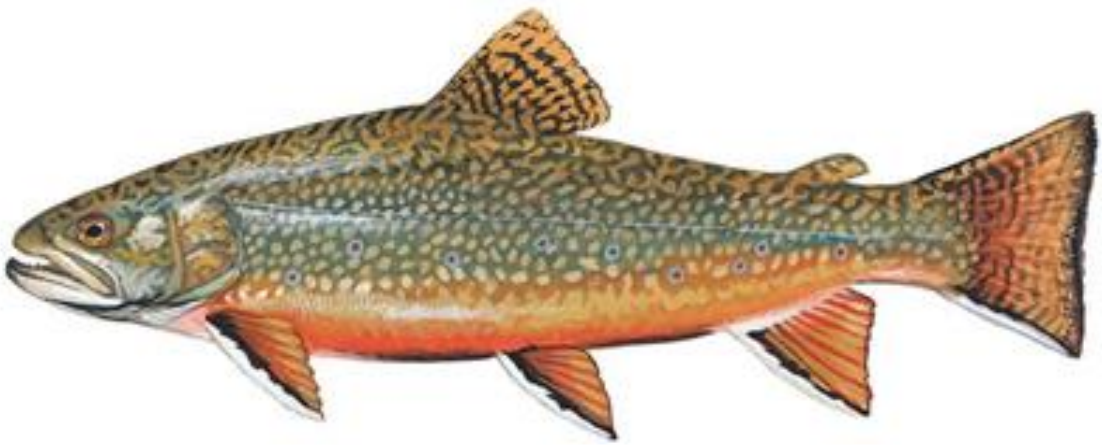

Eastern Brook Trout Monitoring Protocols for Headwater Streams in Virginia's Piedmont

A guidebook prepared for the Piedmont Environmental Council

By Dana Ek and Callee Manna, PEC Fellowship Program July 27, 2017

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Eastern Brook Trout Monitoring Protocols for Headwater Streams in Virginia's Piedmont

This guidebook includes the necessary information to perform standard fish monitoring on streams and rivers in the Upper Rappahannock Watershed. This document was formed from the combination of fish population monitoring data and methods from several organizations in Virginia and data from West Virginia, North Carolina, Maine, and Vermont. At the request of PEC, this guidebook establishes standard protocols for fish monitoring so Virginia's Piedmont restoration projects can measure success with accuracy and robustness. PEC seeks to understand the full story of "restoring to what?," a popular question among conservationists and scientists. Understanding the scope of a stream's health is critical, as well as relating data for water quality monitoring, biological (population) monitoring, and habitat to observe overall ecosystem health.

With this in mind, PEC and regional partners seek to develop successful stream restoration projects that include the rehabilitation of brook trout population dynamics in a concerted effort to become better conservationists for Virginia's Piedmont, and to create models of success that can be transferrable throughout the state. Monitoring long-term success for associated benefits of habitat improvement and water quality is the keystone for this goal, and is a deliverable for PEC restoration projects to expand and improve upon. In collaboration with Claire Catlett, a land conservation officer at PEC, this guidebook draws its focus on four categories of monitoring; water quality, population monitoring, qualitative data, and photo point monitoring. Each of these monitoring standards is an important aspect of fish population monitoring that will provide a clearer estimate of Eastern brook trout populations' habitat and health for future restoration projects.

This guide presents an overview of basic trout monitoring methods collected from across Eastern brook trout habitat in the US. Data has been surveyed from out-of-state monitoring protocols to fill in gaps in Virginia's existing monitoring protocols, as practiced by Virginia's Department of Game and Inland Fisheries (DGIF). Virginia currently dedicates many resources to trout conservation, but trout monitoring protocols are lacking across the state. Although Virginia is a critical location for brook trout habitat, the state has no universal standard and data for fish monitoring. By presenting current initiatives in Virginia and using fish monitoring protocols from others states to fill in the gaps in Virginia protocol, PEC and its partners can monitor trout populations throughout the upper watersheds of the Piedmont, specifically the Upper Rappahannock and James Rivers. These waterways are critical conservation areas for PEC, and there is need for a universal consensus in trout monitoring for accuracy and the continued preservation of wildlife in the Piedmont region.

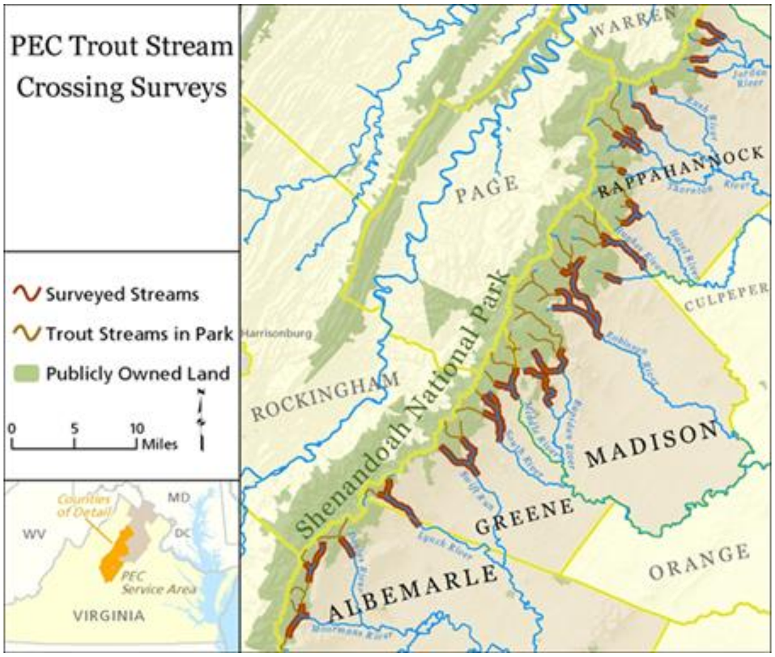

Figure 1: Map of trout streams and rivers in the Piedmont

Species Account: The Eastern brook trout, *Salvelinus fontinalis* is the only trout native to much of the eastern United States. The original range of the brook trout encompasses much of the northeastern corner of North America, including the streams of the high Appalachians as far south as Georgia, and extending west to the Hudson Bay and Great Lakes Basins. It is also known as the "brookie," speckled trout, spotted trout, or squaretail. This unique fish species is considered an indicator for water quality in streams, rivers, and lakes. Brook trout require cold and clear water with low temperatures, preferably below 68 degrees Fahrenheit, with higher levels of dissolved oxygen. Its sensitivity to water quality makes its presence indicative of healthy aquatic ecosystems.

Figure 2: Example of native Eastern brook trout

Brookies are a visually iconic fish, with dark, olive green backs with pale, worm-like markings. Its sides are bluish with yellow and red spots. It has a pale, yellowish-orange belly; in breeding males, the belly is more vivid red or orange. The brook trout's lower fins are orange-red with a white front edge followed by a black streak. They typically range in size from 6-12 inches. Brook trout feed on insects, tadpoles, salamanders, small crayfish and small water snakes. Natural predators of the brook trout are larger water snakes, fish, otters, minks, and birds, such as osprey. Spawning occurs in autumn, mainly in late October to early November. The eggs incubate over the winter and hatch in early spring. Brook trout reach maturity in 2 to 3 years and live to about 6 years of age ("Brook Trout," Chesapeake Bay Program). The Eastern brook trout is not only important biologically, but is also significant to American culture. It is the state fish for seven different states, including Virginia. For anglers, this charismatic native fish has drawn respect and sport for generations.

Conservation: The brook trout faces many threats from human activity and environmental changes. The brook trout requires clean and cold water to thrive, conditions that are threatened throughout its habitat range. Climate change and the resulting increase in water temperature and flood events threaten the delicate aquatic balance brook trout require for survival. Additionally, agricultural and industrial pollutants alter stream water quality and habitat conditions. Stream health can become endangered through higher yields of sediment and channelization, and unnatural changes in local hydrology due to the manipulation of streams and rivers via development of culverts, dams, and other structures. These changes have significant watershed effects on the range of habitat for the brook trout. The result of these environmental effects has been fragmentation and habitat loss for the brook trout, a negative process that ultimately is detrimental to the long-term genetic stability and survival of the population. To protect and conserve this special species, these isolated brook trout populations should be reconnected within sub-watersheds, at a minimum, to ensure the long-term survival of the Eastern brook trout (Heft, 2006). Monitoring these brook trout populations and their habitat range is key to determining the implementation and location of conservation initiatives.

Current Trout Habitat Initiatives in VA: Virginia is one of the southernmost homes of Eastern brook trout. Brook trout are the only trout native to Virginia and make up 80% of all trout in the state ("Wild Brook Trout").The Virginia Department of Game and Inland Fisheries (VDGIF) identifies over two thousand miles of freshwater trout streams, more trout streams than located in all southeastern states combined. The state's extensive population is key for brook trout preservation. With Chesapeake Bay conservation already a state focus for Virginia, headwater streams within the habitat range of brook trout serve as priority zones for water quality and stream restoration. VDGIF is the primary agency responsible for trout monitoring in Virginia. VDGIF monitors trout populations by implementing fishing regulations in an effort to combat the current data shortage on trout stream population and fishing statistics. VDGIF also utilizes electrofishing as a way to monitor fish populations in the state, and is one of the only entities in the state that collects official fish population monitoring data, along with the National Park Service. VDGIF has independent stream prioritization models to determine its monitoring sites. The department monitors a few hundred cold water trout streams annually across the state. Most of these sites are monitored in southwest Virginia, with less than ten trout streams in the Rappahannock Watershed. Notable streams include Kinsey Run, Garth Run, the Rapidan River, and the Conway River.[1]

Shenandoah National Park monitors priority trout streams annually, and other trout streams in the park on an eight year cycle.[2] Priority is given to the largest trout streams. The Park monitors roughly eighty-five fish streams, with most streams having a monitoring site at the Park boundary. Some have additional sites further into the park due to fluctuating water levels at the boundary, although every site is different. Streams of note include Jeremy's Run and Piney River. Electrofishing sites are roughly 100 meters (ranging from 80 meters to 120 meters).

Shenandoah's brook trout monitoring on streams running through the park is usually in line with state protocols from VDGIF, with notable variations including the NPS using greater precision and more people in its electrofishing. Another notable difference is the NPS employs the use natural breaks instead of block nets during electrofishing runs. All fish sites are co-located with UVA water quality work within the park. Shenandoah and VDGIF often share data and co-locate monitoring sites to ensure park efforts are not duplicated. It is also noteworthy that Department of Environmental Quality (DEQ) monitors some sites in Virginia, but their monitoring protocol follows EPA electrofishing methods. Their monitoring sites are randomly selected throughout the state, and are no co-located with any sites from the NPS or VDGIF.

Most nonprofits and other entities appeal to or partner with VDGIF for population monitoring data, and fall in line with VDGIF fish monitoring protocols. It should be noted that not all streams in the state are monitored for trout population data, and that secondary sources of data from citizen scientists serve to bolster the datasets maintained by VDGIF. Some of the most accurate population data in Virginia comes from anglers and local fisherman, as these citizen scientists (some prefer to be known as angler scientists), work through initiatives run by local chapters of Trout Unlimited and other sportsmen's and environmental groups. Beyond VDGIF, fish stocking is also a widely implemented tool from state and federal agencies (such fishing oas the National Park Service) across the northern and mid-Atlantic regions, to control recreational fishing of the brook trout.

Nonprofit organizations play an active role in Virginia brook trout conservation and monitoring. In Virginia's Piedmont, partnerships between Trout Unlimited (TU), Friends of the

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[1] For a detailed case study on trout populations in Kinsey Run, see "Evaluation of Brook Trout (Salvelinus fontinalis) Introductions and Re-introductions Into Four Virginia Blue Ridge Mountain Streams" by Michael Isel.
[2] For specific data on Shenandoah National Park's monitoring protocols and population reports, see Appendix B

Rappahannock (FOR), Piedmont Environmental Council (PEC), the Krebser Fund for Rappahannock County Conservation, Eastern Brook Trout Joint Venture (EBTJV), and Ecosystem Services, LLC devote energy and resources to trout conservation efforts. Primary conservation efforts in Virginia include planting riparian buffers, using GIS information to survey trout habitat, and efforts to remove and restore streams affected by man-made barriers such as culverts that impair fish passage. Notable areas within the Rappahannock watershed include restoration efforts along the Spruce Pine Branch, Robinson River, and Kinsey Run. These conservation efforts are widely utilized across nonprofit and state agencies to improve brook trout habitat in Virginia.

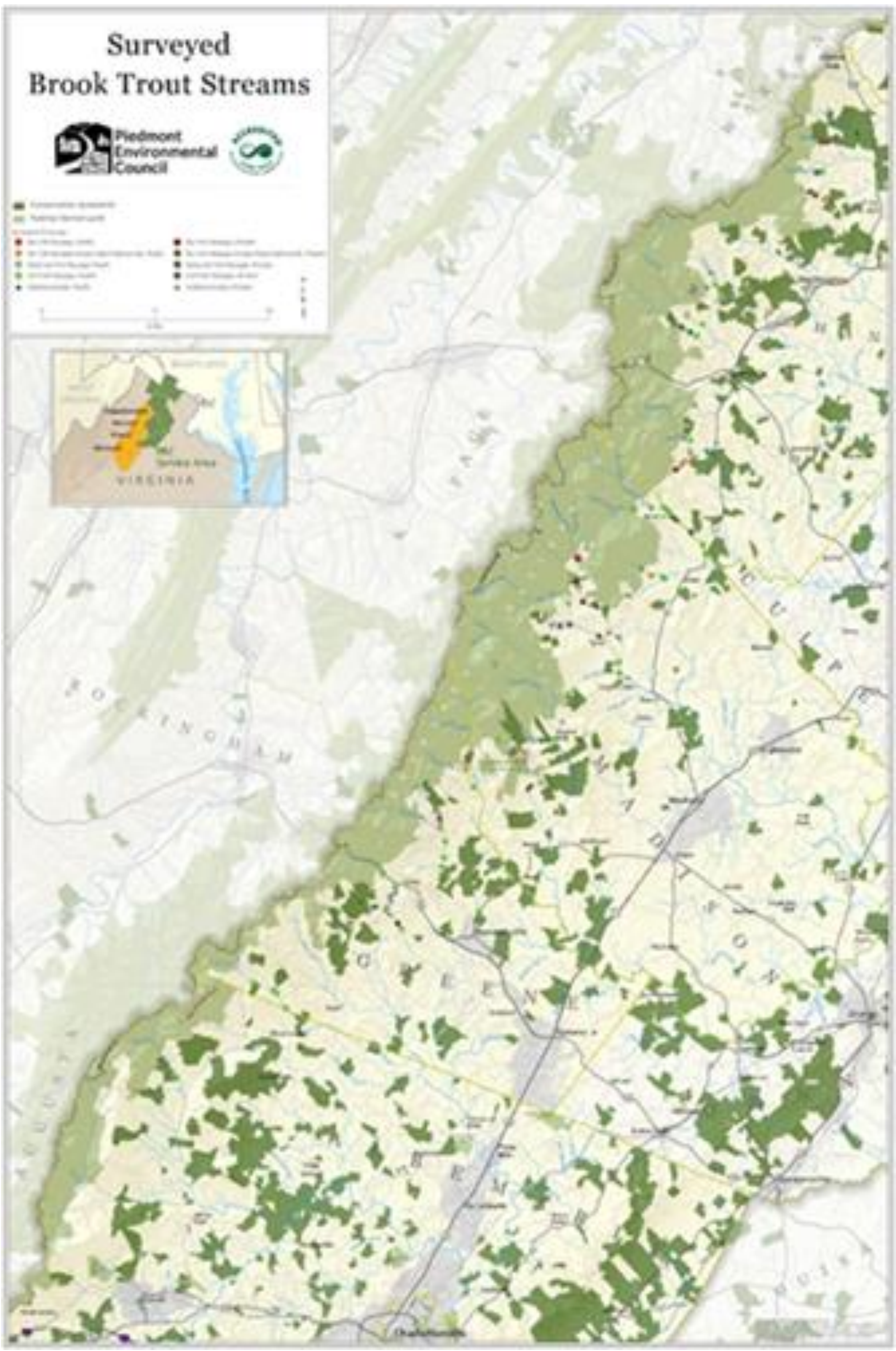

Figure 3: PEC map of surveyed EBT streams in the Virginia Piedmont

Figure 4: Robinson River before PEC restoration and culvert removal

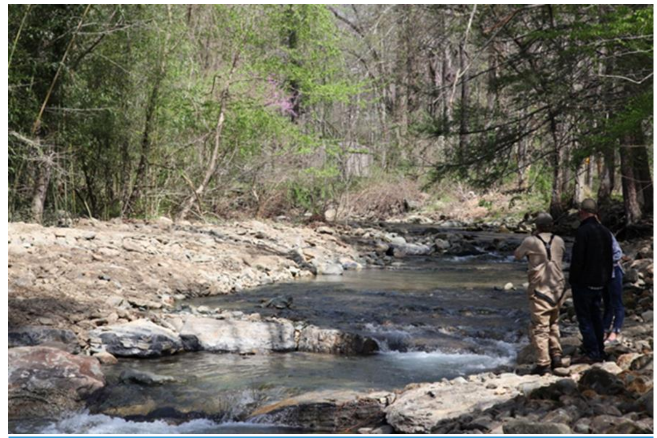

Figure 5: Robinson River after PEC restoration and culvert removal

Figure 6: Spruce Pine Branch before stream restoration and culvert removal

Figure 8: Sprucepine Branch after stream restoration and culvert removal

Figure 7: Sprucepine Branch after stream restoration and culvert removal

Introduction to scientific protocols for Eastern brook trout

Fish monitoring is critical for assessing population health. Trout Unlimited defines monitoring as observing something continuously over time at regular intervals to evaluate conditions in population trends and habitat (Williams et al., 2006). Assessing baseline conditions of the monitoring site(s) and resulting project impacts, or data before and after restoration projects, is a critical component of population monitoring. Furthermore, legal compliance is an important component of monitoring projects, as stream projects are regulated by both state and federal agencies.[3]

Fish population monitoring is comprised of many different components. The most common method for fish population monitoring is electrofishing, where trout are shocked, measured, weighed, and the stream population recorded. In general, fish population monitoring uses a variety of methods to assess quality and quantity of fish in monitoring sites. However, there are many other components to assessing a trout monitoring site that are important for assessing site health for brook trout populations. For example, water quality monitoring, biological, and habitat data are also recorded to observe overall ecosystem health.[4]

The quality of a stream is effected by its surrounding habitat. Habitat incorporates the physical, chemical, and biological components that influence the structure and function of the aquatic community in a stream (Williams et al., 2006). This is important to observe when selecting a site for a trout restoration project. To monitor stream quality, the stream habitat is visually assessed and scored for features such as how much of the stream bottom is affected by sediment, what percentage of the stream bank is covered by native vegetation, the frequency of riffles and bank stability. A stream with good habitat quality will have a wide, healthy forest growing along both stream banks, small amounts of erosion on the stream banks, cobbles and pools throughout the stream and vegetative debris in the stream. Chemical tests for water quality are also key to analyzing stream health. Temperature, Stream Flow, Turbidity, Dissolved Oxygen, pH, and Macroinvertebrates should be monitored in order to analyze the water quality of brook trout habitats.

Scope is also an important component to monitoring protocol. Short and long-term data are essential to developing a well-rounded study. Short-term data provides immediate results, but brook trout populations are known to naturally fluctuate through a variety of environmental factors, including floods, droughts, land use practices, changing temperatures, availability and quality of overwintering habitat, invasive species, and more. While more expensive and time-consuming, long term monitoring provides more reliable data. Data over this longer period

[3] For details regarding Virginia-specific regulations, see Dauwalter et al.'s "Stream Temperature Monitoring: a handbook for Trout Unlimited Chapters"

[4] For additional resources involving trout restoration, see Williams, Jack E. et al.'s "A Guide to Native Trout Restoration: Science to Protect and Restore Coldwater Fishes and their Habitats"

observes a variety of biological, intensive habitat, fish surveys, and water quality components. Trout Unlimited and Eastern Brook Trout Joint Venture hosted a workshop that found monitoring brook trout populations per square mile is an ideal representation of the whole. In general, it is recommended that long-term monitoring stations be sampled bimonthly every 5 years to establish a baseline. After the establishment of a baseline, monitoring once every 2-3 years is recommended for potential changes in acid mine drainage, acid rain, sediment, and nutrient enrichment.

Water Quality Monitoring

Monitoring aspects of the water quality of a stream or river is key to analyzing the stream health, and also can indicate the viability of fish populations. Brook trout require a habitat that is cold and clear, and populations decline when streams do not meet those requirements. Strong wild brook trout populations demonstrate that a stream or river ecosystem is healthy and that water quality is optimal.

Temperature: Brook trout may be found in waters with a temperature range between 0 - 24º C. However, the optimal water temperature range for growth and survival is from 11 - 16ºC (Heft, 2006). Indirect influence of water temperature on fish affects growth rate by limiting food abundance, altering toxicity of waterborne pollutants, and changing oxygen concentration and biochemical oxygen demand. There is general agreement from field and laboratory studies that water temperature is the single most important factor limiting the geographic distribution of brook trout.

Research conducted through Maine's department of fisheries suggest targeted temperature monitoring using thermometers or temperature data loggers at high priority streams, rivers, and watersheds. Late June and early September is the ideal time for temperature monitoring, and biweekly recording of the maximum, minimum, and ambient sampling temperatures is recommended.

Following methods based on USGS Temperature Field Manual

1. Calibrate thermometer based on type of thermometer in use.
2. Measure temperature in those sections of the stream that represent most of the water flowing in a reach. Do not make temperature measurements in or directly below stream sections with turbulent flow or from the stream bank. Practice consistency in the temperature reading location
3. Record on field forms the temperature variation from the crosssectional profile, and the sampling method selected.
4. Immerse the sensor in the water to the correct depth and hold it there for no less than 60 seconds or according to the manufacturer's guidelines until the sensor equilibrates thermally.
5. Read the temperature to the nearest 0.5°C for liquid-in-glass and 0.2°C for thermistor readings—do not remove the sensor from the water.

6. Record Data
7. Remove the temperature sensor from the water, rinse it thoroughly with deionized water, blot it dry, and store it.

Trout Unlimited utilizes several types of temperature monitoring (West Virginia DEP), including:

- Baseline monitoring: Assessing temperature patterns across time at single or multiple sites.
- Long-term monitoring: Tracking trends across watersheds using year-round, long-term monitoring over multiple years and across a large network of sites.
- Impact monitoring: Monitoring pre- and post-restoration treatment water temperature regimes.
- Water quality compliance: Ensure compliance with temperature standards set by state and federal laws; determine effects of warm water discharges

Stream flow: The stream flow rate is the volume of water that moves over a designated point over a fixed period of time. A base flow >55% of the average annual daily flow is considered excellent, a base flow of 25 to 50% is considered fair, and a base flow of < 25% is considered poor for maintaining quality trout habitat.

The following formula is used to calculate flow:

Flow = ALC/T

A= average cross-sectional area of the stream (stream width x average water depth)

L = length of the stream reach measured (usually 20 ft.)

C = a coefficient or correctional factor (0.8 for rocky-bottom streams or 0.9 for muddy-bottom streams). This allows you to correct for the fact that water travels faster at the surface than near the bottom due to resistance from gravel, cobble, etc. Multiplying the surface velocity by a correction coefficient decreases the value and gives a better measure of the stream's overall velocity

T = time (in seconds) for the float to travel the length of L

Methods based on protocol from the EPA "Volunteer Stream Monitoring: A Methods Manual"

1. Select area of interest: at least 6 inches deep, no slow water such as a pool, unobstructed riffles or runs are ideal. The length that you select will be equal to L in solving the flow equation. 20 feet is a standard length used by many programs.
2. Mark transect 1 and 2 (total length L)
3. Determine average cross sectional area of stream, A (stream width x average depth)
4. Time trials of floating object passing from transect 1 to transect 2, (T)
5. This "time of travel" measurement should be conducted at least three times and the results averaged--the more trials you do, the more accurate your results will be. (T)
6. Calculate Flow by plugging in variable values found
7. Record data

Turbidity: Turbidity is the cloudiness or haziness of a fluid caused by large numbers of individual particles that are generally invisible to the naked eye. Brook trout are sight feeders and feeding can be impaired by high or persistent water turbidity. Optimum turbidity values for brook trout growth are approximately 0 - 30 Jackson Turbidity Units (Heft, 2006). An accelerated rate of sediment deposition in streams may also reduce local brook trout production because of the adverse effects on production of food organisms, smothering of eggs and embryos in the redd, or egg nest, and loss of escape and overwintering habitat. Turbidity can be measured using either an electronic turbidity meter or a turbidity tube. In streams where neither vertical nor horizontal methods are effective, a transparency or Secchi tube can be used to measure turbidity and water clarity.

Methods based on Maryland DNR Trout Management Plan

1. Fill tube with water from the sample site, sampling upstream
2. Hold the tube in one hand near the bottom and look into the open end with your head about 10 to 20 centimeters above the tube, so that you can clearly see the secchi tube at the bottom
3. Slowly pour the water sample into the tube, waiting for air bubbles to rise if necessary, until the mark on the bottom of the tube just disappears.
4. Stop pouring the water sample into the tube and look at the level of water in the tube. For turbidity tubes which have a turbidity scale marked on the side, read the number on the nearest line to the water level. This is the turbidity o f the water. If the tube does not have a scale marked, measure the distance from the bottom of the tube to the water level with a tape measure and look up or calculate the turbidity of the water sample using the instructions provided with the tube.
5. Record Data
6. Clean tube and properly store

Dissolved Oxygen: Dissolved oxygen refers to the amount of oxygen (O) dissolved in water. Because fish and other aquatic organisms cannot survive without oxygen, dissolved oxygen is one of the most important water quality parameters. Dissolved oxygen is usually expressed as a concentration of oxygen in a volume of water (mg/L). Brook trout normally require high oxygen concentrations with optimum conditions at dissolved oxygen concentrations near saturation. Local or temporal variations should not decrease to less than 5 mg/l. As temperature rises and dissolved oxygen decreases, fish begin to experience stress. These stresses begin to set in well before the water temperature reaches lethal limits. Optimal oxygen levels for brook trout are not well documented but appear to be >7 mg/l at temperatures < 15° C and >9 mg/l at temperatures >15° C. Dissolved oxygen concentrations should not fall below 50% saturation in the redd for embryo development. Methods for measuring dissolved oxygen include the Winkler Method (a simple titration using a LaMotte dissolved oxygen test kit) or use of a dissolved oxygen probe (Vernier or YSI).

Winkler Method Basic Procedures

1. Fill a 300-mL glass stoppered bottle brim-full with sample water.
2. Immediately add 2mL of manganese sulfate to the collection bottle by inserting the calibrated pipette just below the surface of the liquid. Avoid bubbles.
3. Add 2 mL of alkali-iodide-azide reagent in the same manner.
4. Stopper the bottle with care to be sure no air is introduced. Mix the sample by inverting several times. Check for air bubbles; discard the sample and start over if any are seen. If oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settled to the bottom, mix the sample by turning it upside down several times and let it settle again.
5. Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully stopper and invert several times to dissolve the floc. At this point, the sample is "fixed" and can be stored for up to 8 hours if kept in a cool, dark place. As an added precaution, squirt distilled water along the stopper, and cap the bottle with aluminum foil and a rubber band during the storage period.
6. In a glass flask, titrate 201 mL of the sample with sodium thiosulfate to a pale straw color. Titrate by slowly dropping titrant solution from a calibrated pipette into the flask and continually stirring or swirling the sample water.
7. Add 2 mL of starch solution so a blue color forms.
8. Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue color. Be especially careful that each drop is fully mixed into the sample before adding the next. It is sometimes helpful to hold the flask up to a white sheet of paper to check for absence of the blue color.
9. The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used. Each mL of sodium thiosulfate added in steps 6 and 8 equals 1 mg/L dissolved oxygen.

For a dissolved oxygen probe, consult the methods stated by the provider.

pH: The acidity of a stream is another important factor for the overall health of the ecosystem. Brook trout appear to be more tolerant of low pH than other trout species. The optimal pH range for brook trout is 6.5 – 8.0 with a tolerance range of 4.0 – 9.5. Measurements of pH can be taken via a pH meter.[5]

[5] For an additional resource on pH monitoring, see the Water Resources Research Center's "Analysis Method for pH and Alkalinity"

Methods based on Water Resource Center Analysis of pH

1. Samples should be taken from representative, flowing water. The water must be deeper than the sample bottles and free of surface scum and debris.
2. Carefully wade into the stream, walking upstream and avoiding the stirring up of bottom sediment.
3. To take the sample, dip bottle completely under water, filling to overflowing. Cap bottle while it is still underwater, in order to eliminate any air from the sample bottle. Return to shore and place sample in cooler with ice.
4. After calibrating your meter with the buffers, rinse the electrode(s) and glassware with distilled or deionized water.
5. Carefully measure 100ml of your sample and place in a 150ml beaker
6. It may take up to 3 minutes for the reading to become stable. When stable, but not in excess of 5 minutes, record the sample pH to the nearest 0.01 pH unit

Macroinvertebrates: Macroinvertebrates are animals without backbones, large enough to be seen by the unaided eye, and live at least part of their life cycles within a waterbody. They all occupy all stream habitats and display a wide range of functional feeding preferences. They inhabit the middle of the aquatic food web and are a major source of food for fish and other aquatic and terrestrial animals. Macroinvertebrates form permanent, relatively immobile stream communities and they can be easily collected in large numbers. Data derived from aquatic macroinvertebrate samples provide valuable information on the biological and physical condition of streams, which along with stream habitat and fish community data permits a comprehensive assessment of stream health.

Methods taken from "Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Waters"

1.Collect samples seasonally (Quarterly)
2. Samples should not be collected during periods of excessively high or low flows or within two weeks of a known scouring flow event.
3. Collect samples in riffles (shallow areas where water breaks over rocks)
4. A 600-micron mesh kick net should be used for collecting baseline macroinvertebrate samples
5. Sample with a kick net by holding the net frame firmly against the stream bottom and disturbing the substrate upstream
6. More than 100 macroinvertebrates should be collected per sample.
7. Low numbers of organisms may be indicative of water quality or habitat problems and should be noted on the field sheet.
8. Large sticks and leaves are washed, inspected for organisms and discarded.

Make sure to label all samples with the site number, stream name, location, county, date sampled and the collector's initials.

After collection, samples need to be analyzed in order to determine species richness and diversity. Several scoring methods and indices exist to measure stream health based on macroinvertebrate samples.[6]

Macroinvertebrate Count

Sensitive	Somewhat Sensitive	Tolerant
☐ ____ caddisfly larvae	☐ ____ beetle larvae	☐ ____ aquatic worms
☐ ____ hellgramite	☐ ____ clams	☐ ____ blackfly larvae
☐ ____ mayfly larvae	☐ ____ crane fly larvae	☐ ____ leeches
☐ ____ gilled snails	☐ ____ crayfish	☐ ____ midge larvae
☐ ____ riffle beetle adult	☐ ____ damselfly larvae	☐ ____ lunged snails
☐ ____ stonefly larvae	☐ ____ dragonfly larvae	
☐ ____ water penny larvae	☐ ____ scuds	
	☐ ____ sowbugs	
	☐ ____ fishfly larvae	
	☐ ____ alderfly larvae	
	☐ ____ watersnipe larvae	
boxes checked x 3 = ______ index value	boxes checked x 2 = ______ index value	boxes checked x 1 = ______ index value

WATER QUALITY RATING ☐ Excellent (>22) ☐ Fair (11-16)

Total Index Value = ______ ☐ Good (17-22) ☐ Poor (<11)

Figure 9: Example of macroinvertebrate scoring chart (Ken Pitts, Water Quality Chart)

Total Maximum Daily Load: Total maximum daily load (TMDL) is an indicator of water quality used to gather information about water pollution levels and specific watershed of concern. Brook trout need cold, clear streams to thrive, and are sensitive to a wide variety of water pollutants and quality standards.

Given this, it is important to note that most Virginia streams are impaired and do not meet federal TMDL standards (Final 2014 305(b)/303(d) Water Quality Assessment Integrated Report), and brook trout are sensitive to a host of water quality indicators. We identify e.coli, sediment, nitrogen, and phosphorus as the most important concentrations to monitor, but a more comprehensive study of water quality is needed to provide more accurate information. TMDL is important to note, but too broad for the purpose of this study to be covered comprehensively.

To monitor the following pollutants in a target stream, water quality samples, one-time biological samples, and intensive habitat assessment are taken monthly over the course of one year. Then, this data is used to calibrate pollution models for streams listed on the Clean Water

[6] For an additional resource, see William E. Sharpe's "Biotic Index Card," and "Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Waters"

Act Section 303(d) list, a federal list of impaired streams that fail to meet federal clean water standards.

Below are the general pollution thresholds to monitor for the selected pollutants:

Pollutant	Pollutant standards	Additional notes
E. coli	**The Chesapeake Bay Foundation identifies Virginia's state standard for e.coli in freshwater streams as 235 colonies per 100 ml of water for general aquatic ecosystem health.**	**This threshold is monitored and maintained in most Virginia waterways by the Chesapeake Bay Foundation**
Sediment	**Trout Unlimited recommends less than 10 percent clay and silt compared to gravels and cobble as a healthy sediment load**	**This figure is not specific to the brook trout, but is in line with the species' needs for gravel to spawn and clear water for insects and sufficient oxygen.**
Nitrogen	**Levels of nitrogen should ideally be between 2 to 3 mg/liter to support healthy aquatic ecosystems**	**n/a**
Phosphorus	**Levels of phosphorus should ideally be under 0.05 mg/liter, as this is considered the threshold for phosphorus pollution in streams**	**n/a**

Figure 10: (chart credit: Dana Ek, data from EPA)

*Note: these figures are not exclusive to EBT health, but were taken from studies with pollution recommendations for Virginia freshwater ecosystems. While not exclusive to the EBT, water quality and species health is interrelated, particularly with the brook trout's high sensitivity to water quality, so these general pollution guidelines are relevant to monitoring EBT species' health.

Population Monitoring

Measuring populations is a key way to determine the health and range of brook trout in streams and rivers in Virginia. Collecting and identifying individuals, taking measurements of length/mass, and also taking tissue samples are important aspects of population monitoring. This data can show growth trends, age ranges, and also the overall health of the fish. Fish sampling uses a multi habitat approach. Various habitats are sampled in proportion to their representation within the sampling reach. Each reach should contain riffle, run, and pool habitat samples, if possible. Prior to sample collection, a habitat assessment should be performed to gather qualitative data on the area of interest. Physical and chemical parameters should be measured at the same time as fish sampling to specifically characterize the habitat. Monitoring should be conducted pre-restoration and post-restoration, followed by monitoring annually from July through September for the next 3 years. After 10 years, it can be conducted once every third year (Heft, 2006).

Electrofishing: Electrofishing is a technique commonly used by scientists and fisheries biologists to survey rivers and assess what fish are present. When done properly, it stuns fish for a short period allowing them to be removed carefully with a net, examined and then returned alive with no permanent harm done. Electrofishing is ideally performed between July and early August, when streamflow is not too high and yoy (young of year) are large enough to sample. Population monitoring via electrofishing should be conducted annually if possible and before/after restoration work. The reach that is being monitored should be shocked starting at the bottom, working upstream to properly collect fish. At least one biologist with training and experience in electrofishing techniques and fish taxonomy *must* be involved in each sampling event. Any fish collected that are under 20 millimeters total length should not be identified.[7]

Figure 11. Photo of electrofishing team in a small stream (photo credit: Williams, et al. in "Recovery: Rehoming Brook Trout, the Dweller of Springs")

1. A habitat assessment and physical/ chemical characterization of water quality should be performed within the same sampling reach

[7] For additional electrofishing data, see West Virginia Department of Environmental Protection's "Fish Collection Protocols for Wadeable Streams"

2. Collection via electrofishing begins at a shallow riffle, or other physical barrier at the downstream limit of the sample reach, and terminates at a similar barrier at the upstream end of the reach.
3. Survey sections are recommended to be approximately 250 ft.
4. *Many* options exist for electrofisher configuration and field team organization; however, procedures will always involve pulsed DC electrofishing and a minimum 2-person team for sampling streams and wadeable rivers.
5. Fish collection procedures commence at the downstream barrier. A minimum 2-person fisheries crew proceeds to electrofish in an upstream direction using a side-to-side or bank-to-bank sweeping technique to maximize area coverage.
6. All wadeable habitats within the reach are sampled via a single pass, which terminates at the upstream barrier. Fish are held in livewells (or buckets) for subsequent identification and enumeration.
7. Use 3-pass depletion sampling
    - Each pass with the backpack, and temporary removal of the stunned fish, will remove fewer fish until the final pass results in 0
8. For small streams: use one backpack unit for each 10 ft. (3 m) of stream width, with no section deeper than 3 ft. (1 m)
9. Have at least 1 or 2 team members net fish with dip nets and are responsible for specimen transport and care in buckets or livewells
10. All fish (greater than 20 millimeters total length) collected within the sample reach must be identified to species or subspecies. Recommended to distinguish between yoy and yearling and older fish (distinguish based on length distribution)
11. Specimens that can be identified as brook trout in the field are counted, measured, weighed, examined for external anomalies (deformities, eroded fins, previous tags, or previously clipped fins), and recorded on field data sheets.
12. Field identifications of fish *must* be conducted by qualified/trained fish taxonomists, familiar with local and regional ichthyofauna

Stream Width	Stream Depth	Number/ Type of Electrofishers	Number of Netters
≤ 4 m	Shallow (< 2')	1 backpack	2
≤ 4 m	Deep (> 2')	2 backpacks	2 – 3
4 – 8 m	Shallow	2 backpacks	3 – 4
4 – 8 m	Deep	2 backpacks or barge	2 – 4
> 8 m	Shallow	2 backpacks	3 – 4

> 8 m	Deep	Barge	2 – 4

Figure 12. Personnel and equipment required to effectively electroshock various types of streams/rivers (figure credit: "Fish Collection Protocols - Wadeable Streams")

Mark and Recapture: This method of population monitoring involves marking a number of individuals in a natural population, returning them to that population, and subsequently recapturing some of them as a basis for estimating the size of the population at the time of marking and release. For brook trout, individuals can be marked while being identified after electrofishing and then released. The most commonly used method for marking is clipping the adipose fin or the top of the caudal fin. These clippings can be used for further analysis of the genetics of the population.

Tagging: Fish are often tagged with PIT (passive integrated transponder) tags. PIT tags emit a numbered code that is picked up by a receiver as a fish passes by it. Each pit tag has a unique number so individual fish can be tagged and tracked from their tagging point to a monitoring point that is either mobile or stationary. Tracking the tagged fish will help fish biologists address the habitat use, home range, movement patterns, residence times, survival and growth of these important native fish.[8]

Figure 13: A fish is tagged with a PIT tag (photo credit: "Brook Trout")

Methods based on Biomark Fish Tagging Methods

1. The fish should be held abdomen up with the tail pointing away from you.
2. The needle should be inserted posterior of the tips of the pectoral fins, when the fins are laid along the side of the fish
3. The insertion should be on the abdomen of the fish to the right or left of the mid-ventral line at the tips of the pleural ribs.
4. The needle should be directed posteriorly so the tag is injected away from the heart and other vital organs.

[8] For an additional resource on fish tagging, see Biomark's "Fish Tagging Methods"

5. The needle angle should be inserted at an angle of approximately 10 to 20 degrees from the axis of the fishes body.
6. The depth of penetration of the needle should vary depending on the size of the fish being tagged. The depth should be deep enough to place the tag as far away from the needle hole as possible so tag rejection is minimized.
7. The needle bevel should be facing down against the fish. This will help reduce the depth of penetration required to implant the tag into the body cavity. This will also help to prevent the needle tip from coming in contact with vital organs.
8. The tag should lie between the pyloric caeca and the pelvic girdle

Qualitative Data

Qualitative data on the stream is equally important to the quantitative water quality data. Observations and simple data collections are valuable for a full-scale analysis, such as collecting data on habitat characteristics (e.g. wetted width, substrate type, riparian composition, temperature spot-checks, degree of habitat degradation), and road-crossing dimensions (e.g., diameter, length, type, perch distance, fish passage potential, etc.). Conducting a simple visual assessment with a sketch and description of the area is a recommended start. Further observations about the floodplain, vegetation, tree cover, erosion/sediment, etc. in the area are all important factors to consider in a visual assessment. The flow of the river is also important to observe, a ratio of approximately 1:1 for pools and riffles is the most desirable. There are several different scoring options available to get qualitative data for a reach.[9]

Photo-Point Monitoring

Photo point monitoring is an easy and inexpensive, yet effective, method of monitoring vegetation and ecosystem change. It consists of repeat photography of an area of interest over a period, with photographs taken from the same location and with the same field of view as the original photo. With appropriate site marking and documentation, photos can be replicated precisely by different people many years apart. Monitoring brook trout via photography is another tool that helps conservationists to assess habitat quality and changes over time.

1. Define the objective. To obtain relevant and accurate information, the objective for monitoring must be carefully considered and defined before establishing photo points.
2. Select and establish photo and camera points. Mark each photo point location in the field with a stake or other identifying marker that will hold up to site conditions for the duration of the monitoring effort. Record detailed directions for locating and taking the photo points
3. Photograph the scene or subject. It is best to take photos early in the morning, late in the afternoon, or on slightly overcast days when the sun is less intense. Take photos with the sun at your back. Hold the camera at eye level (~5'). Try to include one-third skyline in

[9] Suggested resource: "Virginia Save Our Streams Assessment Form," located in Appendix A

the photo to help establish the scale of the area being photographed, and to provide reference points for future replication

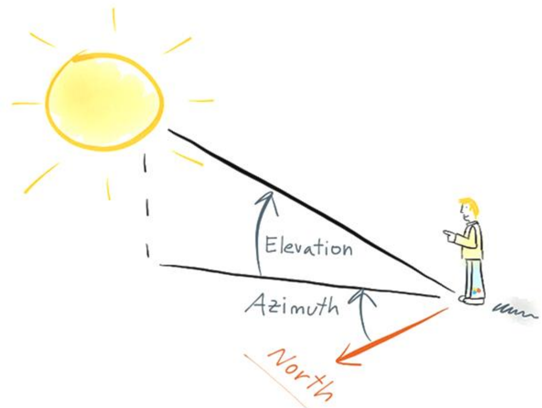

Figure 14: Description of azimuth (photo credit: artwork for article by Raphael Pons)

4. For each picture, take detailed notes on the following:
    a. Photographer name, GPS coordinates, camera height
    b. Site information: site number, date and time of photograph, visual description (rough sketch of photographer's observations and site map)
        i. Calculate the azimuth of the picture's location (photo diagram above)
        ii. Use a compass to identify due North, which has an azimuth of 0 degrees
        iii. Measure the distance (in degrees) between the object of focus, the ground, and the photographer. If the object is east, the distance east is the object's azimuth. If the object is located west, use the formula Z = 360- d; where 'z' represents the azimuth, and 'd' represents distance in degrees from due north.
5. Organize and file the data
    a. Save and record photos in a consistent location, as photo points will be compared and analyzed to show habitat trends and conditions, and to assist in making management decisions
    b. Keep these records on each photo in a photopoint log.[10]

Conclusion and Recommendations:

Virginia is advancing the conservation of its state fish, the Eastern brook trout. The benefit of multiple entities collecting fish population data is shown in the scope of habitat that is accounted for. However, the diversity of partners at local levels can make consensus across the state difficult, let alone the entire range of habitat for the brook trout. When comparing Virginia's state fish data sets to other Eastern brook trout states (most notably West Virginia, North Carolina, Maryland, Maine, and Vermont), it is revealed that the VA state fish data is less robust

[10] See appendix C and D of Oregon Watershed Enhancement Board's "Guide to Photo Point Monitoring"

and may consider more strategic protocol efforts, guided by partnerships that embrace the strengths of shared funding opportunities and citizen and angler scientists.
Monitoring Eastern brook trout populations is critically important to trout conservation efforts as well as its indication of overall ecosystem health. This is especially important in Virginia, where the brook trout habitat is shrinking. As previously mentioned, all trout monitoring for the state of Virginia is conducted by VDGIF. The specific fish monitoring protocols from VDGIF are important to establish a universal protocol, as well as being informed of electrofishing throughout the state. This study was not able to acquire the current standards for fish monitoring specific to VDGIF, and is a goal to build on in the future.

This guidebook serves to familiarize PEC and partner organization with general fish monitoring protocols from across the east coast. The guidebook concludes with several recommended actions for future projects in EBT population monitoring.

- Establish contact with VDGIF to collaborate on future monitoring initiatives, familiarize with specific VDGIF monitoring protocols
- Strengthening old partnerships and building new working relationships with trout conservation groups
- Apply for funding for specific site projects for long-term EBT population monitoring
- Utilization of citizen scientists would be beneficial for increasing community involvement and restoration project feasibility (in addition to time and knowledge of PEC staff)
    - Volunteers can assist with water quality monitoring and visual assessments of stream and trout populations
    - Virginia's avid anglers present a valuable potential resource in the continued work in trout monitoring. Utilizing their passion for brook trout can offer valuable data about populations in specific areas.
- PEC's previous work with citizen volunteer organization indicates volunteer initiatives and community events this would be successful
    - Water quality events at the Goose Creek watershed and Thumb Run were largely successful and well attended, which shows the marketability of PEC to broaden citizen science initiatives. Since PEC already has these successful events, there is considerable room for expansion and broadening of these initiatives.

Afterword

My name is Dana Ek, an undergrad student at George Mason University. I helped write this project, along with Callee Manna and Claire Catlett, as part of our final project in Piedmont Environmental Council's student Fellowship. As an avid outdoors enthusiast, I have always been interested in environmental studies and wildlife conservation. I was immediately struck by PEC's work in brook trout habitat conservation. I grew up in western Salmon country, and became interested in fish conservation efforts in the east when I recognized the natural beauty and cultural significance of eastern trout. After visiting sites along the Rappahannock and Robinson River where PEC and partner organizations fight for improved water quality and fish habitat, I knew I had to focus my project on Virginia's most famous trout. Going forward, I plan to dedicate the rest of my academic studies to fish and wildlife conservation. I am incredibly thankful for PEC giving me the opportunity and support for this project, which I hope environmental organizations in Virginia will find useful in their continued fight to save the Eastern brook trout.

I'm Callee Manna, one of the Piedmont Environmental Council fellows who worked on this guidebook as part of our practicum project for the end of our program. I'm currently a student at the University of Georgia as an undergraduate at the Odum School of Ecology. I hope to pursue graduate school in the next few years and gain a degree in ecosystem management or a similar field of study. I selected this project as my practicum because of my interest in wildlife management and ecology. I've always loved wildlife and recently got into fly fishing, so when I saw the words brook trout I knew I had to get involved. I was also interested in learning how trout protocols are established and how standards can benefit conservation projects at the state level. Throughout the process of organizing and assimilating all of the different data on brook trout, I learned that there is a need for better standards that are shared more openly. Having standards that different organization such as Trout Unlimited, PEC, or the Eastern Brook Trout Joint Venture can use to monitor projects or populations is key to the future of this iconic species. I thoroughly enjoyed learning more about brook trout, their habitat, and what steps it will take in order to properly monitor populations in Virginia's Piedmont and beyond. I'm thankful I was given the opportunity to create something that PEC can use in the future in order to further their conservation initiatives. I'm hopeful that this guidebook can contribute to better management practices of brook trout and serve the organizations who need it.

Appendix

A. HIGH GRADIENT STREAM HABITAT ASSESSMENT FORM VIRGINIA SAVE OUR STREAMS (Modified wording and metric scores from Plafkin et al. 1989)

Site or Reach ID:		**Stream Name:**
Latitude:		**Longitude:**
Watershed:		
Date:	**Time:**	**Investigators:**
Weather last 72 hours		
Description of Site Location		
Description of 100 meter assessed		
Predominant Surrounding Land Use		
Average Stream Width:		**Average Stream Depth:**
Stream Velocity (measured or defined as slow, moderate, or fast):		
Other Notes:		

Instructions:

1. Select 100-meter stretch to be evaluated. You may find it helpful to split the 100 meters up into easily definable sections for evaluation. Note the top and bottom of your stretch to be evaluated.

2. Review the 10 habitat parameters that you will be evaluating in this assessment.

3. Walk or otherwise visually inspect the entire 100-meter stretch to be evaluated. You may find it helpful to sketch your site on the graph paper provided, making note of the riffle areas, pools, runs, glides, and other features (log jams/debris, etc)

4. Begin the habitat assessment. You may want to use the graph paper to help estimate percentages needed to make the assessment. You may also want to use a process of elimination – eliminating the condition categories that do not describe your site.

5. Add all of the sub scores together out of the total to get a final score at the end of the tables.

Site or Reach ID:		**Stream Name:**		
Latitude:		**Longitude:**		
Date:	**Time:**	**Investigators:**		
Habitat Parameter	**Condition Category**			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover (attachment sites for macro-invertebrates	Greater than 70% stable habitat; mix of snags, submerged logs, undercut banks,	40-70% mix of stable habitat; presence of additional substrate that may not yet be	20-40% mix of stable habitat; habitat availability less than desirable; substrate	Less than 20% stable habitat; lack of habitat is obvious; substrate

and overhead cover for fishes)	cobble or other stable habitat (logs and snags are not new fall).	prepared for colonization.	frequently disturbed or removed.	unstable or lacking.
SCORE	18	13	8	3
Comments:				
2. Embeddedness	Gravel, cobble, and boulder particles in riffles and runs are 0- 25% surrounded by fine sediment (e.g. – sand or silt).	Gravel, cobble, and boulder particles in riffles and runs are 25-50% surrounded by fine sediment (e.g. – sand or silt).	Gravel, cobble, and boulder particles in riffles and runs are 50-75% surrounded by fine sediment (e.g. – sand or silt).	Gravel, cobble, and boulder particles in riffles and runs are >75% surrounded by fine sediment (e.g. – sand or silt).
SCORE	18	13	8	3
Comments:				
3. Velocity/Depth Regime	All four velocity/depth combinations present (slow-deep, slow-shallow, fast-	Only 3 of the 4 combinations are present.	Only 2 of the 4 combinations are present.	Dominated by 1 velocity/depth regime.

	deep, fast-shallow).			
SCORE	18	13	8	3
Comments:				
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increases in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% 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 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	18	13	8	3
Comments:				

Habitat Parameter	**Condition Category**			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
5. Channel Flow Status	Water reaches base of both banks, and minimal amount of channel substrate is exposed.	Water fills over 75% of the available channel; or less than 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	18	13	8	3
Comments:				
6. Channel Alteration	Channel straightening or dredging absent or minimal; stream with normal pattern	Some channel straightening present, usually in areas of bridges; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channel straightening may be extensive. Man-made materials – hard engineering, large rocks, cement channels, pipes, riprap, etc. present on both banks; and 40-80% of stream reach	Banks covered with man-made materials including hard engineering, large rocks, cement channels, pipes, riprap, etc.; over 80% of reach channelized and disrupted. Instream habitat greatly altered

			channelized and disrupted.	or removed entirely.
SCORE	18	13	8	3
Comments:				
7. Frequency of Riffles (or bends) Measure distance between riffles – top of downstream riffle to the bottom of upstream riffle. If there are more than two riffles, take the average distance.	Occurrence of riffles relatively frequent. The distance between the riffles divided by the width of the stream is less than 7.	Occurrence of riffles infrequent. The distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat The distance between riffles divided by the width of the stream is between 15-25.	Generally all flat water or shallow riffles - poor habitat. The distance between riffles divided by the width of the stream is greater than 25.
SCORE	18	13	8	3
Comments:				

8. Bank Stability (score each bank) Note: determine left or right side by facing downstream **SCORE ____Left** **SCORE ____Right**	Banks stable; evidence of erosion or bank failure absent or minimal. Less than 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.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious wearing away of bank; 60-100% of bank has erosional scars.
	9	6.5	4	1.5
	9	6.5	4	1.5
Comments:				

Habitat Parameter	**Condition Category**			
	Optimal	**Suboptimal**	**Marginal**	**Poor**

9. Bank Vegetative Protection (score each bank) **SCORE ____Left** **SCORE ____Right**	More than 90% of the streambank surfaces and immediate riparian zone covered by vegetation, including trees, understory shrubs, wetland plants; vegetative disruption through grazing or mowing minimal or not evident.	70-90% of the streambank surfaces covered by vegetation but one class (trees, shrubs, grasses) of plants is not well represented.	50-70% of the streambank surfaces covered by vegetation; patches of bare soil or closely cropped vegetation common.	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 height – ex. Mowed or grazed.
	9	6.5	4	1.5
	9	6.5	4	1.5
Comments:				
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roads, clear-cuts,	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	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.

SCORE ____Left SCORE ____Right	lawns, or crops) have not impacted zone.		zone a great deal.	
	9	6.5	4	1.5
	9	6.5	4	1.5
Comments:				

TOTAL SCORE: ___________ What does this mean?

You can compare the total score to itself each year. You may also want to compare the habitat score of your site to the habitat score at a "pristine" stream within your watershed. General habitat conditions

Total Score greater than 153 = Optimal Habitat Conditions

Total Score between 130 and 152 = Suboptimal Habitat Conditions

Total Score between 80 and 129 = Marginal Habitat Conditions

Total Score less than 80 = Poor Habitat Conditions

B.) Shenandoah National Park's brook trout monitoring protocols and population reports

1. The following are links to NPS databases and reports to provide background for specific monitoring practices in Shenandoah National Park

- Links to a USGS report on surface water quality and aquatic biotic data in Shenandoah NP.
  https://pubs.usgs.gov/sir/2013/5157/
- This resource is from the National Park data store; the database provides reports, information, and previous studies on Shenandoah's fisheries program and fish population reports.
  https://irma.nps.gov/DataStore/Reference/Profile/2190356

- This resource is National Park data on Shenandoah's population monitoring protocol. https://irma.nps.gov/DataStore/Reference/Profile/2197660
- Links to a summary report from fish monitoring in Shenandoah National Park (2010). https://irma.nps.gov/DataStore/Reference/Profile/2189017
- This resource has NPS inventory and monitoring reports and data on fish streams located in the park. https://science.nature.nps.gov/im/units/midn/monitor/fish.cfm

References:

"Analysis Method for PH and Alkalinity." Water Resources Research Center. Web. <https://wrrc.umass.edu/research/projects/acid-rain-monitoring-project/analysis-method-ph-and-alkalinity>.

"Annual Virginia Save Our Streams Habitat Assessment:." Virginia Save Our Streams. Web. <http://www.vasos.org/monitors-page/data-sheet-downloads/>.

"Biological Monitoring." West Virginia Department of Environmental Protection. Web. <http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/WBFish.pdf>

"Brook Trout." Chesapeake Bay Program. N.p., n.d. Web. <http://www.chesapeakebay.net/fieldguide/critter/brook_trout>.

"Brook Trout." Falmouth Rod and Gun Club. N.p., n.d. Web. <http://www.falmouthrodandgunclub.com/BrookTrout.html>.

Bruckner, Monica Z. "The Winkler Method - Measuring Dissolved Oxygen." Microbial Life Educational Resources. N.p., n.d. <https://serc.carleton.edu/microbelife/research_methods/environ_sampling/oxygen.html>

"Chapter 8: Fish Protocols." United States Environmental Protection Agency. Web. <https://archive.epa.gov/water/archive/web/html/ch08main.html>.

Dauwalter, Dan, Kurt Fesenmyer, and Paul Holden. "Stream Temperature Monitoring." Trout Unlimited. Web. <http://www.tu.org/sites/default/files/water-temperature-monitoring-v3-final.pdf>.

EPA. "Chesapeake Bay Total Maximum Daily Load (TMDL)." *Environmental Protection Agency*, 30 June 2017. Web. https://www.epa.gov/chesapeake-bay-tmdl>.

EPA. "Volunteer Stream Monitoring: A Methods Manual." United States Environmental Protection Agency. Web. <https://www.epa.gov/sites/production/files/2015-06/documents/stream.pdf>.

"Final 2014 305(b)/303(d) Water Quality Assessment Integrated Report." Virginia Department of Environmental Quality. N.p., 2014. Web. <http://www.deq.virginia.gov/Programs/Water/WaterQualityInformationTMDLs/WaterQualityAssessments/2014305(b)303(d)IntegratedReport.aspx>.

"Fish Collection Protocols - Wadeable Streams." West Virginia Department of Environmental Protection. 2009. N.p., n.d. Web. <http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/WBFish.pdf>.

"Fish Tagging Methods." Biomark. Web. <http://www.biomark.com/Documents%20and%20Settings/67/Site%20Documents/PDFs/Fish%20Tagging%20Methods.pdf.>

Fondriest Environmental, Inc. "Measuring Turbidity, TSS, and Water Clarity." Fundamentals of Environmental Measurements. Web. <http://www.fondriest.com/environmental-measurements/equipment/measuring-water-quality/turbidity-sensors-meters-and-methods/>.

Heft, Alan A. "Maryland Brook Trout Management Plan 2006." Maryland Department of Natural Resources. N.p., n.d. Web. http://dnr.maryland.gov/fisheries/Documents/MD_Brook_Trout_management_plan.pdf

Isel, Michael. "Evaluation of brook trout (Salvelinus fontinalis): introductions and re-Introductions into four Virginia Blue Ridge mountain streams." 2011.

"Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Waters." Commonwealth of Kentucky Energy and Environmental Cabinet. Web. <http://water.ky.gov/Documents/QA/Surface%20Water%20SOPs/Methods%20for%20Sampling%20Benthic%20Macroinvertebrate%20Communities%20in%20Wadeable%20Waters.pdf>.

"OWEB Guide to Photo Point Monitoring." Oregon Watershed Enhancement Board. Web. <http://www.oregon.gov/OWEB/docs/pubs/PhotoPoint_Monitoring_Doc_July2007.pdf>.

Pitts, Ken. "Water Quality Chart." http://kenpitts.net/bio/ecology/water_quality_chart.htm.

Pons, Rafael. "Understanding Azimuth and Elevation." PhotoPills. N.p., n.d. Web. <http://www.photopills.com/articles/understanding-azimuth-and-elevation>.

Sharpe, William E., William G. Kimmel, and Anthony R. Buda. "Biotic Index Card."Department of Ecosystem Science and Management. Web. http://ecosystems.psu.edu/youth/sftrc/lesson-plan-pdfs/BioticIndexCard.pdf

West Virginia DEP. "Watershed Assessment Branch 2015 Field Sampling Standard Operating Procedures." *West Virginia Department of Environmental Protection*. 2015. Web.

http://www.dep.wv.gov/WWE/watershed/wqmonitoring/Documents/SOP%20Doc/2015WABSOP/2015%20WAB%20Field%20Sampling%20SOP.pdf>.

"Wild Trout Program." *Wild Trout Program | VDGIF*. N.p., 2017. Web. < https://www.dgif.virginia.gov/fishing/trout/wild-trout-program/.>

Wilde, Franceska D. "Temperature." USGS Field Manual. Web. https://water.usgs.gov/owq/FieldManual/Chapter6/6.1_ver2.pdf

Williams, Jack, et al., "A Guide to Native Trout Restoration." Trout Unlimited. 2006. Web. http://www.tu.org/sites/default/files/NativeTroutRest_Williams.pdf

Williams, Ted et al., "Recovery: Rehoming Brook Trout, the Dweller of Springs." Cool Green Science. N.p., 27 May 2016. Web. 25 July 2017.