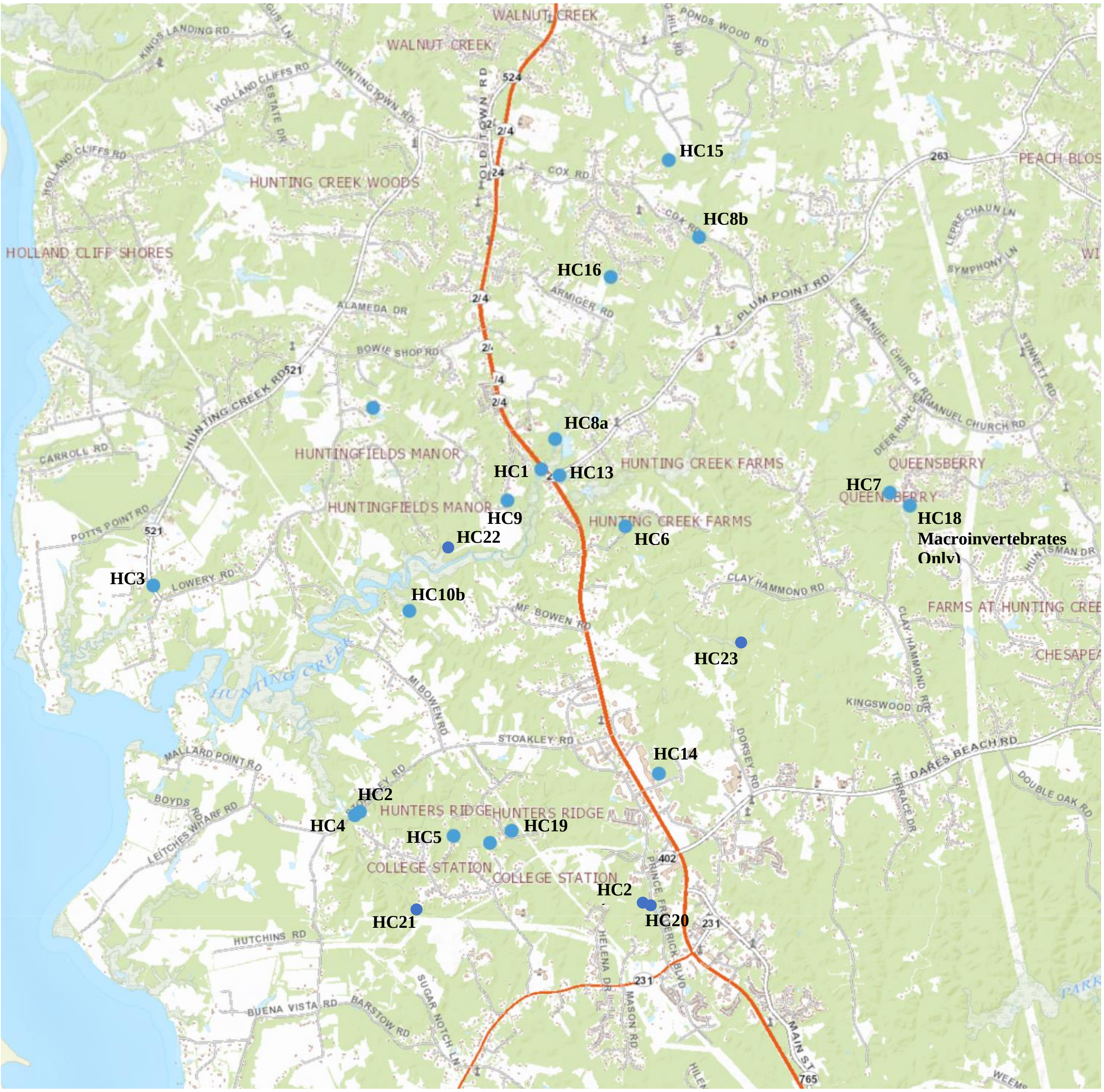


Table F1. 2017 Land Use/Land Cover Data for Selected NHD v.2.1 Catchments in the Hunting Creek Watershed (Percentages of Total Catchment Acres Minus Water).

Catchment Name (acres)	Natural Vegetation	Agriculture	Developed	Impervious
College Creek (748.9)	66.1	5.5	28.4	8.6
Upper Mill Creek (1693.0)	56.6	4.7	38.8	14.6

Lower Mill Creek (479.8)	64.4	26.3	9.4	2.5
Fox Point Creek (710.8)	58.3	13.4	28.3	9.4
Fox Run/East Fox Run (2262.5)	72.3	2.3	25.4	9.8
Chingaware Run (741.4)	49.0	5.9	45.1	9.9
Barberry Branch (301.4)	56.5	12.5	31.0	6.0
Quail Ridge Run (731.7)	49.9	5.4	44.7	12.0
Reits Branch (1155.1)	43.0	4.1	53.0	12.0
Winterberry Creek (415.8)	58.9	14.2	26.8	6.4
Little Lyons Creek (1577.1)	47.6	31.3	21.1	5.7
Total Hunting Creek Watershed (19,126.8)	58	11	31	9

- **Natural Vegetation** represents tree canopy, forest cover, forest succession, and wetlands.
- **Agriculture** represents farm fields and pastures.
- **Developed** represents mining operations, sidewalks, driveways, buildings, roads, and lawn.
- **Impervious** represents sidewalks, driveways, buildings, and roads.

StreamStats Site Catchments

The USGS online application StreamStats was used to produce the following tables and illustrations. From the USGS website (address current January 2024): https://www.usgs.gov/streamstats StreamStats is a Web-based tool that provides streamflow statistics, drainage-basin characteristics, and other information for USGS stream gaging stations and for user-selected ungagged sites on streams. When users select the location of a stream gaging station, StreamStats provides previously published information from a database.

Figure F2 provide the two StreamStat reports that cover the Hunting Creek watershed. The report contains a picture of each basin but this was left out since it duplicates the images in the pictures in Figures 3, 4a, 4b, and 5. The Basin Characteristics FOREST, IMPERV, LC11DEV, AND PRECIP are based on data over 10 years old but are included here for reference.

The application was also used to create reports showing the basin and select characteristics (drainage area, % forest, % impervious, % developed, mean annual precipitation). The application allows a file type (.KMZ) that can be read by Google Earth. The KMZ files were opened in Google Earth to make the Figures 3 and 4a below. Figure 4b was created by individual catchments imported into the County GIS application. The Figure 3 shows the Hunting Creek Watershed including the Little Lyons Creek region basin (Small outlined area middle left. StreamStats counts this region as separate from the main Hunting creek basin). The designation HCx denotes only nontidal sites tested by Friends of Hunting Creek (FOHC) volunteers. Sites designated HUN-x are tidal sites tested by Chesapeake Biological Lab (CBL) and are included for reference. Figure 4a shows some of the individual drainage basins sampled during the 2023 water testing blitz. There were far more test sites than those shown but those shown in Figure 4a are the largest subcatchments that make up the Hunting Creek watershed. Figure 4b is similar to 4a and shows the larger catchments imported into a County GIS map which provides greater detail for roads and subdivisions. The circles show the exit point location for StreamStat calculations for each test site noted in the figure. Figure F5 shows additional catchments which generally lie within the catchments shown in Figure F4a. The figures after Figure F5 show the catchments of the individual sampling sites. Figures beginning with Figure 6 includes information and graphics for each individual catchment for all sites tested for chemistry. Each site's set of information includes text from the site ID chart as well as the coordinates used by StreamStats. StreamStats maps for selecting a catchment definition point are lower resolution than those in the Calvert GIS system and Google Earth which were used as the source for site coordinates. The small difference in the catchment coordinates is trivial in the impact to the reports. Select sites with large catchment areas such as HC1 and HC13 include the catchment boundary imported into GIS.

NOTE: Creek names in *Italics* denote unnamed tributaries unofficially named by FOHC.

Figure F2. Two catchments used by StreamStats to characterize the total flow from Hunting Creek

Hunting Cr Main Basin
StreamStats Report Collapse All

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	27.5	square miles
FOREST	Percentage of area covered by forest	60.1	percent
IMPERV	Percentage of impervious area	9.79	percent

LC11DEV	Percentage of developed (urban) land from NLCD 2011 classes 21-24	16.9	percent
PRECIP	Mean Annual Precipitation	45.1	inches

Little Lyons Creek Region
StreamStats Report Collapse All

Basin Characteristics

Parameter Code	**Parameter Description**	**Value**	**Unit**
DRNAREA	Area that drains to a point on a stream	2.49	square miles
FOREST	Percentage of area covered by forest	41.2	percent
IMPERV	Percentage of impervious area	5.46	percent
LC11DEV	Percentage of developed (urban) land from NLCD 2011 classes 21-24	11.7	percent
PRECIP	Mean Annual Precipitation	45	inches

Figure F3. Hunting Creek Watershed (includes Little Lyons Creek region)

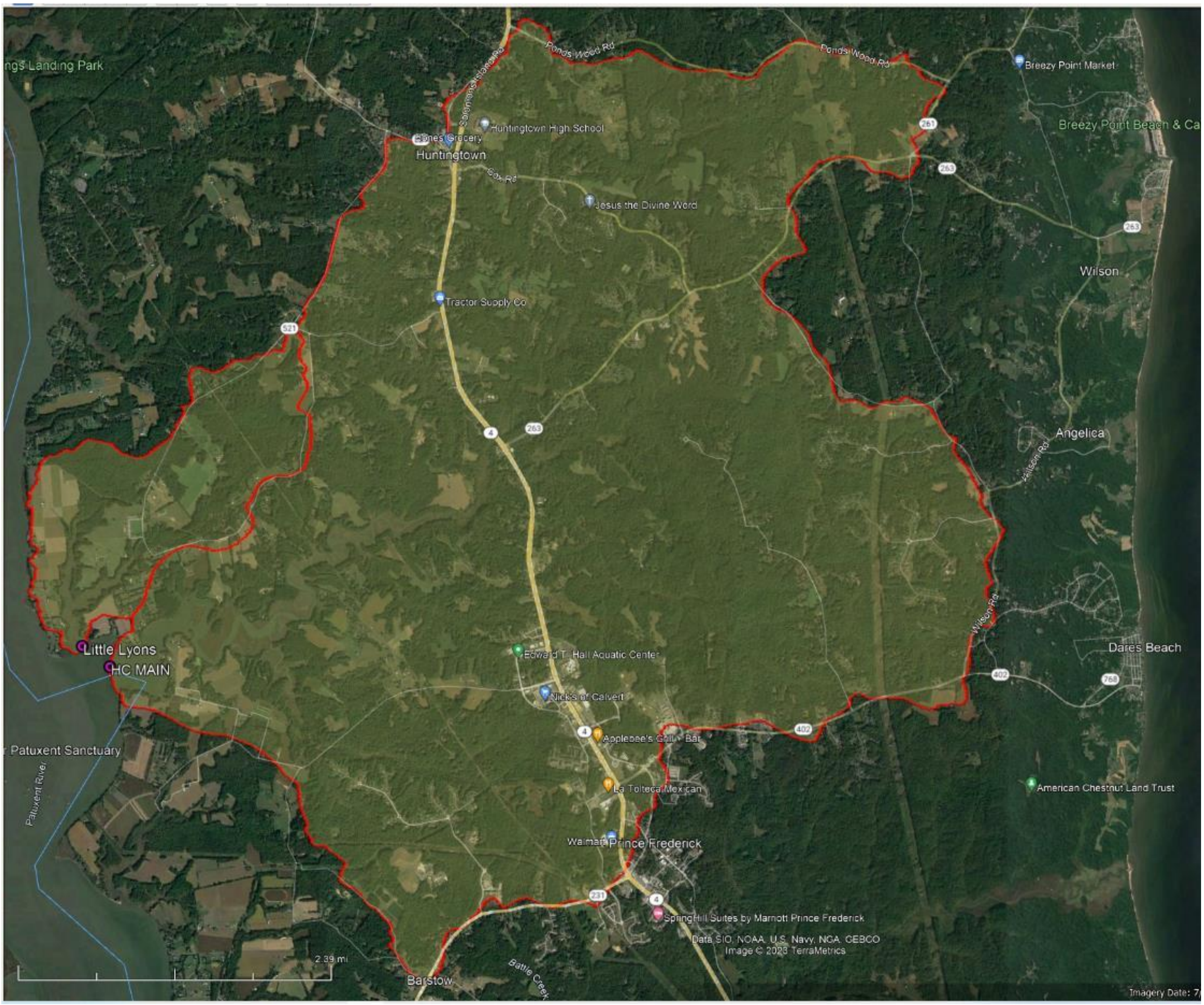


Figure F4a. Select 2023 Blitz sites overlaid on watershed. The tidal portion of Hunting Creek is from the Patuxent River almost to Rt 4. Note the more transparent shading of tidal extent. Test sites designated by HUN-x are tidal sites tested by CBL.

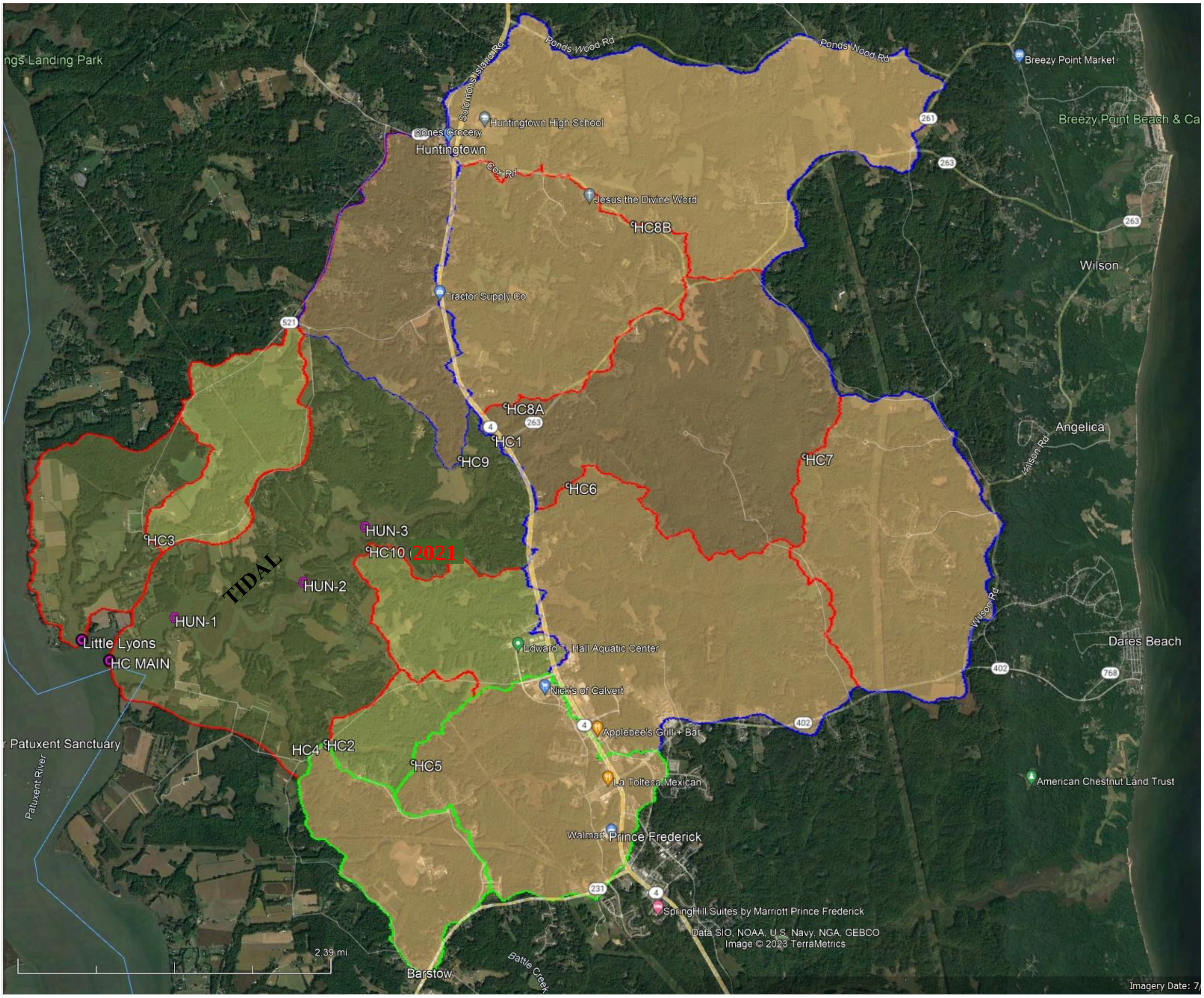


Figure F4b. Major Catchments imported into GIS

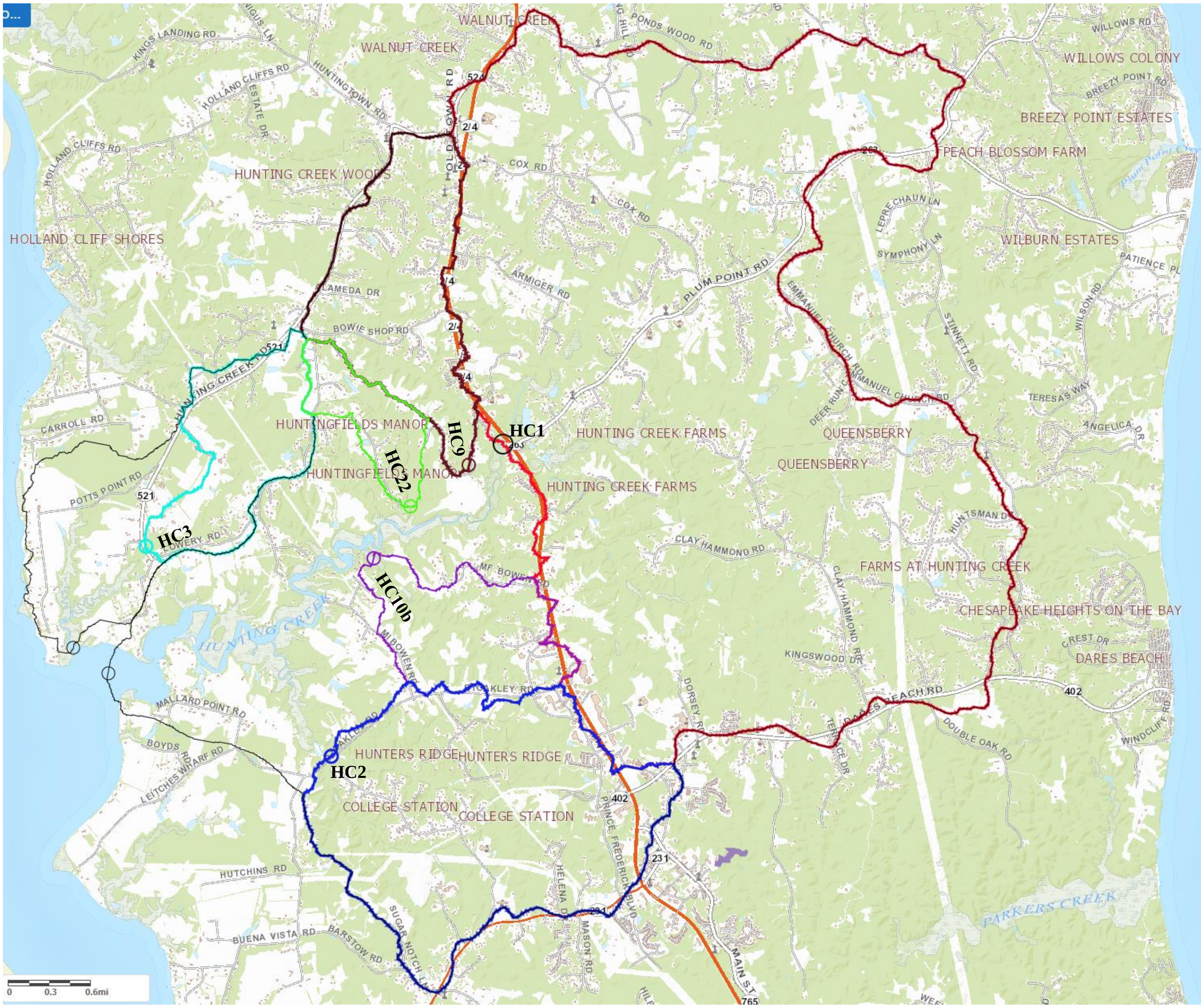


Figure F5. Outer Catchments

(Note the border of the Hunting Creek watershed with the Little Lyons Creek sub-watershed is shown in black)

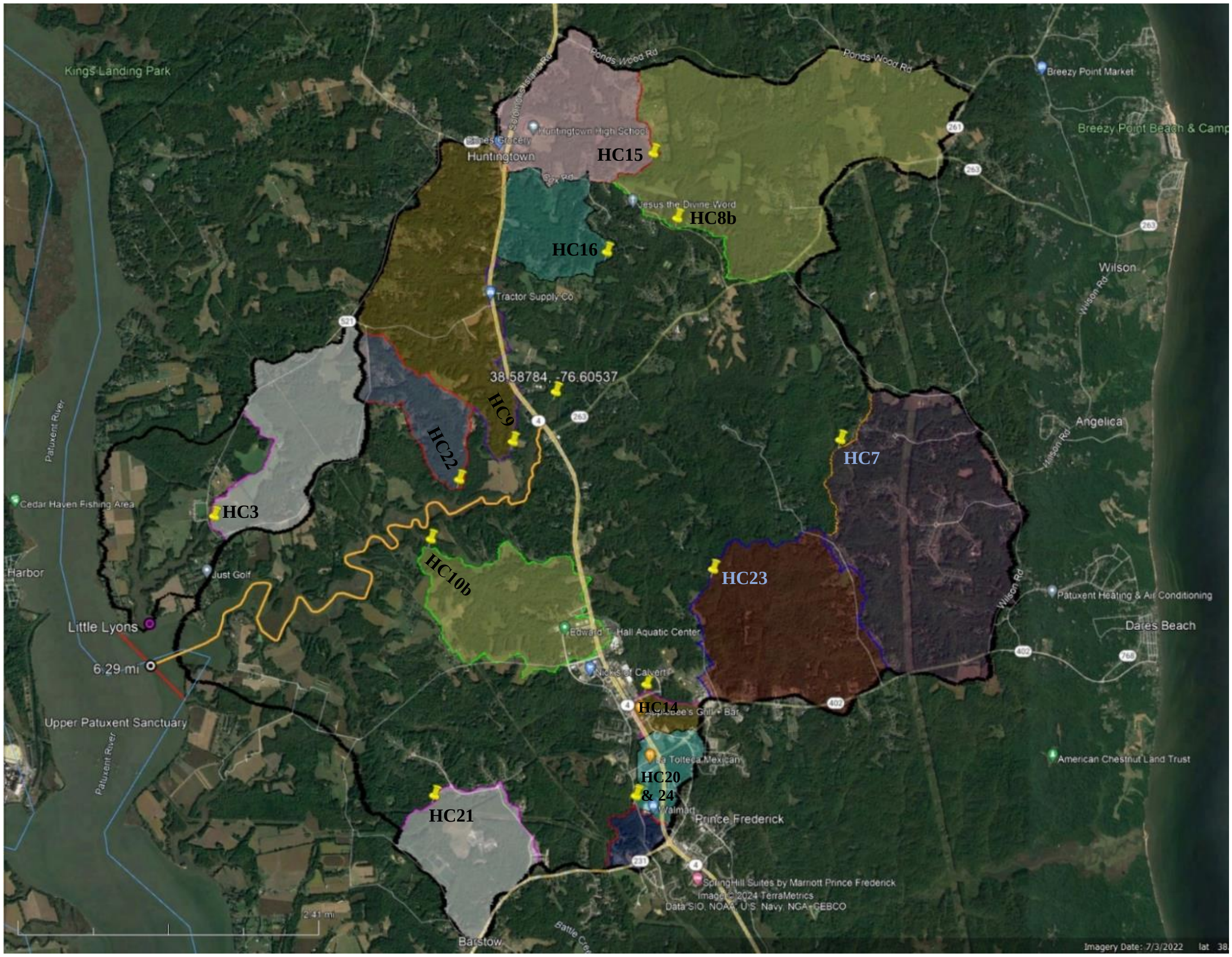


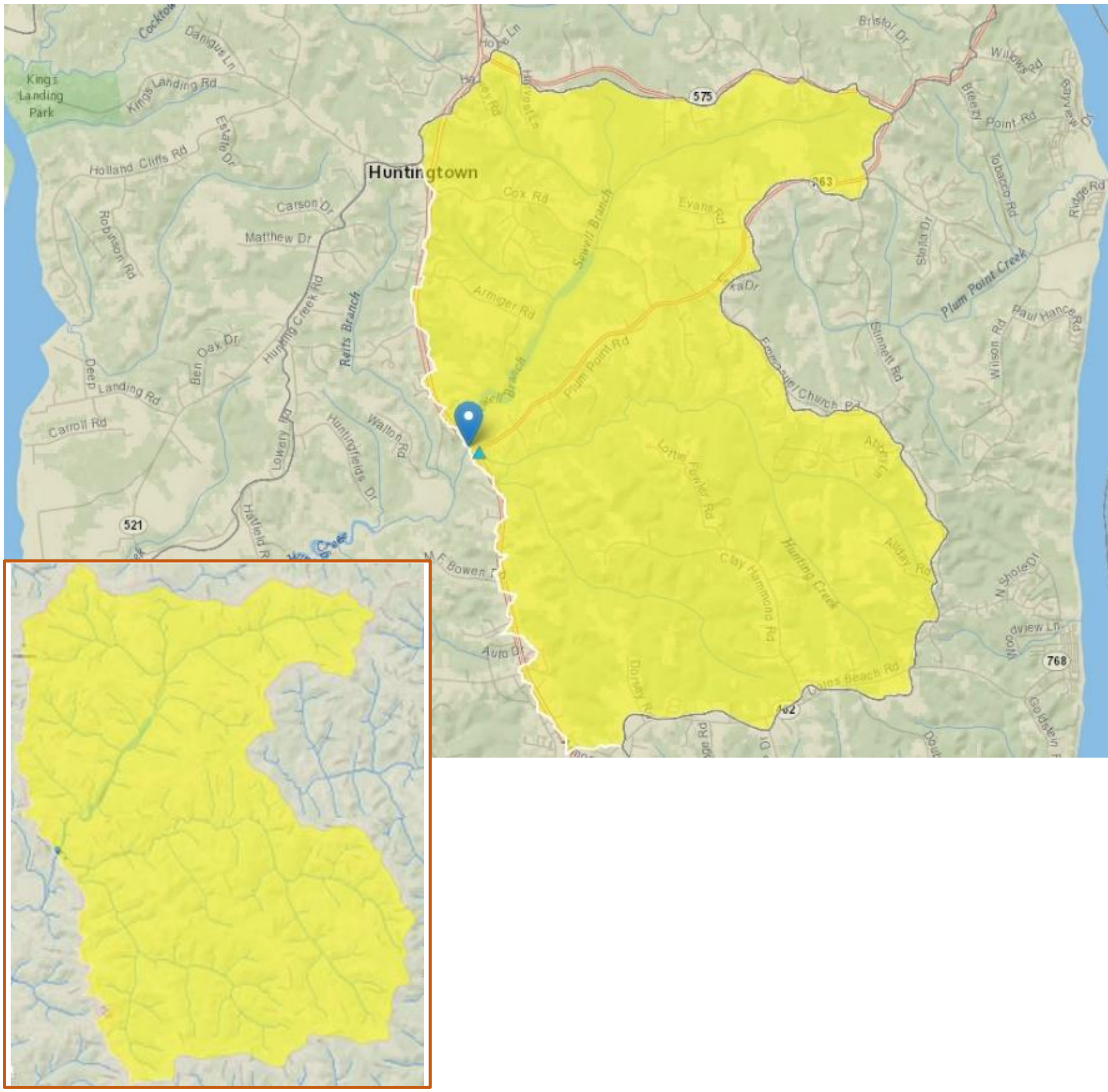
Figure F6. HC1 **(Note imported image into GIS for clarity and detail)**

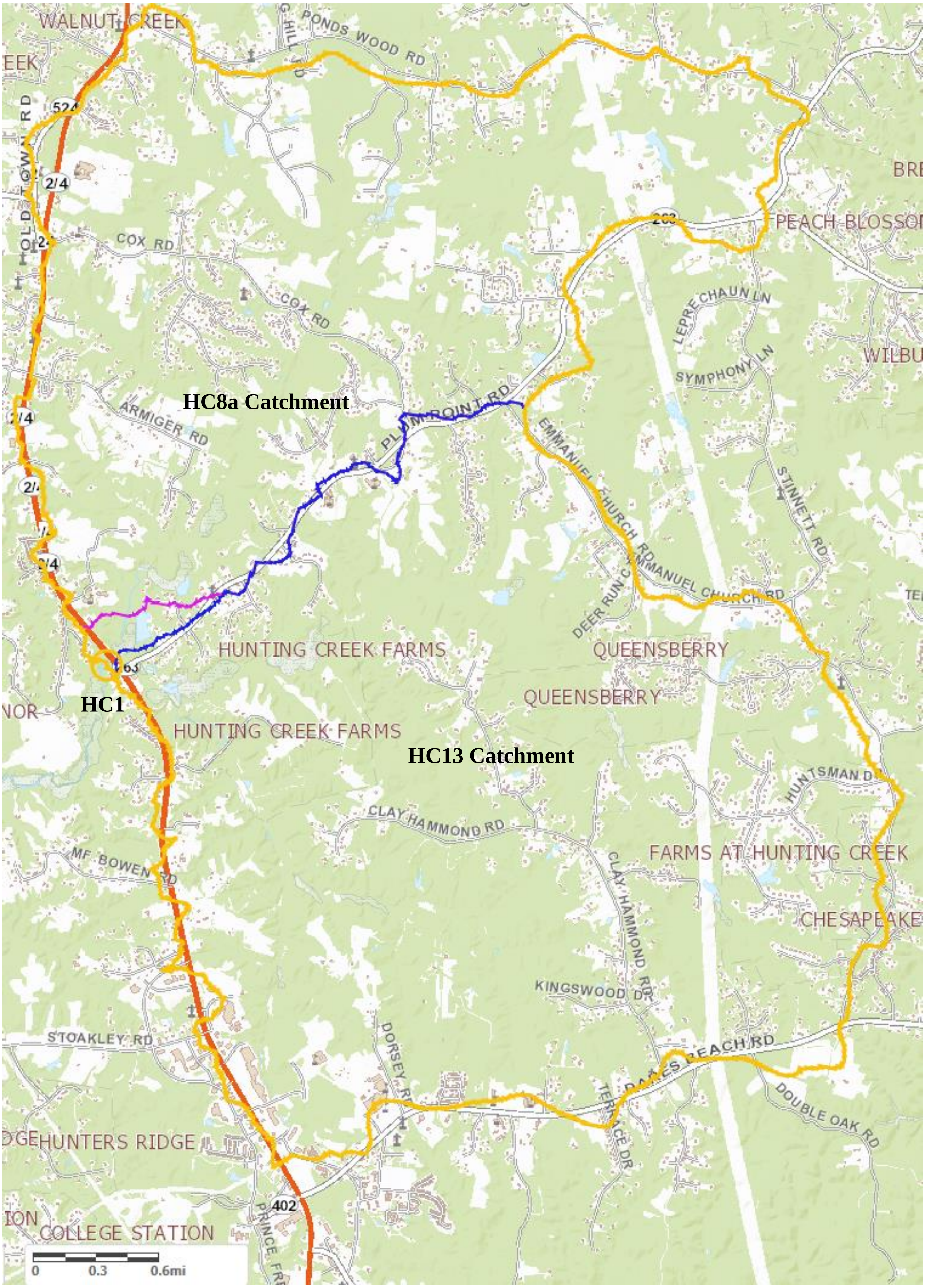
HC1	38.584843	-76.607017	Hunting Creek, Rt 2/4 Bridge

Coordinates used by StreamStats: 38.584843, -76.60700

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	15.9	square miles
FOREST	Percentage of area covered by forest	65.7	percent





HC8a Catchment
HC1
HC13 Catchment
WALNUT CREEK
PONDS WOOD RD
OLD TOWN RD
COX RD
ARMIGER RD
PLUM POINT RD
EMMANUEL CHURCH RD
DEER RUN CT
HUNTING CREEK FARMS
QUEENSBERRY
PEACH BLOSSOM
LEPRECHAUN LN
SYMPHONY LN
STINNETT RD
HUNTSMAN DR
CLAY HAMMOND RD
MF BOWEN RD
FARMS AT HUNTING CREEK
CHESAPEAKE
KINGSWOOD DR
STOAKLEY RD
DORSEY RD
DARES BEACH RD
TERRACE DR
DOUBLE OAK RD
HUNTERS RIDGE
COLLEGE STATION
PRINCE FR
0 0.3 0.6mi

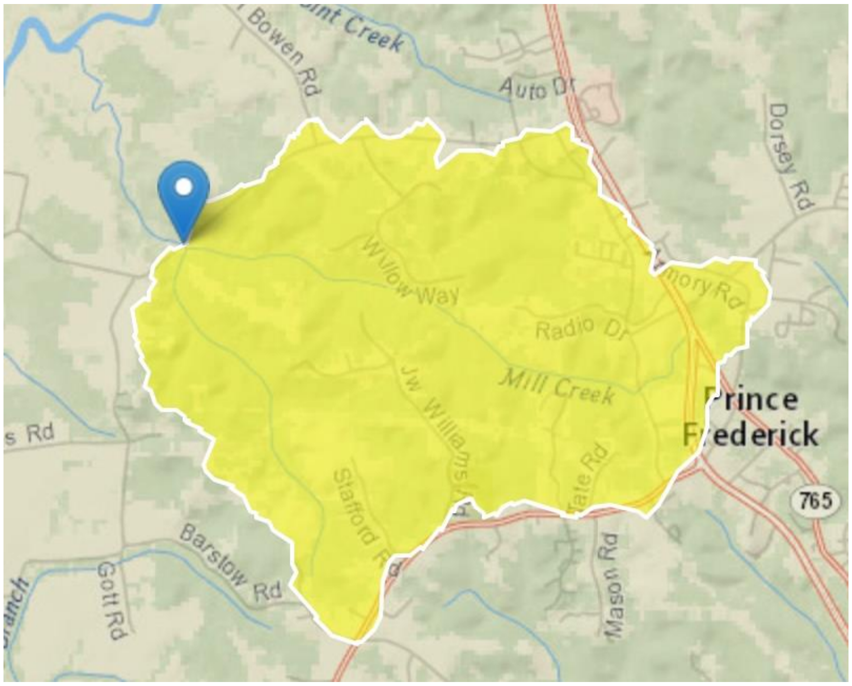
Figure F7. HC2

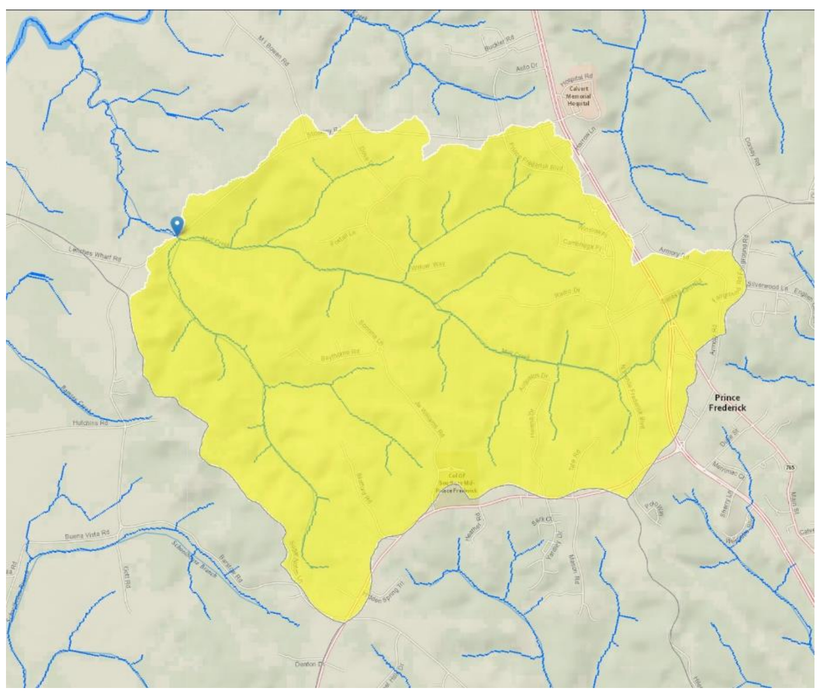
HC2	38.550865	-76.630076	Mill Creek, Stoakley Rd

Coordinates used by StreamStats: 38.55075, -76.63058

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	3.84	square miles
FOREST	Percentage of area covered by forest	67.7	percent





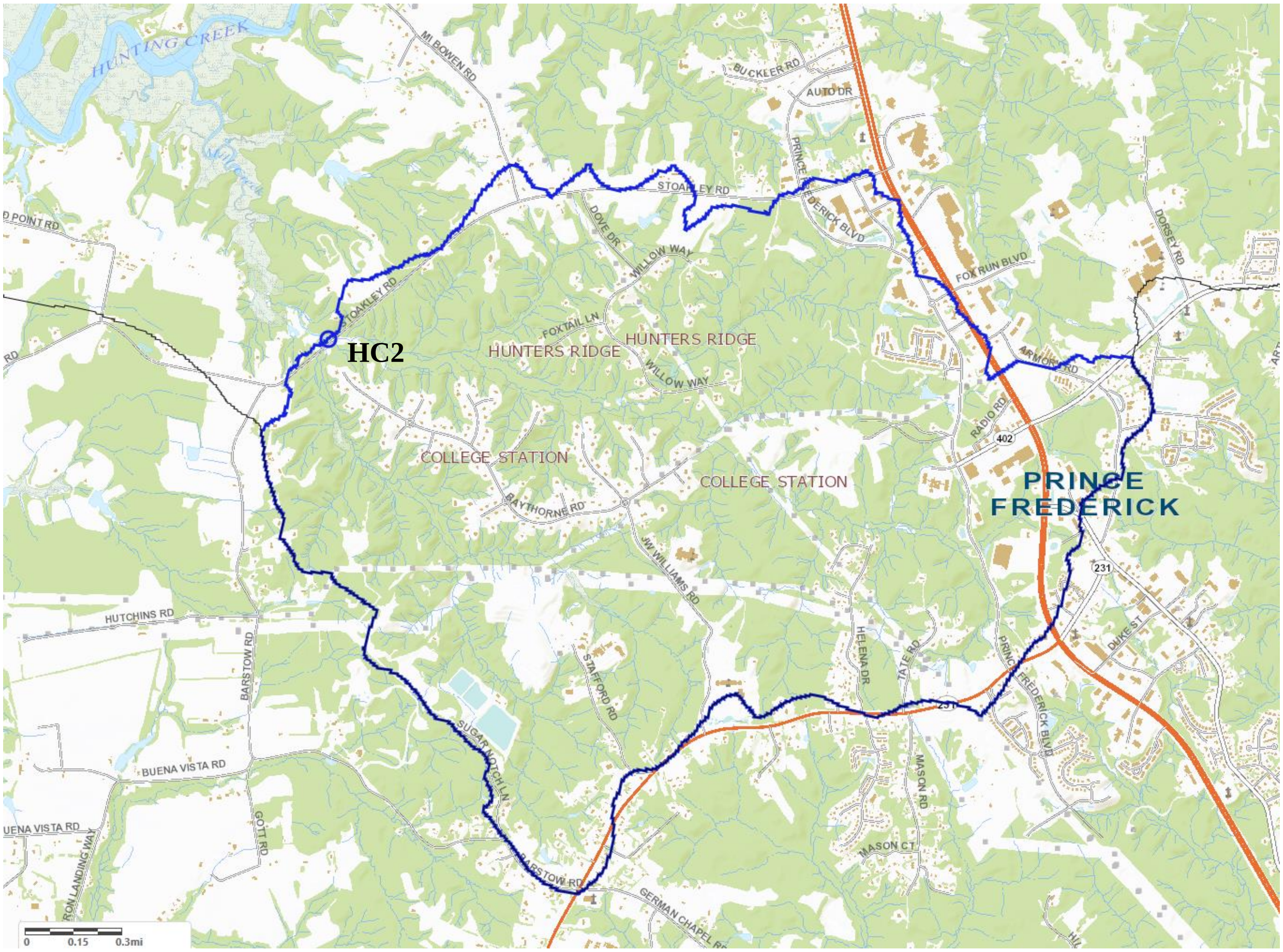

HUNTING CREEK
MI BOWEN RD
BUCKLER RD
AUTO DR
STOAKLEY RD
PRINCE FREDERICK BLVD
DOVE DR
WILLOW WAY
FOX RUN BLVD
DORSEY RD
D POINT RD
HC2
FOXTAIL LN
HUNTERS RIDGE
HUNTERS RIDGE
ARMORY RD
RADIO RD
402
COLLEGE STATION
COLLEGE STATION
PRINCE FREDERICK
BAYTHORNE RD
JW WILLIAMS RD
231
HUTCHINS RD
BARSTOW RD
STAFFORD RD
HELENA DR
TATE RD
DUKE ST
SUGAR NOTCH LN
MASON RD
BUENA VISTA RD
GOTT RD
MASON CT
BARSTOW RD
GERMAN CHAPEL RD
0
0.15
0.3mi

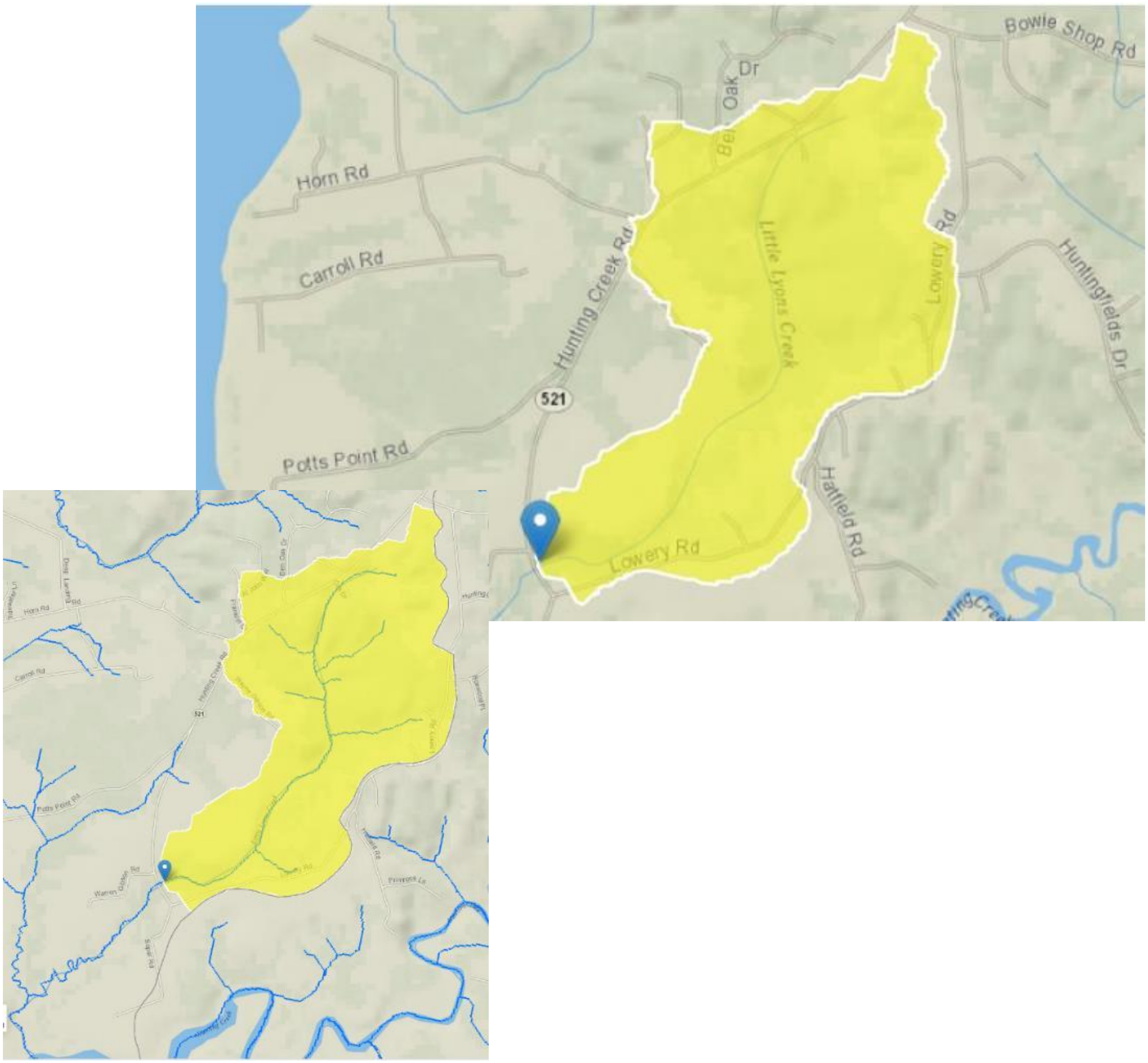
Figure F8. HC3

HC3	38.573407	-76.656031	Little Lyons Creek, Hunting Creek Rd

Coordinates used by StreamStats: 38.567347, -76.65590

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	1.13	square miles
FOREST	Percentage of area covered by forest	52	percent



HC3
HUNTINGFIELDS MAN
Twirly Hole
127.5
50.7
25.2
33.9
0
500
1000ft

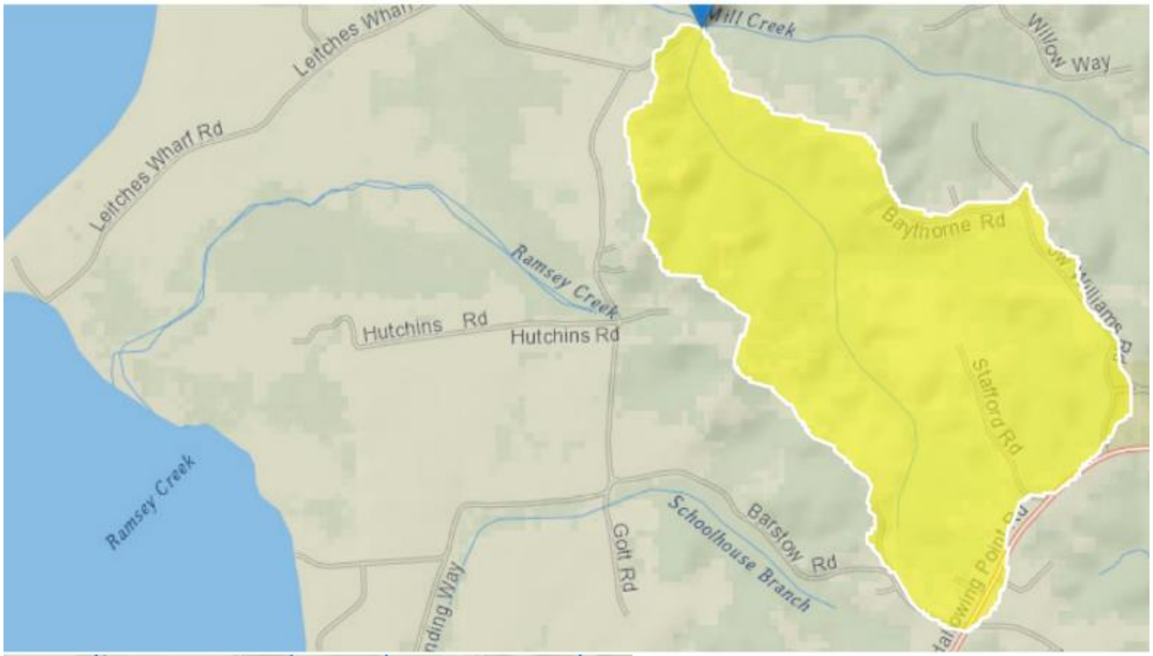
Figure F9. HC4

HC4	38.550589	-76.630649	*College Creek*, Stoakley Rd

Coordinates used by StreamStats: 38.55035, -76.63063

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	1.19	square miles
FOREST	Percentage of area covered by forest	69.9	percent



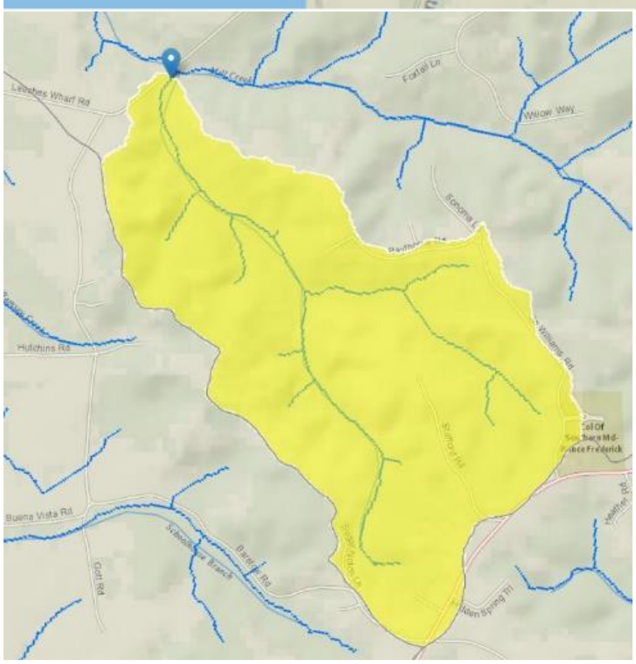


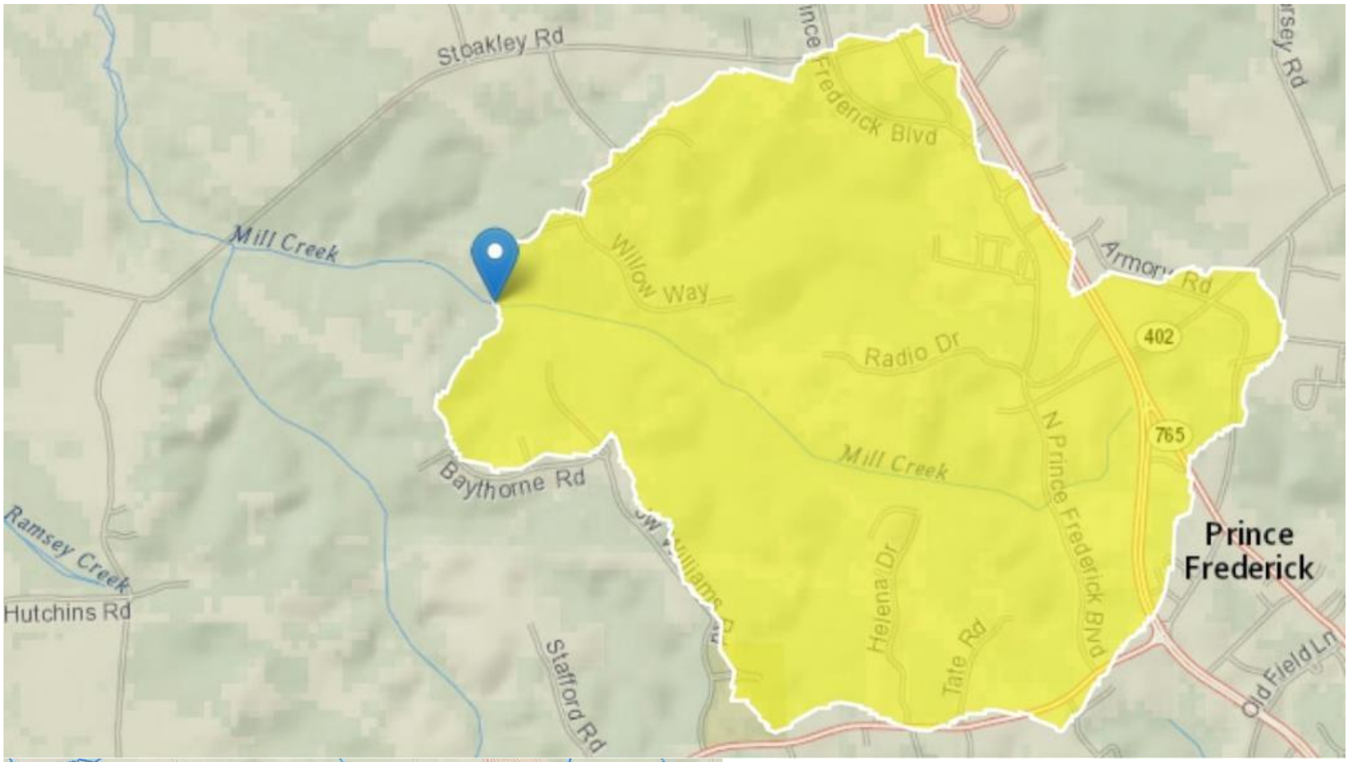
Figure F10. HC5

HC5	38.548495	-76.618217	Mill Creek, Hunters Ridge

Coordinates used by StreamStats: 38.54864, -76.61829

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	2.1	square miles
FOREST	Percentage of area covered by forest	65.8	percent



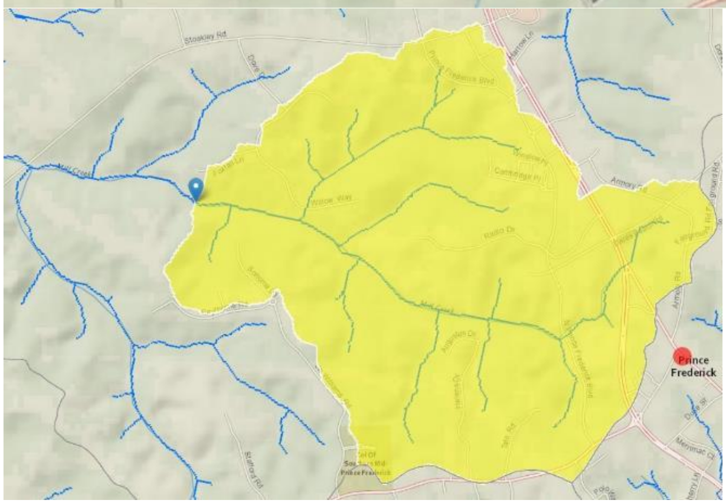


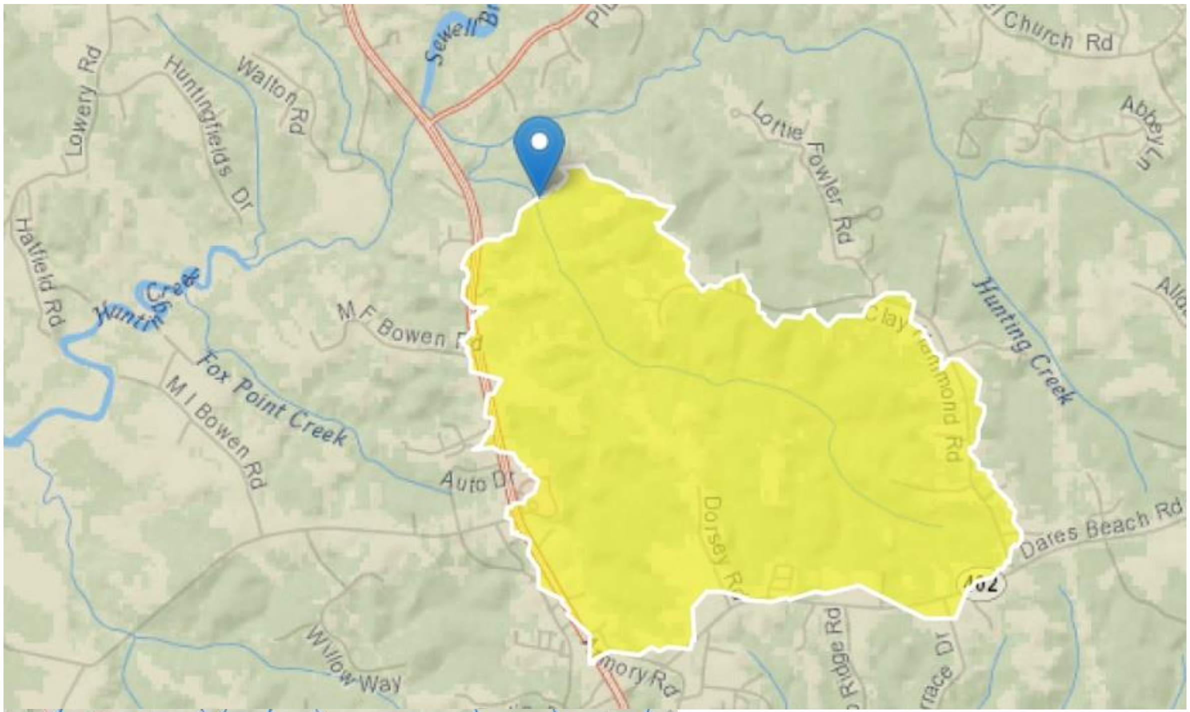
Figure F11. HC6

HC6	38.579128	-76.596507	Fox Run, Hunting Farms Ln

Coordinates used by StreamStats: 38.578914, -76.59657

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	3.42	square miles
FOREST	Percentage of area covered by forest	71.9	percent



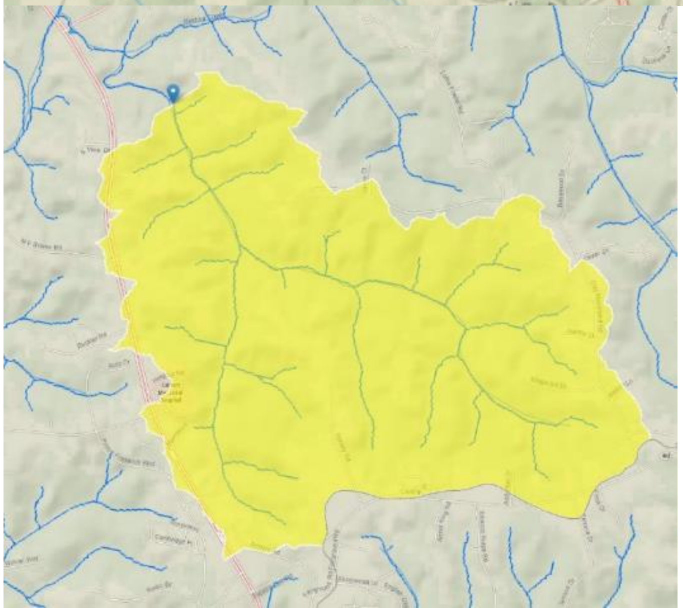


Figure F12. HC7

HC7	38.582335	-76.563076	Hunting Creek, Queensberry

Coordinates used by StreamStats: 38.58219, -76.56327

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	2.65	square miles
FOREST	Percentage of area covered by forest	66.6	percent

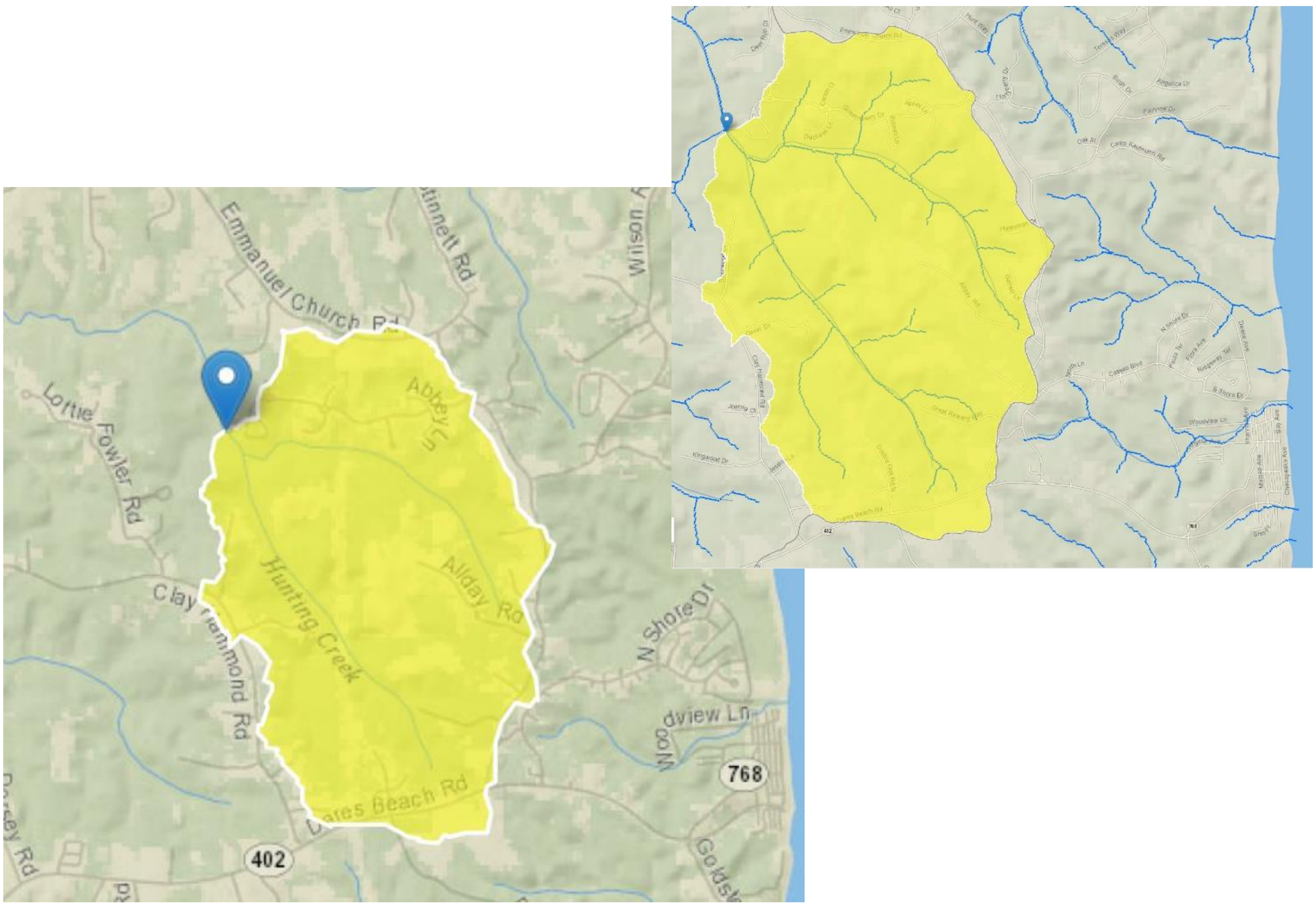


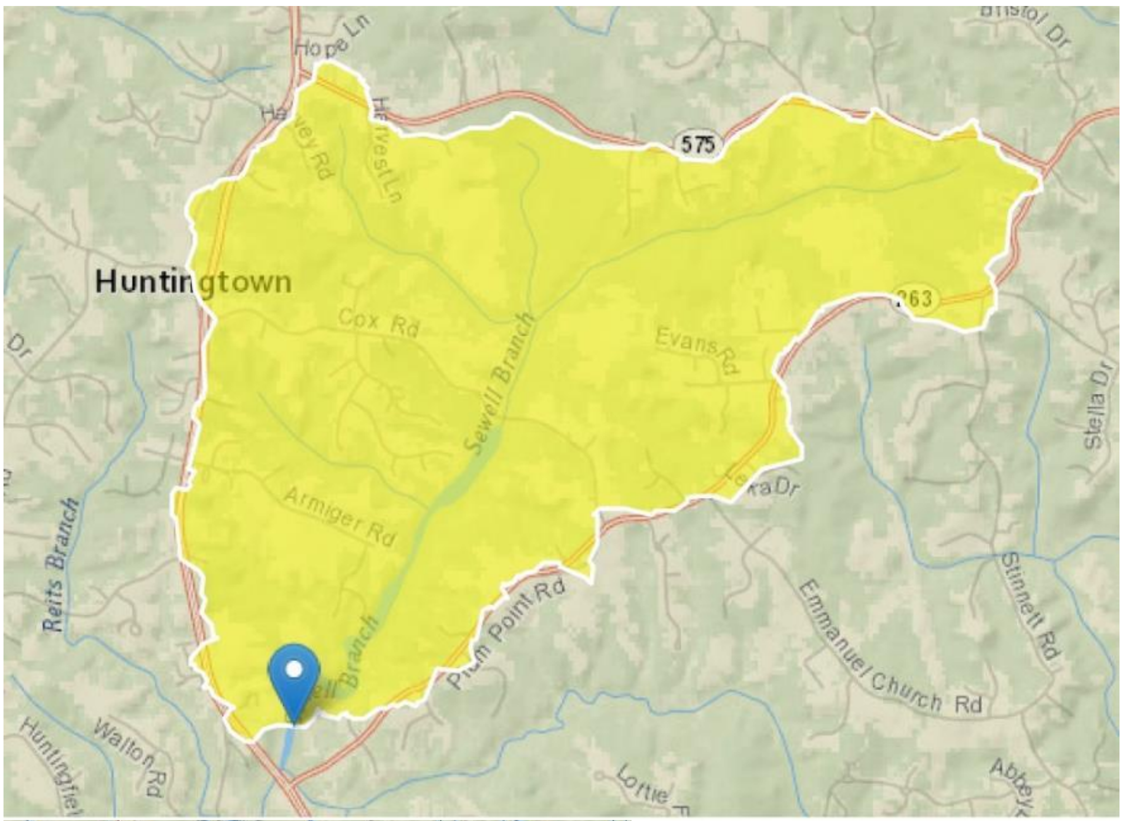
Figure F13. HC8a

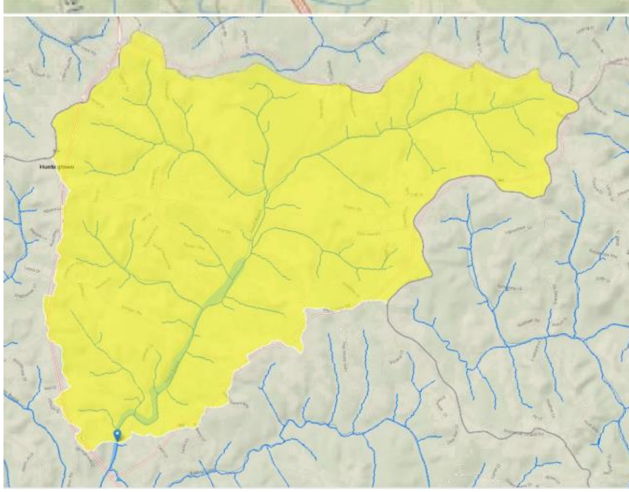
HC8a	38.587877	-76.605287	Sewell Branch, near Calverton school

Coordinates used by StreamStats: 38.58789, -76.60533

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	6.5	square miles
FOREST	Percentage of area covered by forest	59.9	percent





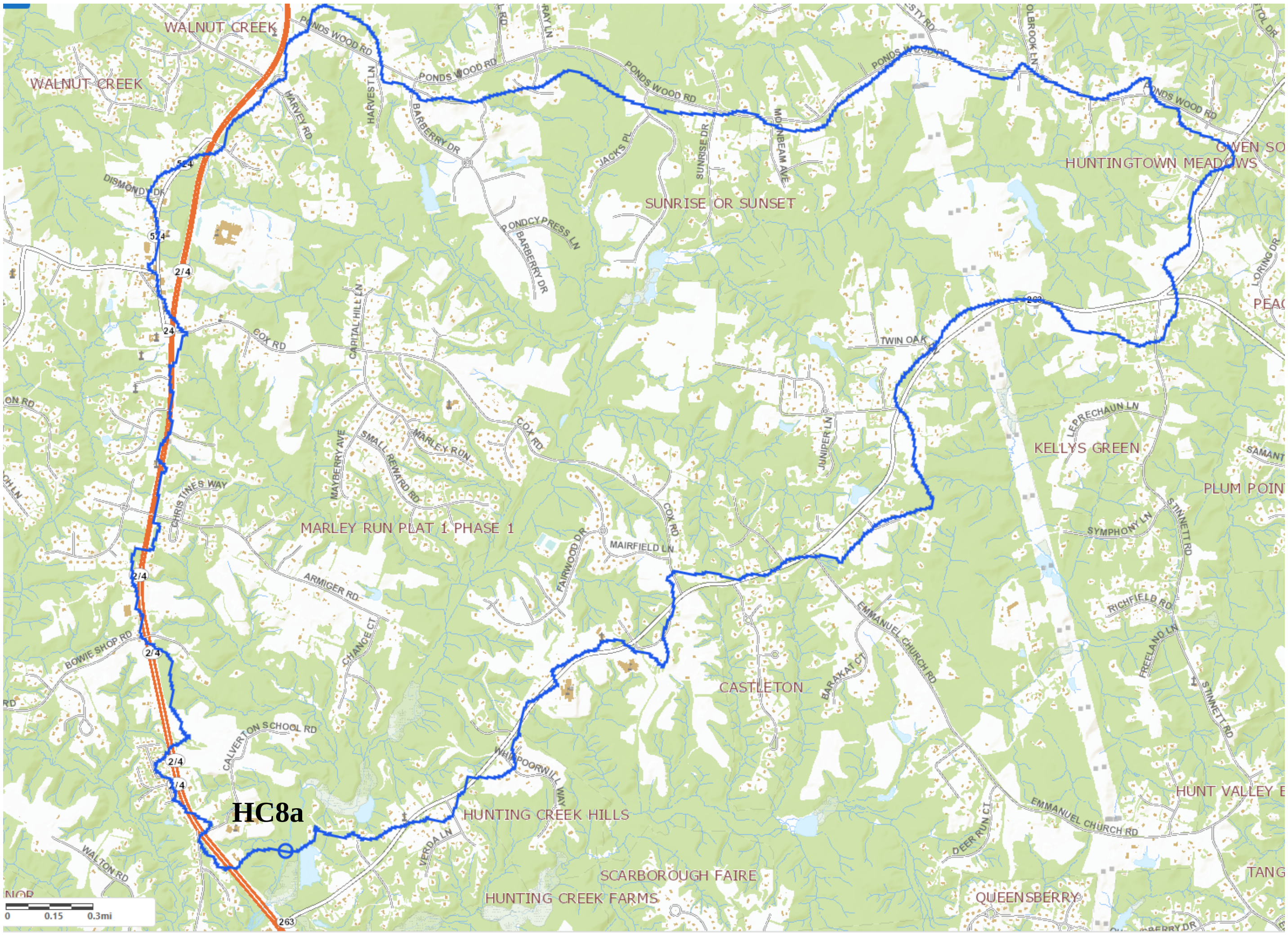

HC8a
WALNUT CREEK
HUNTINGTOWN MEADOWS
SUNRISE OR SUNSET
KELLYS GREEN
MARLEY RUN PLAT 1 PHASE 1
CASTLETON
HUNTING CREEK HILLS
SCARBOROUGH FAIRE
HUNTING CREEK FARMS
QUEENSBERRY
PLUM POINT
PONDS WOOD RD
COX RD
EMMANUEL CHURCH RD
CALVERTON SCHOOL RD
0 0.15 0.3mi

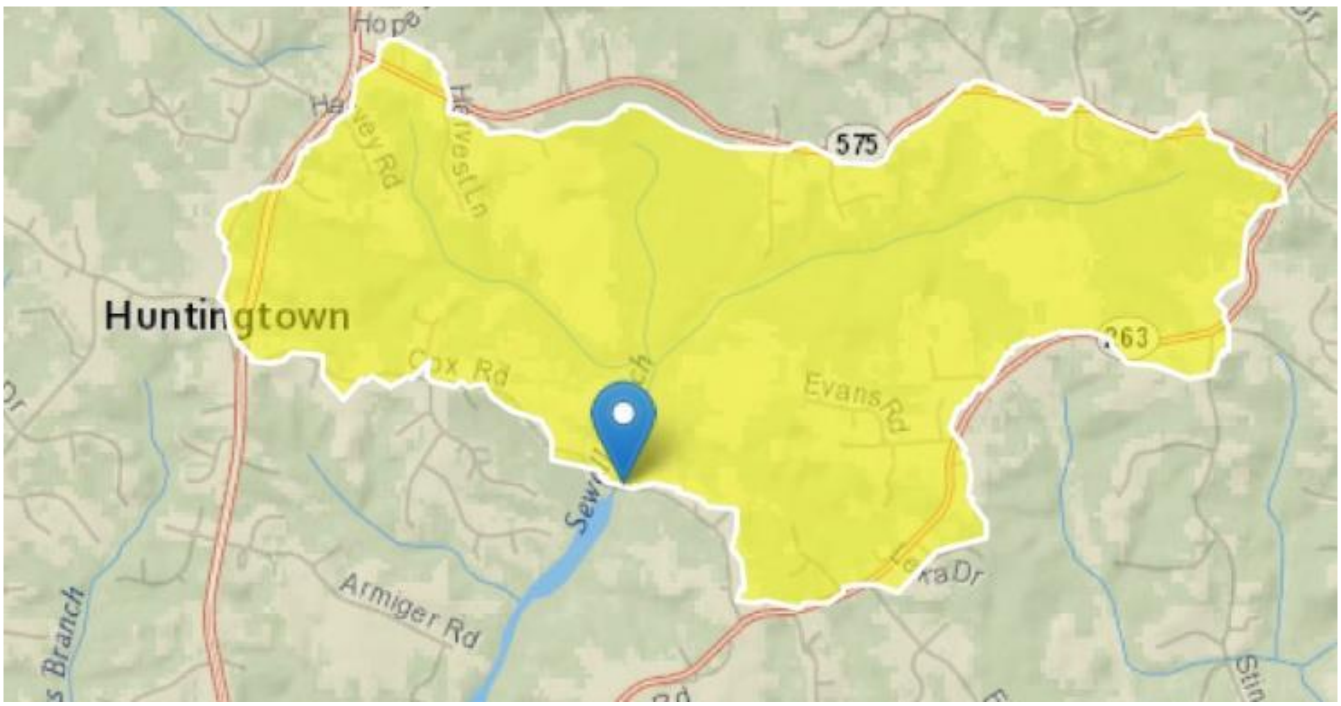
Figure F14. HC8b

HC8b	38.607776	-76.587049	Sewell Branch, Cox Rd.

Coordinates used by StreamStats: 38.60793, -76.58741

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	4.06	square miles
FOREST	Percentage of area covered by forest	59.7	percent



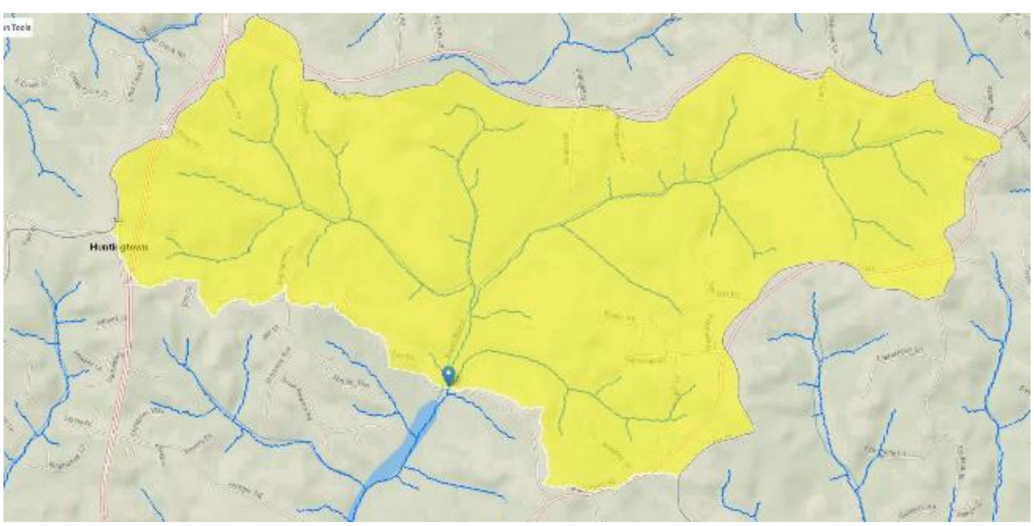


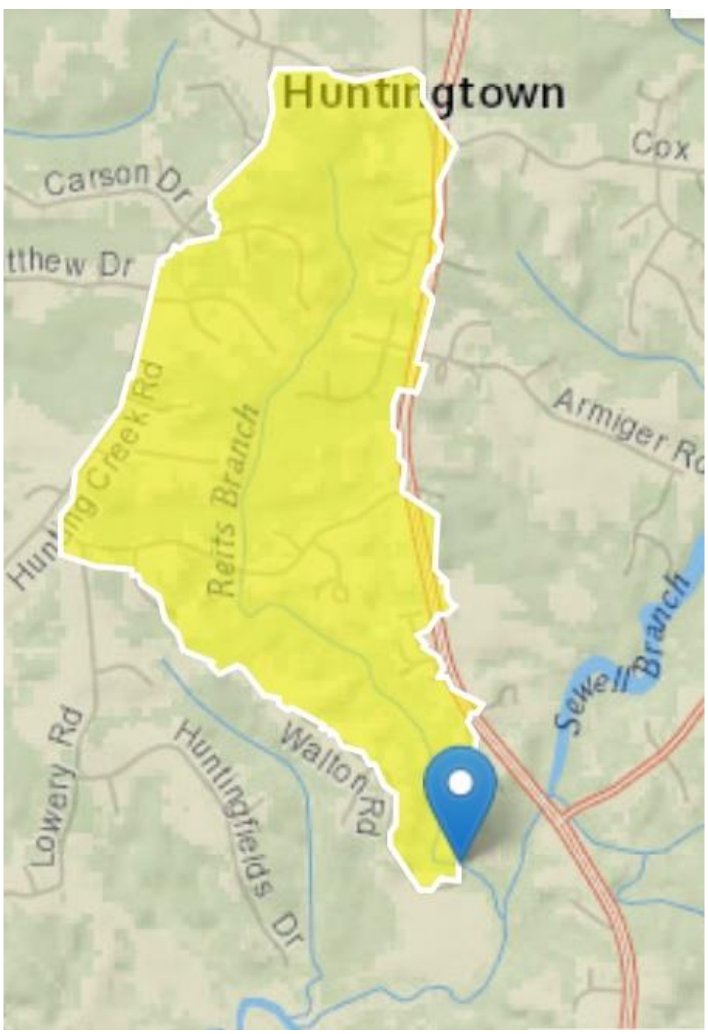
Figure F15. HC9

HC9	38.581740	-76.611284	Reits Branch, Walton Rd

Coordinates used by StreamStats: 38.58201, -76.61140

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	1.76	square miles
FOREST	Percentage of area covered by forest	57.1	percent



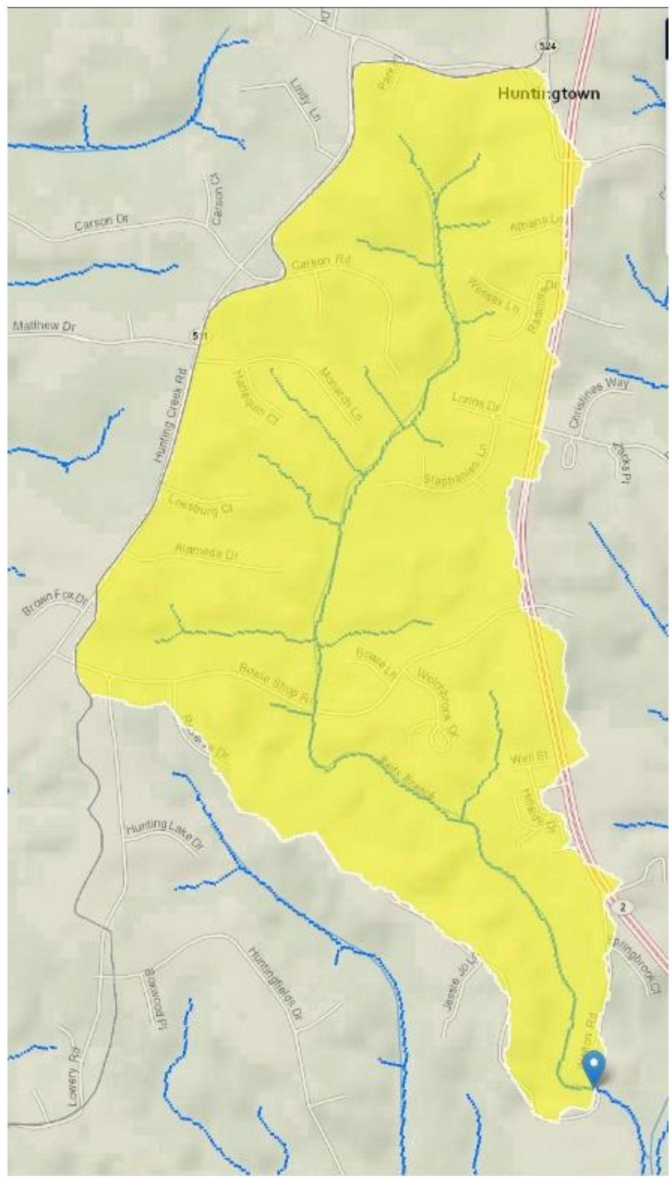


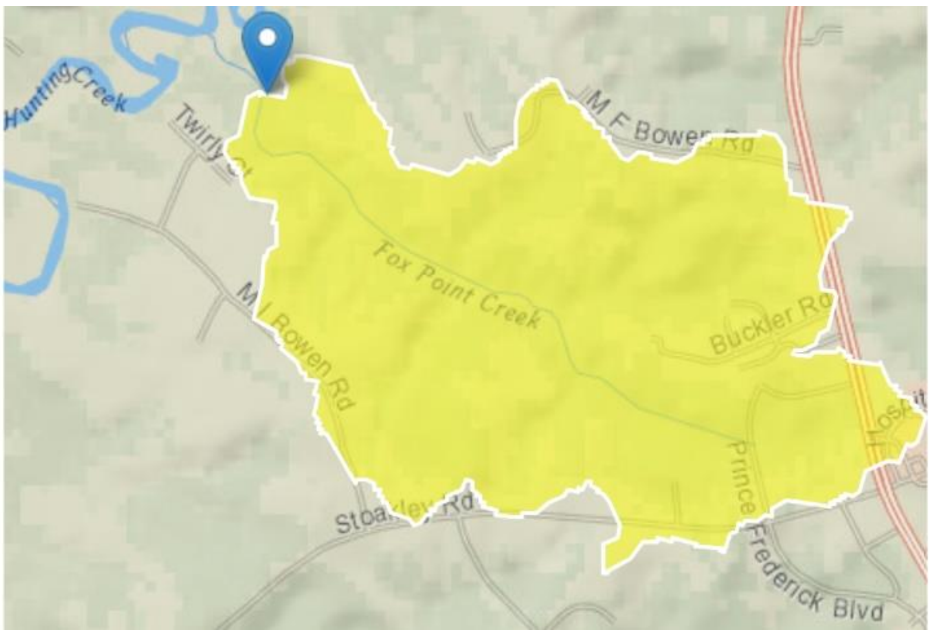
Figure F16. HC10b

HC10b	38.570806	-76.623676	Fox Point Creek, upstream of Hunting Creek

Coordinates used by StreamStats: 38.57114, -76.62378

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	1.08	square miles
FOREST	Percentage of area covered by forest	57.5	percent



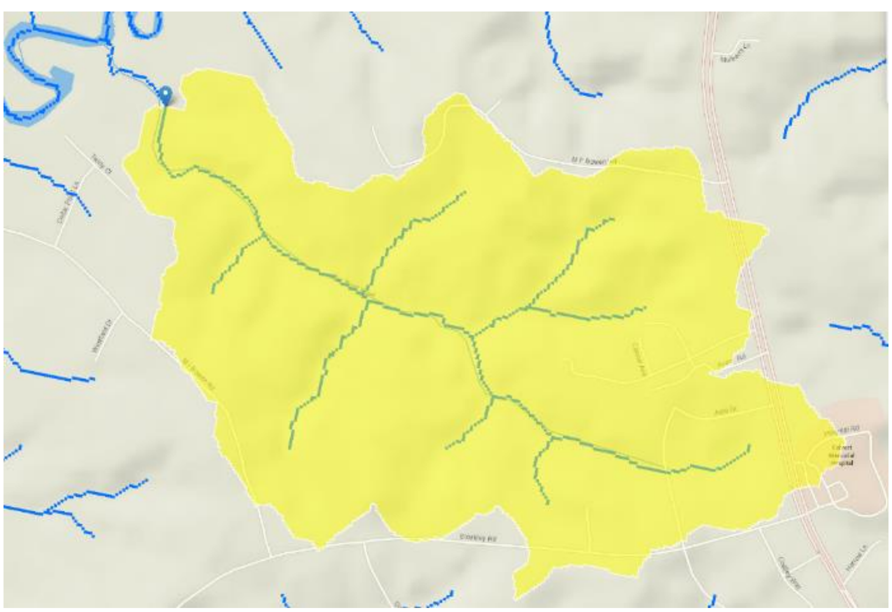


Figure 17. HC12 **(Note: Site HC12 not sampled for 2023, included for reference)**

HC12	38.547489	-76.610592	Mill Creek, Hunters Ridge

Coordinates used by StreamStats: 38.54603, -76.60878

> Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	1.16	square miles
FOREST	Percentage of area covered by forest	59.4	percent

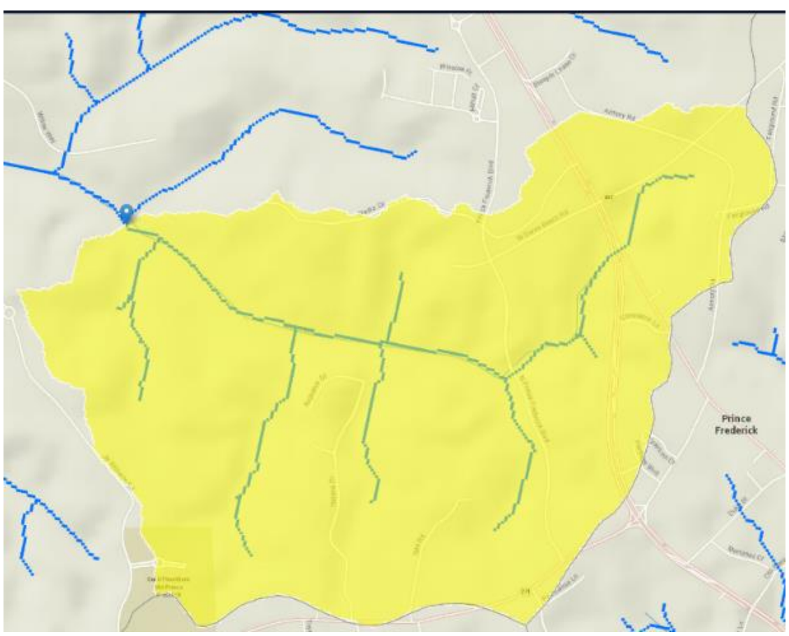


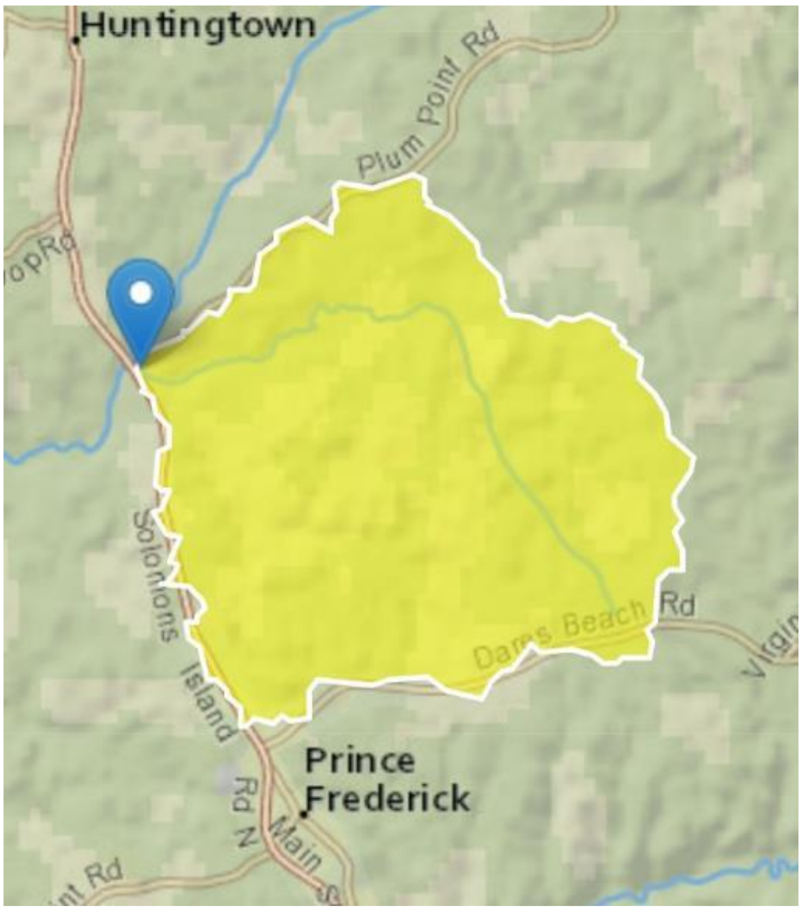
Figure F18. HC13

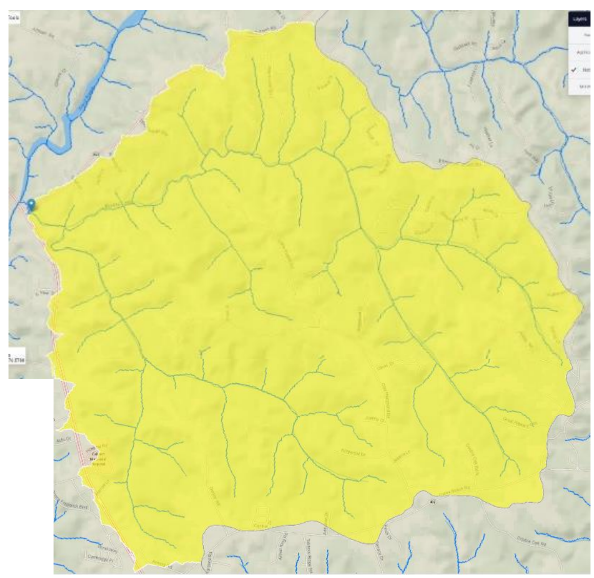
HC13	38.584250	-76.604804	Hunting Creek, Plum Pt. Rd.

Coordinates used by StreamStats: 38.58395, -76.60488

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	9.3	square miles
FOREST	Percentage of area covered by forest	69.9	percent





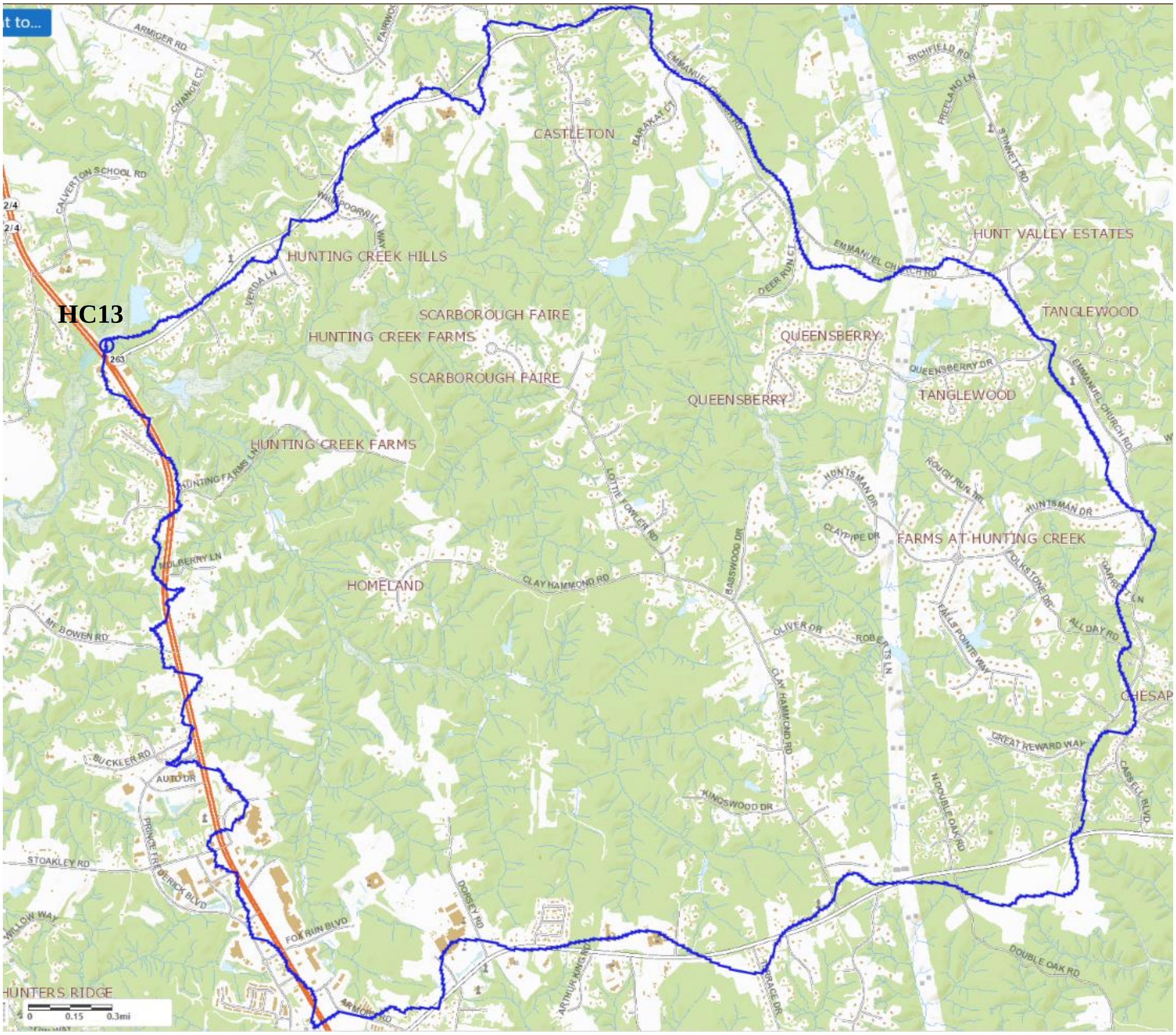

HC13
CASTLETON
HUNTING CREEK HILLS
SCARBOROUGH FAIRE
HUNTING CREEK FARMS
QUEENSBERRY
TANGLEWOOD
HUNT VALLEY ESTATES
FARMS AT HUNTING CREEK
HOMELAND
HUNTERS RIDGE
ARMIGER RD
CALVERTON SCHOOL RD
EMMANUEL CHURCH RD
CLAY HAMMOND RD
STOAKLEY RD
BUCKLER RD
AUTO DR
PRINCE FREDERICK BLVD
FOX RUN BLVD
DORSEY RD
DOUBLE OAK RD
GREAT REWARD WAY
KINGSWOOD DR
OLIVER DR
BASSWOOD DR
LOTTIE FOWLER RD
QUEENSBERRY DR
HUNTSMAN DR
CLAYPIPE DR
ALLDAY RD
RICHFIELD RD
FREELAND LN
VERDA LN
MULBERRY LN
MT BOWEN RD
WILLOW WAY
0 0.15 0.3mi

Figure F19. HC14

HC14	38.554528	-76.592331	Fox Run, Fox Run Blvd.

Coordinates used by StreamStats: 38.55453, -76.59226

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	0.14	square miles
FOREST	Percentage of area covered by forest	47.9	percent

Figure F20. HC15

HC15	38.615396	-76.590811	*Barberry Branch*, Ponds Woods

Coordinates used by StreamStats:38.61543, -76.59093

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	1.04	square miles
FOREST	Percentage of area covered by forest	58.8	percent

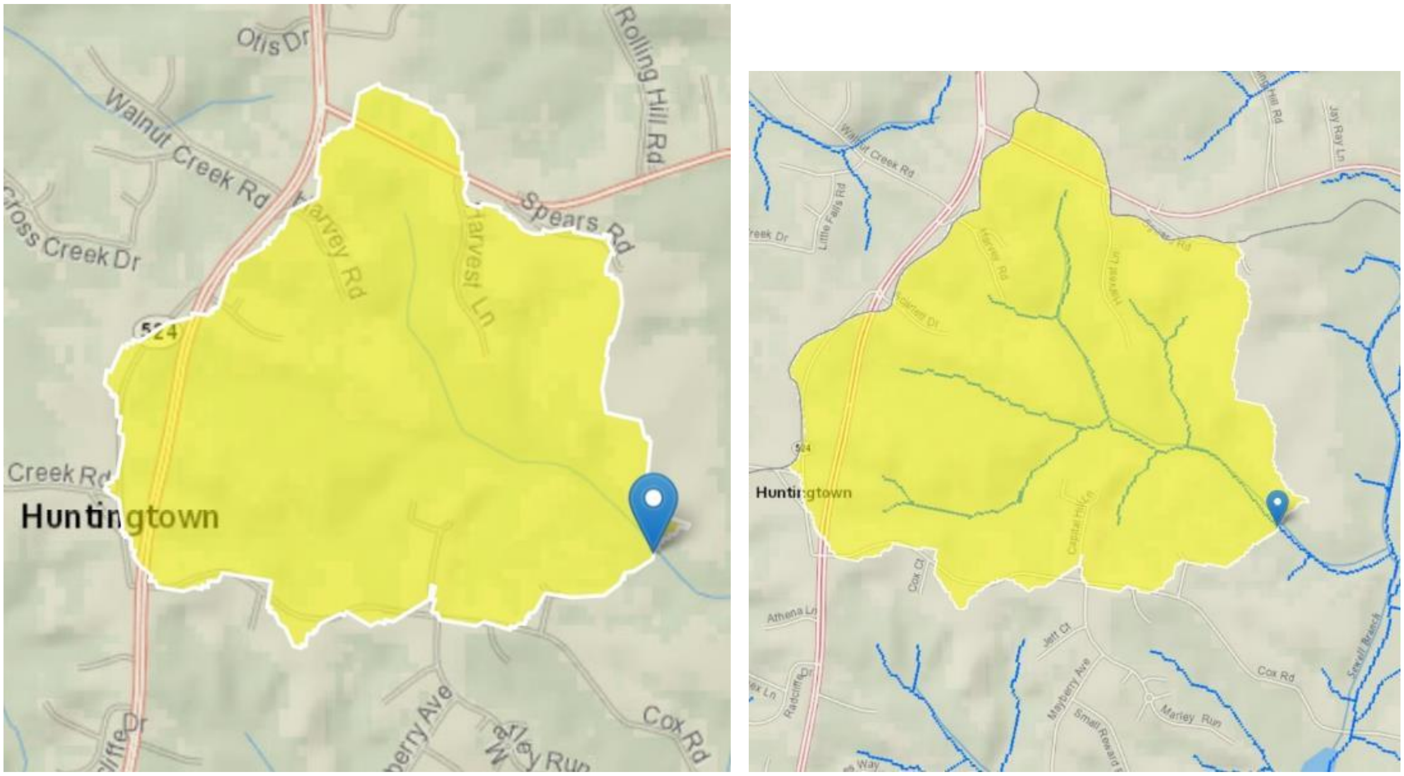


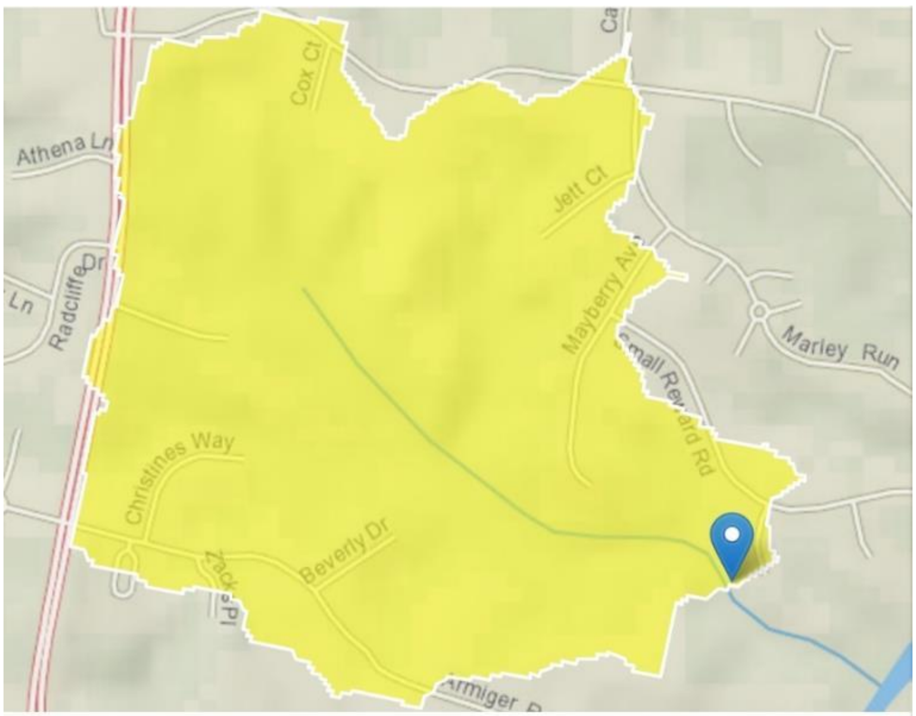
Figure F21. HC16

HC16	38.603900	-76.598206	*Quail Ridge Run*, Marley Run

Coordinates used by StreamStats: 38.60383, -76.59777

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	0.58	square miles
FOREST	Percentage of area covered by forest	57.3	percent



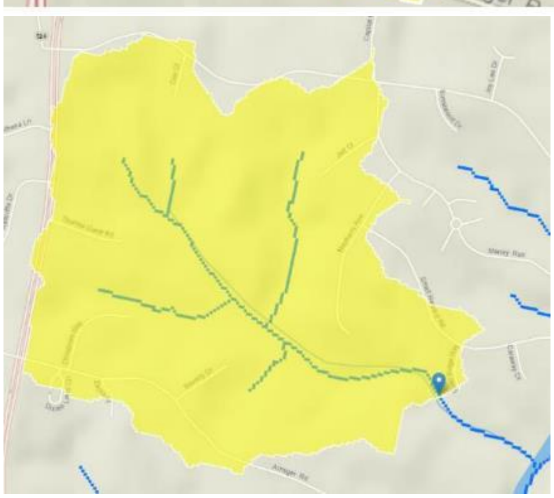


Figure F22. HC18

HC18	38.581099	-76.560572	*Chingaware Run*, Queensberry (Macroinvertebrates and eDNA sampling only)

Coordinates used by StreamStats: 38.58045, -76.5606

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	1.15	square miles
FOREST	Percentage of area covered by forest	63.5	percent

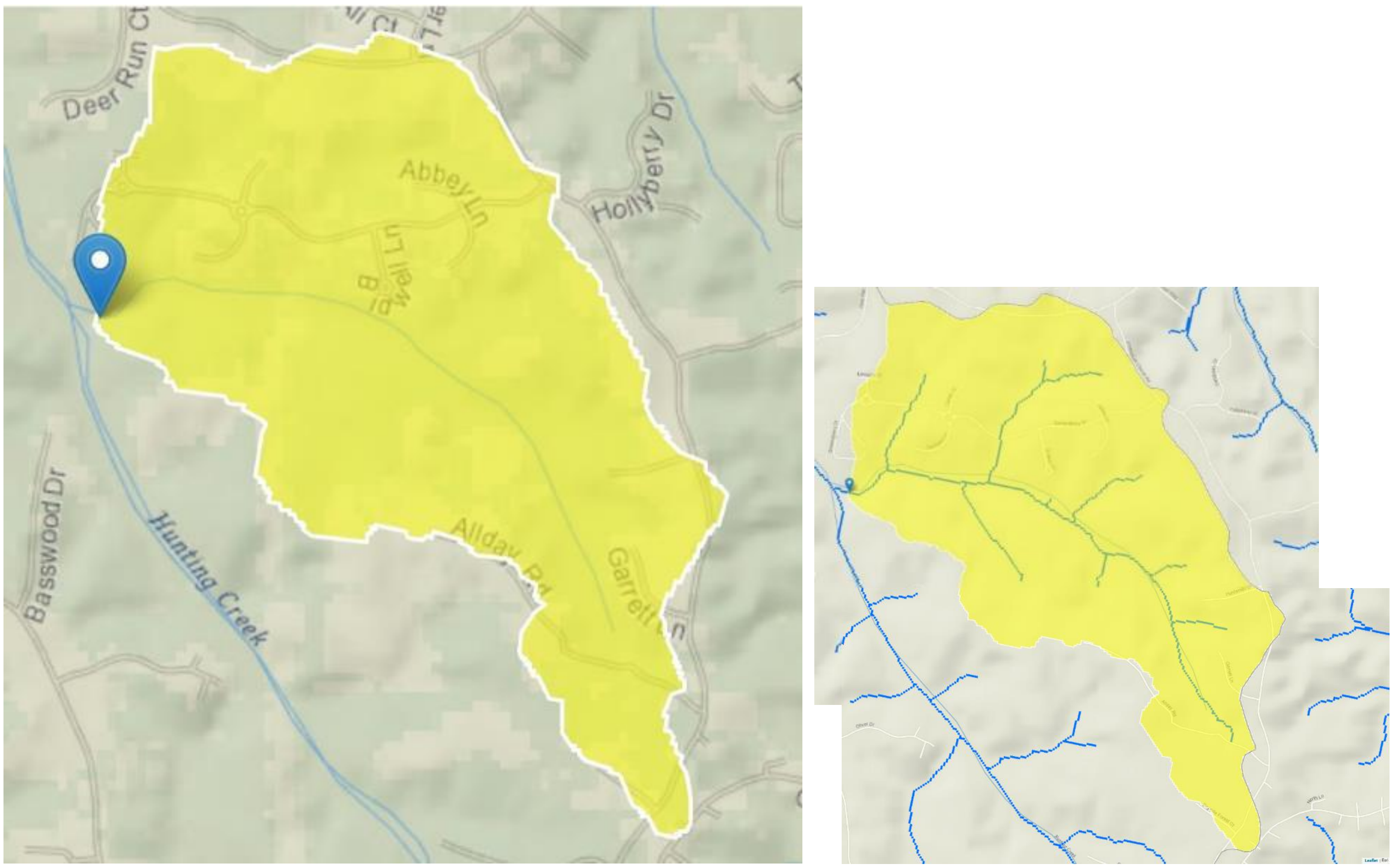


Figure F23. HC19

HC19	38.548962	-76.610882	*Willow Run,* Tributary to Mill Creek, Hunters Ridge

Coordinates used by StreamStats: 38.54902, -76.61091

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	0.49	square miles
FOREST	Percentage of area covered by forest	63.8	percent

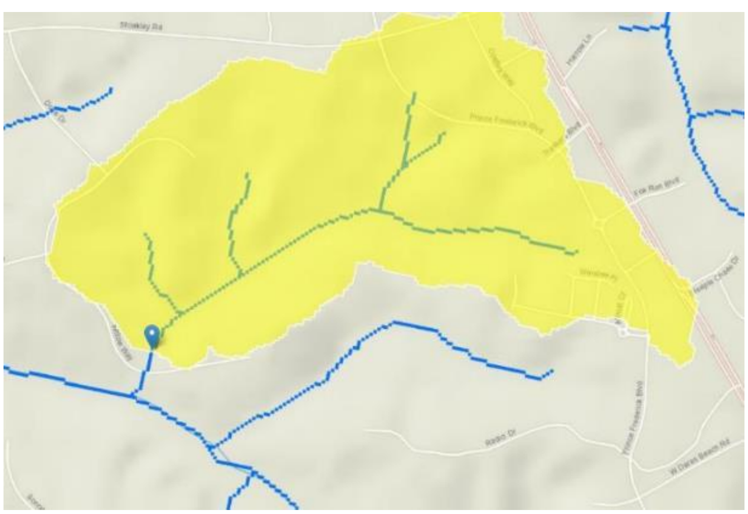


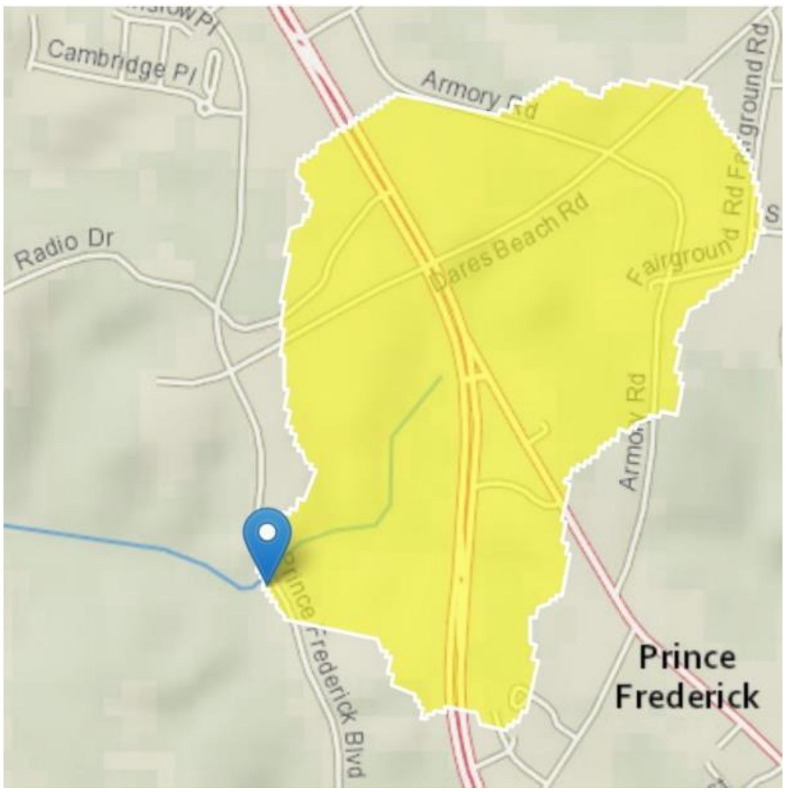
Figure F24. HC20

HC20	38.541126	-76.594114	upper Mill Creek, Prince Frederick Blvd

Coordinates used by StreamStats: 38.54139, -76.59331

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
FOREST	Percentage of area covered by forest	33	percent
FOREST_MD	Percent forest from Maryland 2010 land-use data	35.1	percent



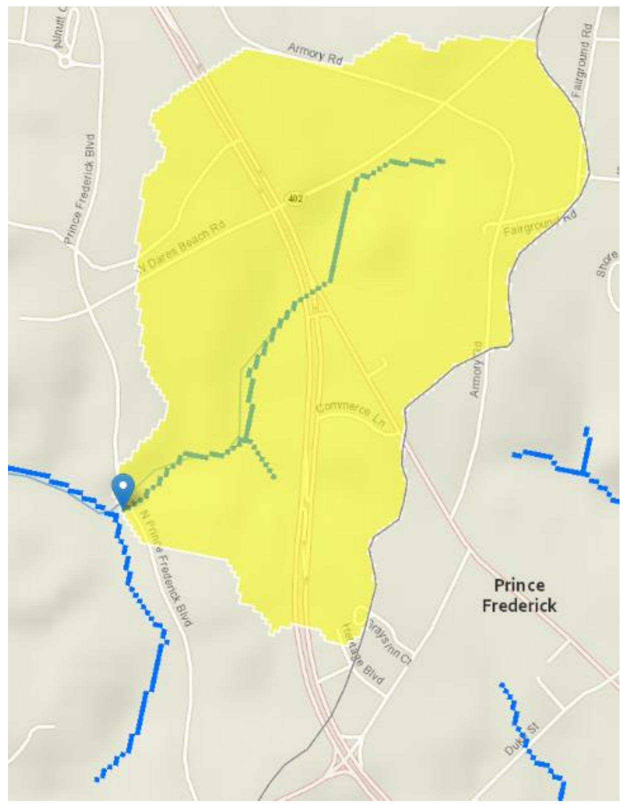


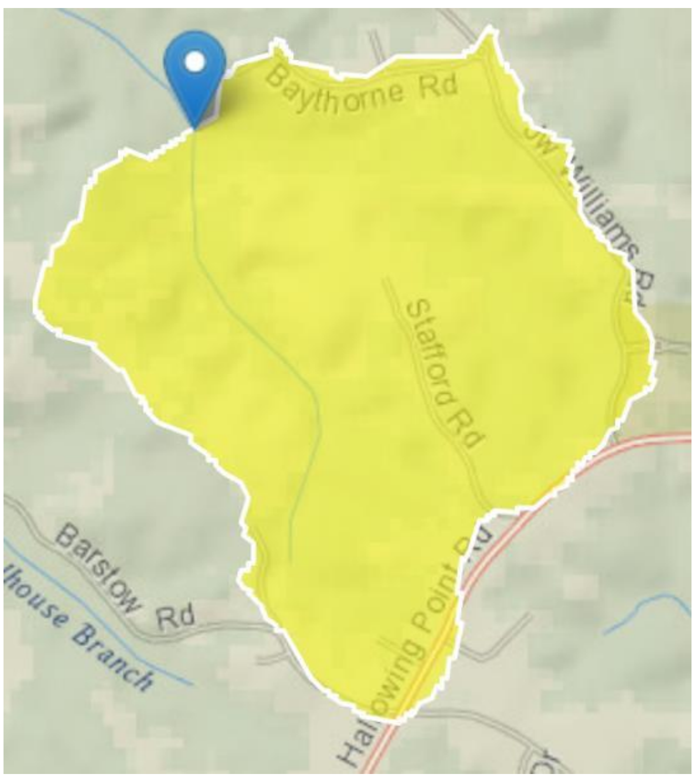
Figure F25. HC21

HC21	38.541040	-76.62333	*College Creek*, College Station,

Coordinates used by StreamStats: 38.54108, -76.62322

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	0.83	square miles
FOREST	Percentage of area covered by forest	63.2	percent



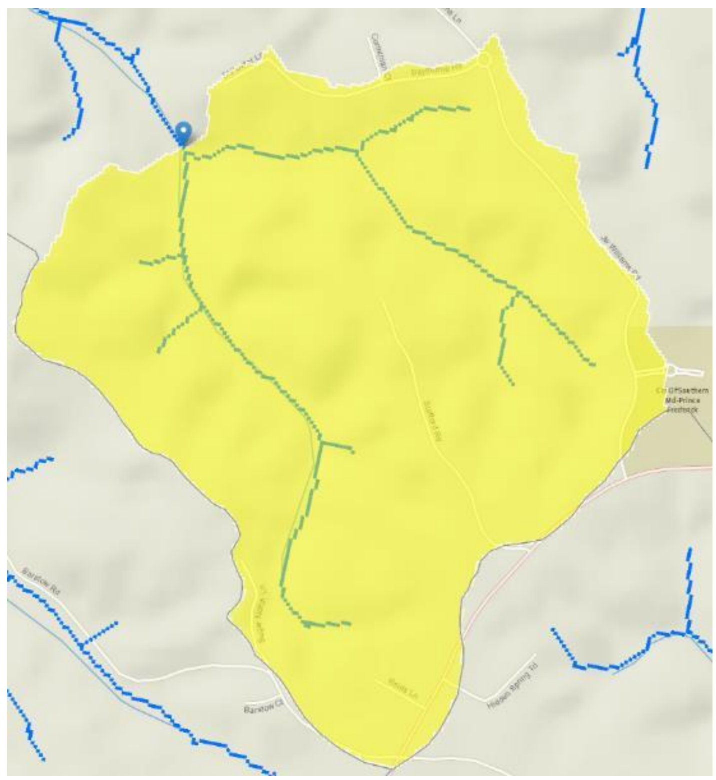


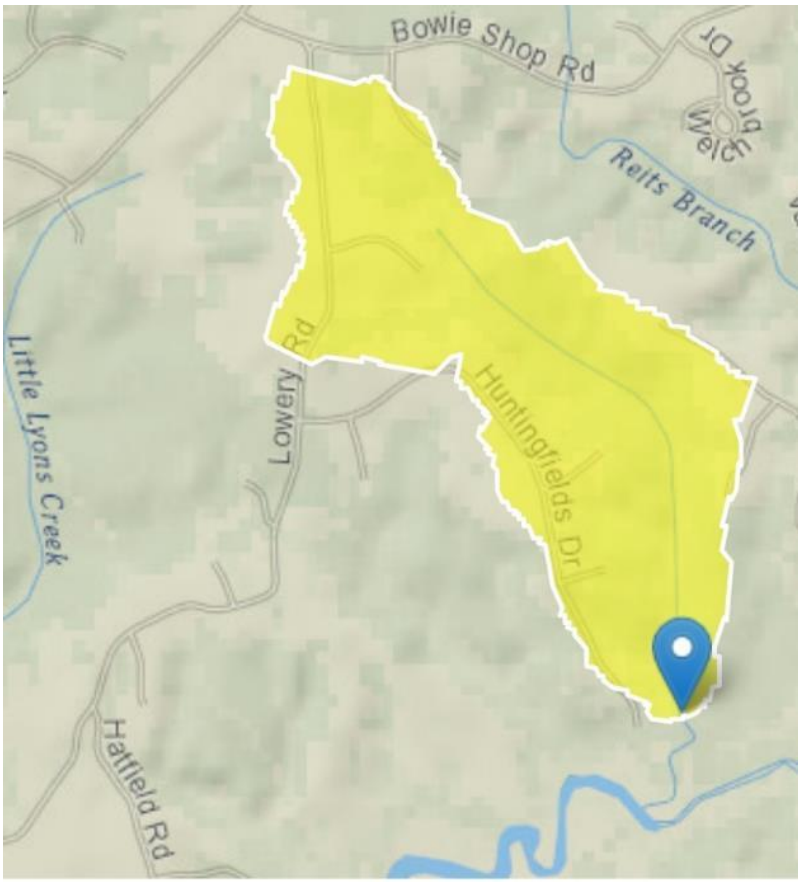
Figure F26. HC22

HC22	38.577751	-76.61970	*Winterberry Creek*, Hunting Fields Manor

Coordinates used by StreamStats: 38.57781, -76.61973

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	0.51	square miles
FOREST	Percentage of area covered by forest	57.3	percent



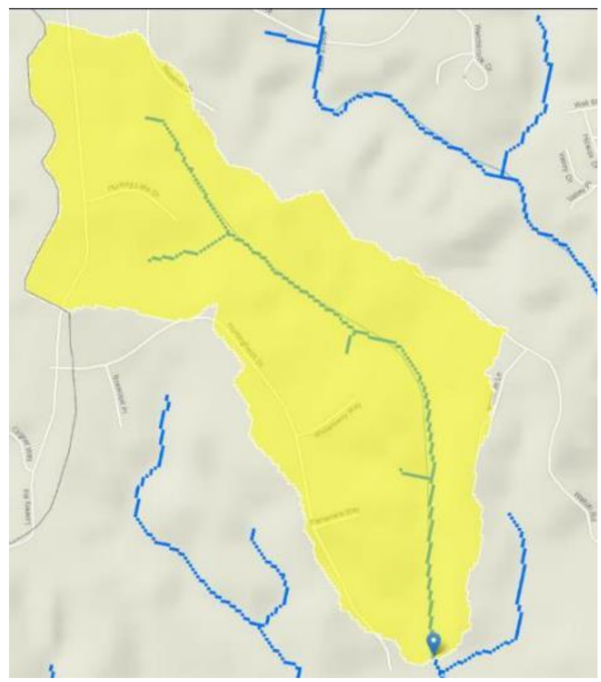


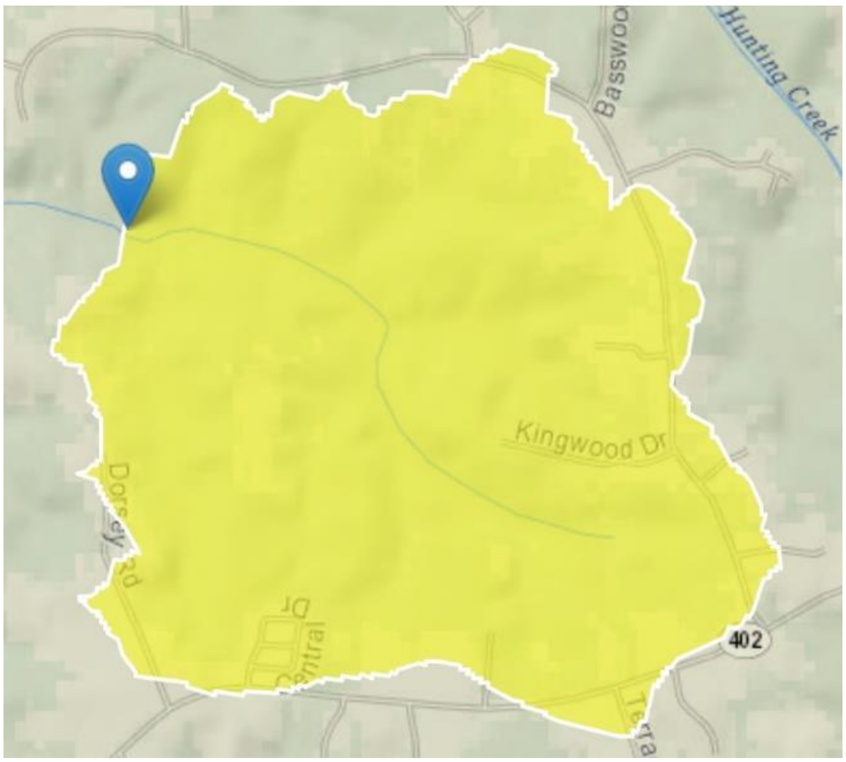
Figure F27. HC23

HC23	38.56750	-76.58208	East Fox Run, Hughes Tree Farm

Coordinates used by StreamStats: 38.56744, -76.58217

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	1.62	square miles
FOREST	Percentage of area covered by forest	78.8	percent



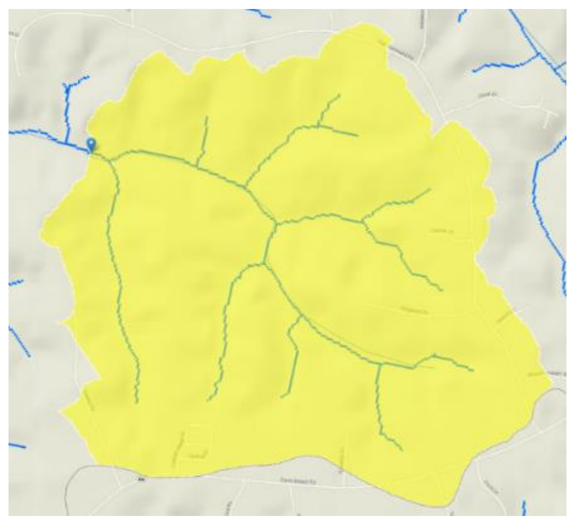


Figure F28. HC24

HC24	38.541467	-76.595070	*Boulevard Branch* (Tributary to upper Mill Creek)

Coordinates used by StreamStats: 38.54117, -76.59346

Basin Characteristics

Parameter Code	Parameter Description	Value	Unit
DRNAREA	Area that drains to a point on a stream	0.16	square miles
FOREST	Percentage of area covered by forest	53.5	percent

(Note: The Latitude used by StreamStats was chosen such that the catchment for HC24 was defined as upstream of the confluence with upper Mill Creek. The actual confluence is about 100m further downstream compared to where StreamStats shows it. The actual catchment is likely only slightly larger.)