Standard Operating Procedures Manual

Fairfax County Biological Stream Monitoring Program

County of Fairfax
Department of Public Works
& Environmental Services
Watershed Planning & Assessment Branch

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A. BENTHIC MACROINVERTEBRATE PROTOCOLS

Benthic macroinvertebrate communities are a major component of any healthy stream system. They are an important link in the aquatic food web, forming the core diet of many stream fishes. These organisms are also useful indicators of water quality, due to their short life spans and their varying tolerances to chemical, organic, and sediment pollution.

1. EPA's Rapid Bioassessment Protocol Multi-habitat Field Sampling Methods

Since Fairfax County contains two different physiographic provinces (Piedmont, and Coastal Plain) that each have a variety of different aquatic habitat types, a sampling method that incorporates all these types of habitats is used. Selected sites are sampled in the early spring between mid-March and mid-April (prior to the spring/summer emergence of many adult aquatic insects). All 100 meter sample sites are sampled using the "20-Jab" or "multi-habitat" MACSW method which was designed by the Mid-Atlantic Coastal Streams Workgroup (US EPA, 1997) specifically for streams with variable habitat structure and adopted for use in EPA's Rapid Bioassessment Protocol III (RBP III) for benthic macroinvertebrate sampling in streams (Barbour et al., 1999). Observed habitats within the sample reach are proportionally sampled using twenty 0.5 meter "jabs" with the D-frame net. Habitats are designated as undercut banks, aquatic vegetation, sand, cobble, and snags. Samples collected in the field have the larger organic debris removed and then are preserved with denatured ethanol (95%) in 1L polyethylene bottles. Labeled bottles are then transported to a laboratory where they are logged in and stored for later sub-sampling and taxonomic identification.

The following field equipment is used for the multi-habitat sampling:

- standard D-frame dip net, 500 µ opening mesh, 0.3 m width (~ 1.0 ft frame width)
- sieve bucket, with 500 µ opening mesh
- Large polyethylene wash tray
- 95% ethanol (denatured)
- 1L HDPE Nalgene sample bottles & labels
- forceps
- pencils, clipboard & calculator
- Benthic Macroinvertebrate Field Data Sheet (Figures A1 & A2)
- Waders and insulated neoprene gloves

The Quality Assurance and Quality Control (QA/QC) methodology defined by the original SPS protocol from the baseline study was followed. Specific procedures are outlined in separate sections where applicable. In accordance with the protocol, 10% of all samples are (randomly) selected to be re-sampled, and/or rechecked for accuracy, consistency, and data repeatability.

Site Code: _____

Benthic Macroinvertebrate Sampling
Data Sheets

Watershed:	Date:	Start Time:
Stream Order:	Recorder:	Finish Time:
Investigators:	Physiographic Province: ____Coastal Plain ____Piedmont ____Triassic Basin	

Habitat Types: # of Jabs:

_____% Sand _____
_____% Snags _____
_____% Cobble _____
_____% Vegetated Banks _____
_____% Submerged Macrophytes _____

*****If habitat type is less than 5% of area, do not count it toward jabs

Water Quality	
Temperature	
% Saturation	
Dissolved Oxygen	
Conductivity	
Specific Conductance	
pH	

Weather		
Today:	storm/heavy rain	showers (intermittent)
	rain (steady)	sunny
	partly cloudy	cloudy
Past 24 hrs	storm/heavy rain	showers (intermittent)
	rain (steady)	sunny
	partly cloudy	cloudy

Riparian Zone/ Instream Features

Predominant Surrounding Landuse	
forest	commercial
field/pasture	industrial
agricultural	other _____
residential	other _____

Local Water Erosion		
none	moderate	heavy

Channelized?	
Yes	No

Canopy Cover		
open	moderate	heavy

Riparian Zone Width	
LB:_____	RB:_____

Possible impairments to benthics (i.e. golf course, industrial area):

Other Comments:

Figure A1: Field data sheet for benthic macroinvertebrate sampling (front).

Cobble (hard substrate) - Cobble will be prevalent in the riffles (and runs), which are a common feature throughout most mountain and piedmont streams. In many high-gradient streams, this habitat type will be dominant. However, riffles are not a common feature of most coastal or other low-gradient streams. Sample shallow areas with coarse (mixed gravel, cobble or larger) substrates by holding the bottom of the dip net against the substrate and dislodging organisms by kicking the substrate for 0.5 m upstream of the net.

Snags - Snags and other woody debris that have been submerged for a relatively long period (not recent deadfall) provide excellent colonization habitat. Sample submerged woody debris by jabbing in medium-sized snag material (sticks and branches). The snag habitat may be kicked first to help dislodge organisms, but only after placing the net downstream of the snag. Accumulated woody material in pool areas are considered snag habitat. Large logs should be avoided because they are generally difficult to sample adequately.

Vegetated banks - When lower banks are submerged and have roots and emergent plants associated with them, they are sampled in a fashion similar to snags. Submerged areas of undercut banks are good habitats to sample. Sample banks with protruding roots and plants by jabbing into the habitat. Bank habitat can be kicked first to help dislodge organisms, but only after placing the net downstream.

Submerged macrophytes - Submerged macrophytes are seasonal in their occurrence and may not be a common feature of many streams, particularly those that are high-gradient. Sample aquatic plants that are rooted on the bottom of the stream in deep water by drawing the net through the vegetation from the bottom to the surface of the water (maximum of 0.5 m each jab). In shallow water, sample by bumping or jabbing the net along the bottom in the rooted area, avoiding sediments where possible.

Sand (and other fine sediment) - Usually the least productive macroinvertebrate habitat in streams, this habitat may be the most prevalent in some streams. Sample banks of unvegetated or soft soil by bumping the net along the surface of the substrate rather than dragging the net through soft substrates; this reduces the amount of debris in the sample.

Figure A2: Field data sheet for benthic macroinvertebrate sampling (back).

2. Laboratory Identification and Analysis

The following laboratory equipment was used to identify, record, and catalog the benthic macroinvertebrate samples:

- previously collected benthic sample in 1L nagene jug(s)
- 8-inch diameter sieve with 500 µ mesh
- sorting grid, (30 squares) with 500 µ mesh (Figure A3)
- polyethylene wash tray
- dissecting microscopes (stereoscopes)
- fiber-optic light source
- 95% ethanol (denatured)
- glass sample vials (with teflon lids) and label tape
- 9-unit laboratory counter with grand total counter
- Petri dishes & extra-fine/jewelers forceps
- sample chain of custody form (Figure A4)
- sample QA/QC log in sheets (Figure A5)
- benthic macroinvertebrate laboratory bench sheet (Figures A6 & A7)

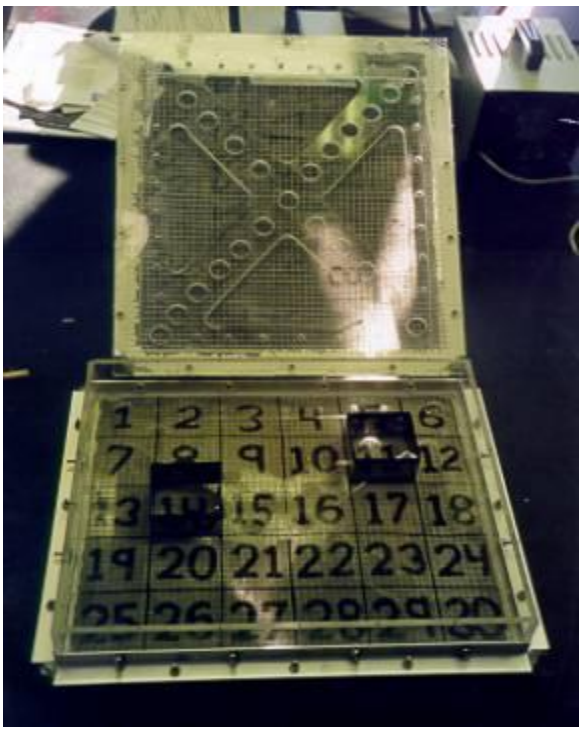

Figure A3: Benthic sample sorting grid.

Upon arrival in the lab, field samples are logged-in on the chain of custody form. The ethanol solution is flushed and replaced in all stored samples typically 1 month after original collection (this increases preservation and flushes out leached chlorophyll in solution).

Invertebrate collections are developed by rinsing each sample and spreading it over the surface of a 30 x 36 cm, 500 µ mesh sorting grid sub-sampler (Caton, 1991) (Figure A3) [very large volume samples may be divided into two sorting grids]. Place the sub-sampler in enough water to cover the sample and allow to hydrate for at least 10 minutes. A sub-sample of individuals is picked or "sorted" from a randomly selected square subdivision marked on the grid's surface (30 total squares). This is accomplished by removing debris and organisms from the randomly-selected square, placing this mixture into a white water-filled plastic tray which is illuminated via fiber optic lights, and carefully removing all organisms (a microscope is not used for subsampling but may be used to verify an organism). It is quite helpful to inspect and remove larger debris from the tray. Once that square is fully picked, another randomly selected square is then picked until a minimum of 200 (not to exceed 240) organisms are obtained. If picking through an entire grid is likely to result in a subsample of greater than 240 organisms, then that grid is subsampled in the same manner as before to decrease the likelihood of exceeding 240 organisms. That is, spread the contents of the last grid into another gridded pan and pick grids one at a time until the desired number is reached. If a specimen lies across 2 squares, it belongs to the square containing its head.

Specimens fall into one of three groups; 1)chironomidae, 2)oligochaeta, and 2)all others. Organisms that are not counted in the sample include vertebrates (salamanders or newts), zooplankton (i.e. copepods) or non-aquatic macroinvertebrates (i.e. adult dipterans). Organisms from each site's sub-sample are tallied by group and transferred to one of three sample vials (one vial for each respective group), preserved with 95 percent ethanol, and labeled with the following information:

- Site code
- Date collected (found on chain of custody form)
- Date sorted
- Sorted by (sorter's initials)
- Particular sample group (C = chironomidae, O = oligochaeta, • = others).
- Number of organisms in the particular group vial
- Total number of organisms in the sub-sample ($200 < n < 240$)

The total number of "squares" picked (from the sorting grid) to reach the 200 organism target number is recorded on the QA/QC sample log-in sheet. In compliance with protocols, after laboratory processing is completed for a given sample, all sieves, pans, trays, etc., that have come in contact with the current sample are rinsed thoroughly, examined carefully, and picked free of organisms or debris. Any organisms found are added to the sample residue, which is then preserved.

Once all site samples are sub-sampled, sorted, and labeled, taxonomic identifications are then made to the genus level (whenever possible) using microscopes. Genus level classification of all macroinvertebrate samples are performed using selected taxonomic keys (Pennak 1989, Peckarsky 1990, Wiggins 1995, Merritt and Cummins 1996). Certain specimens may be physically damaged to an extent such that accurate genus-level identification is not possible. In these situations, the lowest possible taxonomic

identification is noted on the data sheet. Time constraints have prevented the more detailed examinations required to identify taxa such as aquatic worms (Oligochaeta) and midge larvae (Chironomidae) to this level. Therefore, oligochaetes are identified at the class level, and chironomids are identified at the family level. The representatives in each respective taxonomic grouping are enumerated, recorded and summed on the benthic identification bench sheet (Figures A6 & A7). The final total number of organisms is also recorded on the sample identification log-in sheet (Figure A5) along with the date identification was completed and the taxonomist's initials. All individuals from the sub-sample are then returned to the 95 percent ethanol solution and archived. To ensure conformity with protocols, these additional steps are taken:

- Ten percent of the already-processed and identified samples are randomly selected and rechecked (by a different taxonomist) for taxonomic and numerical consistency.
- A voucher collection of all samples and sub-samples is being continuously maintained. These specimens are properly labeled, preserved, and stored in the laboratory for future reference.

Benthic Macroinvertebrate Sample Log-in Sheet					
	Site ID	Watershed	Date collected	Date received by lab	# of containers
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
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50					
51					
52					
53					

Figure A4: Benthic macroinvertebrate sample field-to-laboratory log in sheet (chain of custody form).

QA/QC Sample Log In Sheet for Identification

Sample	Site Code	Identification				QA/QC Site?	QA/QC Identification				
		Started	Finished	Initials	n = ?		Started	Finished	Initials	n = ?	squares
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
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31											
32											
33											
34											
35											

Figure A5: Benthic macroinvertebrate sample QA/QC Log-in sheet.

SITE ID: _____

Benthic Macroinvertebrate Identification Sheet

Taxonomist:		**Identification Start Date:**
		Identification Finish Date:
Watershed:		**Sorting Date(s):**
		Collection Date:
Subsample Target: 200 Organisms		
		Number sorted:
QC Sample? Y N	**QC Site? Y N**	**Number ID'ed:**

Organisms			**#**	**L.S.***	**T.I.**
Order	**Family**	**Genus**			
Oligochaeta				A	
Chironomidae				L	
Hirudinea					
Isopoda					
Amphipoda					
Decapoda					
Ephemeroptera					
Plecoptera					
Odonata					
		Subtotal:			

***Lifestages: A (Adult), P (Pupae), L (Larvae)**

Figure A6: Benthic Identification Bench Sheet (front page).

SITE ID: _____

Benthic Macroinvertebrate Identification Sheet

Organisms			#	L.S.*	T.I.
Order	**Family**	**Genus**			
Trichoptera					
Hemiptera					
Megaloptera					
Coleoptera					
Diptera					
Gastropoda					
Bivalves					
Acariformes					
Other					
		Subtotal:			
		Grand Total:			

***Lifestages: A (Adult), P (Pupae), L (Larvae)**

Figure A7: Benthic Identification Bench Sheet (back page).

3. Development of a Benthic Macroinvertebrate Index of Biotic Integrity

The response of a given biological community to environmental degradation can provide a useful measure of overall system health. Such responses, often evident as changes in community structure and composition, can highlight single-source environmental stressors, or the cumulative impact of multiple stressors. Potential measures of relative tolerance and intolerance to stressors will be identified from within the various subcategories (i.e., genus, functional feeding group, and habitat) of the macroinvertebrate communities.

These attributes, or "metrics," were used to construct the foundation of an Index of Biotic Integrity (IBI) for ranking each study site. The index has two distinct components; (1) a set of criteria which transforms the metric values into scores that can then be used in the aggregate and (2) narrative "integrity" classes (excellent, good, fair, poor and very poor) which reflect relative correspondence to the numeric rating of the "reference" or undisturbed condition streams (Table A1).

Table A1: Classification ratings used on the Benthic Macroinvertebrate Index of Biotic Integrity scores.

INDEX SCORE	RATING	DESCRIPTION
80 to 100	**Excellent**	**Equivalent to reference conditions; High biodiversity and balanced community**
60 to 80	**Good**	**Slightly degraded site with intolerant species decreasing in numbers**
40 to 60	**Fair**	**Marked decrease in intolerant species; shift to an unbalanced community**
20 to 40	**Poor**	**Intolerant species rare or absent, decreased diversity**
0 to 20	**Very Poor**	**Degraded site dominated by a small number of tolerant species**

For the benthic macroinvertebrates, indices were created separately for the Piedmont and the Coastal Plain areas. An index was created for the Coastal Plain province using metrics taken from the Mid-Atlantic Integrated Assessment data report (Table A2), *Assessment Framework for Mid-Atlantic Coastal Plain Streams Using Benthic Macroinvertebrates* (Maxted et al. 1999). For the Piedmont region the Index of Macrobenthic Biotic Integrity (Jones 2000, personal communication) is used since it provides regionally tested metrics and multi-year data for the same reference sites which were used in the baseline study (Table A3). Examples for calculating individual metrics from the taxonomic data for inclusion into the biological indices are given below.

Table A2: Index of Biotic Integrity metric descriptions for benthic macroinvertebrates for Coastal Plain. (Based on Maxted et al. 1999).

METRIC	DESCRIPTION
1. Taxa Richness	**Number of different taxa at a site**
2. EPT Taxa	**Number of Mayfly, Stonefly and Caddisfly taxa at a site**
3. Percent Ephemeroptera	**Percent of sample that was in the order Ephemeroptera**
4. Hilsenhoff Biotic Index	**Hilsenhoff Biotic Index – general tolerance/intolerance of the sample**
5. Percent Clingers	**Percent of individuals whose habitat type is clingers**

Table A3: Index of Macrobenthic Biotic Integrity metric descriptions for benthic macroinvertebrates for the Piedmont (Jones 2000, personal communication).

METRIC	DESCRIPTION
1. Taxa Richness	**Number of different taxa at a site**
2. EPT Richness	**Number of Mayfly, Stonefly and Caddisfly taxa at a site**
3. Percent EPT	**Percent of sample that are Mayfly, Stonefly and Caddisfly excluding the tolerant Net-Spinning Caddisflies (Hydropsychidae)**
4. Percent Trichoptera w/o Hydropsychidae	**Percent of sample that are Caddisflies excluding the tolerant Net-Spinning Caddisflies (Hydropsychidae)**
5. Percent Coleoptera	**Percent of sample that are beetles**
6. Family Biotic Index	**General tolerance/intolerance of the sample**
7. Percent Dominance	**Percent of the most abundant taxa**
8. Percent Clingers + Percent Plecoptera	**Percent of individuals whose habitat type is clingers plus percent of sample that are stoneflies but are not clingers**
9. Percent Shredders	**Percent of individuals that uses shredding as its primary functional feeding group**
10. Percent Predators	**Percent of individuals that uses predation as its primary functional feeding group**

Example 1: For metric values that *decrease* with increasing disturbance (Total Taxa, EPT Richness, % EPT w/o Hydropsychidae, % Trichoptera w/o Hydropsychidae, % Coleoptera, % Clingers plus % Plecoptera, % Clingers, % Shredders, % Ephemeroptera and % Predators).

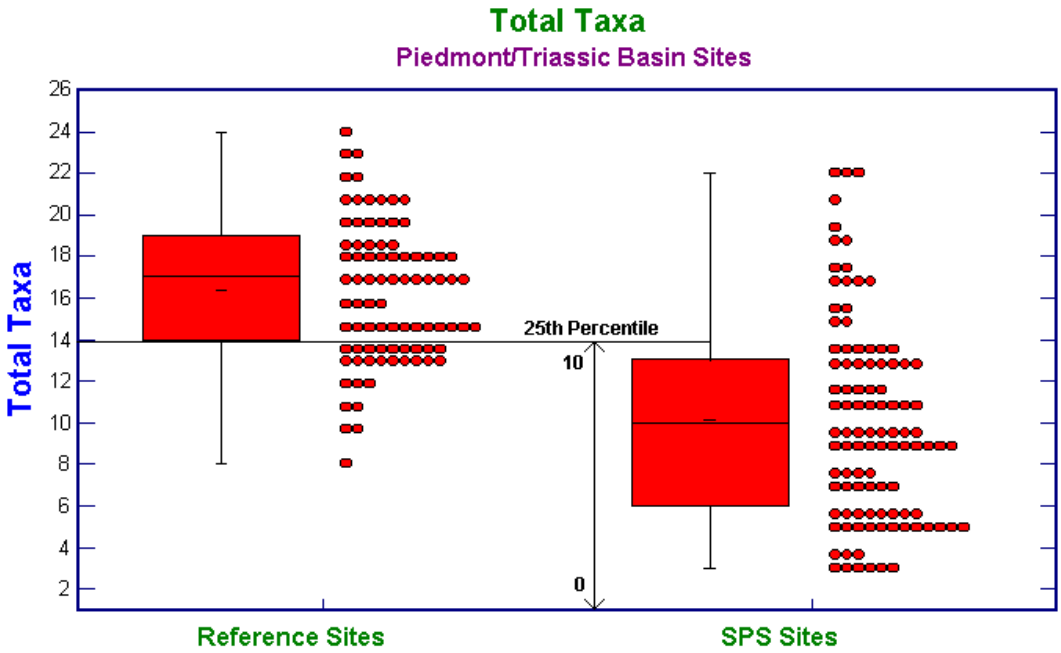

Figure A8: Box and Whisker Plot of Total Taxa for the Piedmont.

The data for total taxa from the Piedmont reference areas and the total taxa data were plotted against each other using a box and whisker plot. The 25th percentile from the reference data was then designated as the "reference condition" value. Therefore, any value above that mark was considered equivalent to reference conditions. The 25th percentile value of the reference data was then divided by 10 to obtain the conversion factor. In this example (Figure 8) the conversion factor would be 14 (the 25th percentile of the reference conditions) divided by 10 (the upper limit of the 10-point scale), which is 1.4.

Table A4: Metric value conversions for Example 1.

Site Values	Converted Values	Final Value
7	5	5
10	7.14	7.14
22	15.71	10
13	9.29	9.29
8	5.71	5.71
5	3.57	3.57
4	2.86	2.86
14	10.00	10
6	4.29	4.29
3	2.14	2.14
17	12.14	10

All the county site values for total taxa were then divided by the conversion factor to convert them to the final 0 to 10 scale (Table B4). If the resulting value was more than 10, it was rectified to 10. The resulting values for all metrics were then summed to give each site a rating between 0 – 100. Each site was then given a qualitative ranking based on its final rating (Table A1).

These steps were also performed for the Coastal Plain site data. Unlike the Piedmont sites however, for which spatially and temporally broad reference information was available, the Coastal Plain sites were only compared to the two Kane Creek (least impaired/reference) sites. The metric scores for the Kane Creek sites were used in lieu of the 25th percentile of aggregate reference data for inversely-correlated metrics (Total Taxa, EPT Richness, % Ephemeroptera and % Clingers).

<u>Example 2</u>: For metric values that *increase* with increasing disturbance (i.e. FBI, HBI and Percent Dominance).

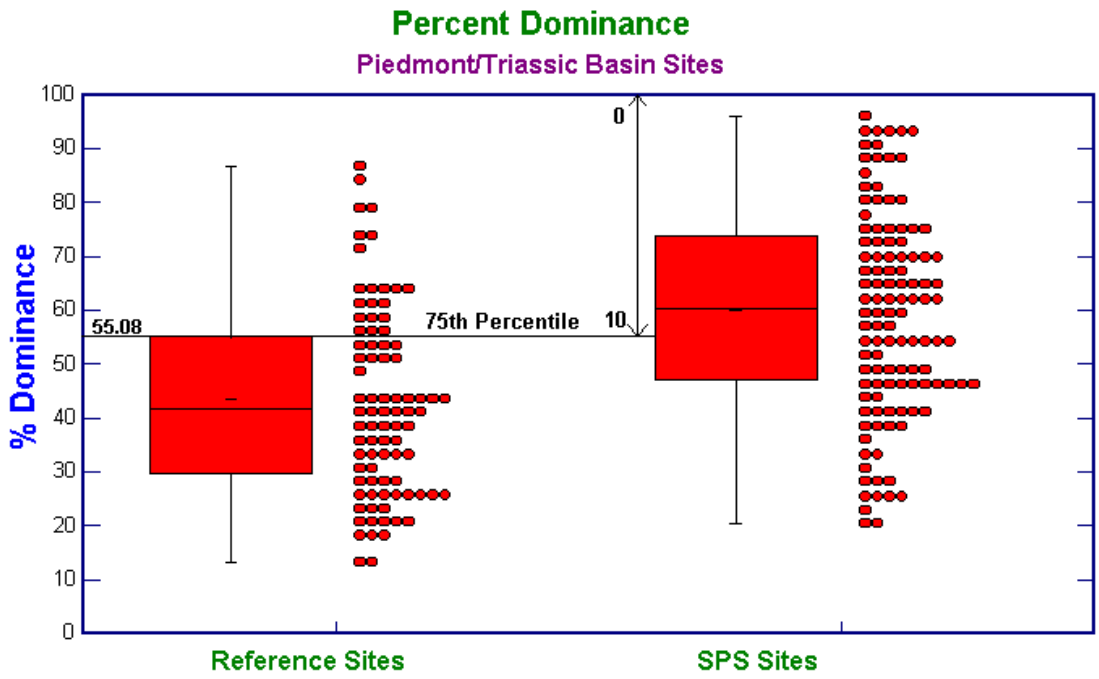

Figure A9: Box and Whisker Plot of Percent Dominance for the Piedmont.

The data for percent dominance from the Piedmont reference areas and the data were plotted against each other using a box and whisker plot. In this case, the 75th percentile from the reference data was designated as the "reference condition" value. The difference between these metrics and those from example 1 is that the best value obtainable is 0 for the metric instead of 100, and the 75th percentile of the reference data, rather than the 25th, is the 10 value on the 0 to 10 scale. In this example (Figure A9), 100 percent dominance is the 0 value and 55.08 is the 10 value. In order to obtain the conversion factor, the 75th percentile value for the reference condition was subtracted from its upper limits. This value was then divided into 10 to arrive at the

conversion factor. So in this example, the 75th percentile (55.08) is subtracted from the upper limit of this metric (100) to give 44.92. The final step to obtain the conversion factor is to divide 44.92 by 10, which yields 4.492. Individual values from the monitoring sites for percent dominance were then taken and subtracted from 100. Each value was then divided by the conversion factor to give the 0 to 10 value for that site (Table A5). If the value exceeded 10, the site was given a value of 10. This procedure was also followed for the coastal plain sites using the coastal plain reference data. The converted values for each site were then summed to form a 0 to 100 scale. Since the coastal plain index consisted of only 5 metrics, the summed total was doubled to give it a 0 to 100 range (Table A1).

Table A5: Metric value conversions for Example 2.

SPS Site Value	100 - SPS site	Converted Value	Final Value
59.38	40.62	9.04	9.04
49.03	50.97	11.35	10
94.44	5.56	1.24	1.24
88.79	11.21	2.50	2.50
82.14	17.86	3.98	3.98
58.74	41.26	9.19	9.19
90.70	9.30	2.07	2.07
95.83	4.17	0.93	0.93
76.87	23.13	5.15	5.15
95.88	4.12	0.92	0.92
50.72	49.28	10.97	10
49.63	50.37	11.21	10

These steps were also performed for the Coastal Plain site data. Unlike the Piedmont sites however, for which spatially and temporally broad reference information was available, the Coastal Plain sites were only compared to Kane Creek reference sites. The averaged metric scores for the two Kane Creek sites were used in lieu of the 75th percentile of aggregate reference data for the one directly correlated metric (Hilsenhoff Biotic Index).

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B. FISH SAMPLING PROTOCOLS

1. Rapid Bioassessment Protocol Field Sampling Methods

Fish assemblages represent the apex of most stream communities. Fish typically are at the top of the food web and are sensitive to both natural and anthropogenic changes within a given system and are, therefore, useful indicators of stream ecosystem health. Fish are also more readily understood and appreciated by the public than are other biological components of streams systems. Therefore, they can be useful tools for developing community interest in environmental and water management issues. The methods employed were based largely upon the EPA's Rapid Bioassessment Protocols V (Barbour et al. 1999). Because of sporadic and sparse occurrence of fish assemblages in first order and intermittent headwater streams, the value and validity of using these assemblages as ecosystem health indicators is questionable. As such, fish communities were sampled from non-tidal freshwater, perennially-flowing, second order (or greater) streams within Fairfax County or streams with greater than 300 acre drainage areas (contributing watersheds).

The following equipment was used for sampling:

- Smith-Root, Models 12-B and LR-24 backpack electro-fishers
- 12-volt DC batteries for electro-fisher(s)
- rubber gloves (high-voltage rated, insulated)
- boot-foot chest waders and belts for all participants
- hand dip-nets, both long- and short-handled (1/8 inch mesh)
- block nets (i.e., seines)
- buckets and live car(s) for fish storage and transport
- data sheets (Figures B2 & B3) printed on waterproof paper & pencils
- 95% ethanol (denatured)
- specimen jars and labels
- species key and field guide (Jenkins and Burkhead, 1994).

2. Fish Sampling, Identification, and Preservation

Using the Smith-Root Inc. backpack electro-fishing units, a single-pass sample is conducted through each selected 100-meter reach (number of electro-fisher units is be dependent upon stream width and depth). Block nets are deployed at the upstream reach boundary, and collection is conducted in the upstream direction. During each pass captured specimens are transported in water-filled buckets and maintained in a portable in-stream live well for subsequent examinations. Fish are identified to the species level and representatives in each category are enumerated and recorded. Upon final identification, the fish are then immediately released back into the stream. To minimize the risks of mortality or injury to fish, electro-fisher unit settings are adjusted to reflect stream water conductivity and corresponding manufacturer recommendations.

As is the standard practice with fish sampling protocols, juvenile or young-of-year (YOY) specimens, determined to be those individuals under 20 mm total length, are not counted towards the species counts. This is due to their higher mortality rates in the first year of life, as well as ambiguities (or incomplete development) in proper morphological characteristics necessary for accurate identifications in certain species. Species in the *Gambusia* genus are excluded from this practice as the adults frequently measure near 20 mm in total length. Therefore *Gambusia* individuals measuring less than 10 mm are considered YOY and are not included in the sample counts.

Positive field identification is particularly difficult with some specimens, and preservation of representative individuals, in some cases, may be needed for more detailed laboratory examinations. All specimen collections are carried out in accordance with the guidelines set forth in the current Virginia Department of Game and Inland Fisheries (DGIF) Scientific Collection Permit issued to Fairfax County Ecologists on a bi-annual basis.

A uniform fish sampling data sheet is used during the fish sampling session (Figures B2 & B3) for all county streams.

Figure B1: Fish survey using backpack electro-fishers

Site ID _____

RBP Coastal Plain Assessment Scores	
Parameter	**Score**
1) Epifaunal Substrate/Available Cover	_____
2) Pool Substrate Characterization	_____
3) Pool Variability	_____
4) Sediment Deposition	_____
5) Channel Flow Status	_____
6) Channel Alteration	_____
7) Channel Sinuosity	_____
8) Bank Stability	RB:_____
	LB:_____
9) Bank Veg. Protection	RB:_____
	LB:_____
10) Rip. Veg. Zone Width	RB:_____
	LB:_____

RBP Piedmont Assessment Scores	
Parameter	**Score**
1) Epifaunal Substrate	_____
2) Embeddedness	_____
3) Velocity-Depth Regimes	_____
4) Sediment Deposition	_____
5) Channel Flow Status	_____
6) Channel Alteration	_____
7) Frequency of Riffles (or Bends)	_____
8) Bank Stability	RB:_____
	LB:_____
9) Bank Vegetative Protection	RB:_____
	LB:_____
10) Rip. Veg. Zone Width	RB:_____
	LB:_____

Water Quality		
Temperature	___.__	°C
% Saturation	___.__	%
Dissolved Oxygen	___.__	mg/l
Conductivity	____	µS/cm
Specific Conductance	____	(µS/cm)/c°
pH	___.__	

Weather		
Today:	storm/heavy rain	showers (intermittent)
	rain (steady)	sunny
	partly cloudy	cloudy
Past 24 hrs:	storm/heavy rain	showers (intermittent)
	rain (steady)	sunny
	partly cloudy	cloudy

Riparian Zone/ Instream Features

Predominant Surrounding Landuse	
Forest	Commercial
Field/Pasture	Industrial
Agricultural	Golf Course
Residential	Other _____

Canopy Cover		
Open	Moderate	Heavy

Channelized?	
Yes	No

Local Streambank and Channel Bottom Erosion			
None	Low	Moderate	Heavy

Riparian Zone Width	
LB	**RB**
0-25	0-25
25-50	25-50
50-75	50-75
75-100	75-100
100+	100+

Possible impairments to fish (i.e. golf course, industrial area):

Other Comments:

Figure B2: Fish sampling field data sheet (front).

Site ID:	**Sampling start time:**	**Finish time:**	**Number of Electorfisher units:**
Date:	**Recorder:**		
Stream Order:	**Investigators:**		

Species	**Number of Individuals**	**Total**
Ameiurus natalis		
Ameiurus nebulosus		
Anguilla rostrata		
Campostoma anomalum		
Catostomus commersoni		
Clinostomus funduloides		
Cyprinella spp.		
Erimyzon oblongus		
Etheostoma blennioides		
Etheostoma flabellare		
Etheostoma olmstedi		
Exoglossum maxillingua		
Fundulus diaphanus		
Gambusia holbrooki		
Hypentelium nigricans		
Lampetra aepyptera		
Lepomis auritus		
Lepomis cyanellus		
Lepomis gibbosus		
Lepomis macrochirus		
Luxilus cornutus		
Micropterus salmoides		
Notemigonus crysoleucas		
Notropis hudsonius		
Notropis procne		
Noturus insignis		
Pimephales notatus		
Rhinichthys atratulus		
Rhinichthys cataractae		
Semotilus atromaculatus		
Semotilus corporalis		
	Grand Total:	

Figure B3: Fish sampling field data sheet (back).

3. Development of a Index of Biotic Integrity for Fish

Fish species were first classified into groups including trophic guilds and tolerance values. Designations of tolerant or intolerant in Fairfax County were based on field observational data. Trophic and habitat classifications were based on the literature (Smogor 1999, and Teels 2001)(Table B1).

An extensive suite of candidate metrics were evaluated based on trophic characteristics, tolerance, and community structure, and each was then assessed for its usefulness in developing an Index of Biotic Integrity for fish. Metrics and scoring criteria that were tested were similar to those tested by Billy Teels whose work was completed in the Occoquan watershed in 2001 (Teels 2001). In addition, metrics and scoring criteria used by the statewide Maryland Biological Stream Survey were also tested (Southerland, personal communication). Metrics were chosen on their ability to distinguish most impaired sites from least impaired sites (Figure B2).

Studies have shown that there is a significant difference in fish assemblages found in the Coastal Plain versus the Piedmont (Smogor 1999, and Roth et al. 2000). A small portion of Fairfax County is considered to be Coastal Plain but the area is small and is highly impacted from anthropomorphic sources. For this reason, all of Fairfax County will be considered as Piedmont. Metrics used for Piedmont streams are similar to those used by Teels. Scoring criteria was based on 1999-2006 Fairfax County bioassessment reference data from Prince William Forest Park and was determined using the tri-sectioning method as detailed by Fausch et al. (1984) and Karr (1986). Further refinement of the metrics and/or scoring criteria may occur in the future as more data is collected.

Classification ratings were based on the maximum and minimum score and five categories were created from the difference.

Table B1: Trophic guilds and tolerance ratings for fish species found within Fairfax County.

Abbreviations for tolerance ratings are as follows: T = Tolerant, M = Moderate, I = Intolerant. Abbreviations for trophic guilds are as follows: AHI –algivore/herbivore/invertivore, DAH – detritivore/algivore/herbivore, INV – invertivore, IP – invertivore/piscivore, PIS – piscivore.

CommonName	Family Type	Tolerance	TrophicGuild	Non-Native	Benthic	Lithophils
Alewife	Herring	M	AHI			
American Eel	Eel	M	IP			
Banded Killifish	Killifish	M	INV			
Black Crappie	Sunfish	M	IP	X		
Blacknose Dace	Minnow	T	AHI			X
Blue Catfish	Catfish	M	IP	X		
Bluegill	Sunfish	T	INV	X		
Bluntnose Minnow	Minnow	T	AHI	X		
Brown Bullhead	Bullhead	M	IP			
Central Stoneroller	Minnow	M	DAH			X
Chain Pickerel	Pike	M	PIS			
Channel Catfish	Catfish	M	IP	X		
Comely Shiner	Minnow	M	INV			X
Common Carp	Minnow	M	AHI	X		
Common Shiner	Minnow	M	INV			X
Creek Chub	Minnow	T	IP			
Creek Chubsucker	Sucker	M	INV			
Cutlips Minnow	Minnow	I	INV			
Cyprinella	Minnow	M	INV			
Eastern Mudminnow	Mudminnow	M	INV			
Eastern Silvery Minnow	Minnow	M	AHI			
Fallfish	Minnow	I	IP			
Fantail Darter	Darter	M	INV		X	
Fathead Minnow	Minnow	M	AHI	X		
Gizzard Shad	Herring	M	AHI			
Golden Redhorse	Sucker	M	INV	X	X	X
Golden Shiner	Minnow	M	AHI			
Goldfish	Minnow	M	AHI	X		
Green Sunfish	Sunfish	T	IP	X		
Greenside Darter	Darter	M	INV	X	X	
Largemouth Bass	Black Bass	M	PIS	X		
Least Brook Lamprey	Lamprey	M	DAH			
Longear Sunfish	Sunfish	M	INV	X		
Longnose Dace	Minnow	I	INV		X	
Margined Madtom	Madtom	I	INV		X	
Mosquitofish	Livebearer	M	INV			
Mummichog	Killifish	M	INV			
Northern Hogsucker	Sucker	I	INV		X	X
Northern Snakehead	Snakehead	M	P	X		
Potomac Sculpin	Sculpin	I	INV		X	
Pumpkinseed Sunfish	Sunfish	M	INV			
Redbreast Sunfish	Sunfish	M	IP			
Redear Sunfish	Sunfish	M	INV	X		
River Chub	Minnow	M	IP			
Rosyside Dace	Minnow	M	INV			X
Shield Darter	Darter	I	INV		X	X
Silverjaw Minnow	Minnow	M	AHI			X
Smallmouth Bass	Black Bass	M	PIS	X		
Spottail Shiner	Minnow	M	INV			
Stripeback Darter	Darter	M	INV		X	X
Swallowtail Shiner	Minnow	M	INV			X
Tessellated Darter	Darter	T	INV		X	
Warmouth	Sunfish	M	IP	X		
White Perch	Striped Bass	M	IP			
White Sucker	Sucker	T	AHI			X
Yellow Bullhead	Bullhead	T	IP			
Yellow Perch	Perch	M	IP			

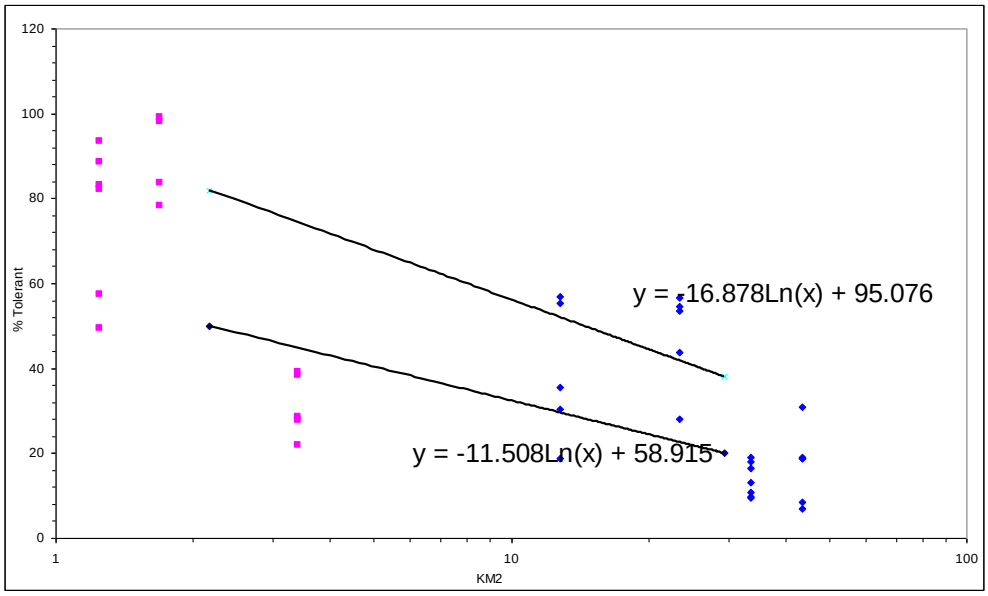

Figure B4: Example of tri-sectioning completed for metrics in the Piedmont. The pink squares represent small watersheds (< 10 km^2) while the blue squares represent larger watersheds (> 10 km^2)

Table B2: Scoring criteria for Fish Index of Biotic Integrity for sites less than 50 km^2.

Piedmont	1	5
1. Number of Species	< 2.8386Ln(x) + 1.8009	> 3.0687Ln(x) + 4.6226
2. Number of Darter Species	0	>=2
3. Percent Tolerant	> -17.262Ln(x) + 103.37	< -12.658Ln(x) + 64.807
4. Number of Intolerant Species	< 0.7393Ln(x)	> 0.6137Ln(x) + 1.1245
5. Percent Generalists (AHI)	> -16.878Ln(x) + 88.076	< -7.6718Ln(x) + 35.944
6. Percent Benthic Invertivores	< 1.9724Ln(x) + 0.01	> 4.5037Ln(x) + 0.77
7. Percent Lithophils - Tolerants	< 1.71	> 14.52

x is watershed area in kilometers squared

Table B3: Scoring criteria for Fish Index of Biotic Integrity for sites greater than 50 km^2.

Piedmont	1	5
1. Number of Species	< 2.8386Ln(x) + 1.8009	> 3.0687Ln(x) + 4.6226
2. Number of Darter Species	0	>=2
3. Percent Tolerant	> 35.84%	< 15.29%
4. Number of Intolerant Species	< 0.7393Ln(x)	> 0.6137Ln(x) + 1.1245
5. Percent Generalists (AHI)	> 22.05%	< 5.93%
6. Percent Benthic Invertivores	< 1.9724Ln(x) + 0.01	> 4.5037Ln(x) + 0.77
7. Percent Lithophils - Tolerants	< 1.71	> 14.52

Table B4: Classification rating for the Fish Index of Biotic Integrity.

Fish IBI	RATING
> 29	Excellent
23 to 28	Good
18 to 22	Fair
13 to 17	Poor
< 13	Very Poor

4. References

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C. BACTERIA MONITORING PROTOCOL

1. Background

To fulfill the US Clean Water Act's mandate to maintain “fishable and swimmable” waters, US EPA developed ambient water quality criteria based on a scientific assessment of the relationship between pollutant concentrations and environmental and human health effects. Ambient water refers to any fresh, marine, or estuarine surface water used for recreation, propagation of fish, shellfish, or wildlife, agriculture, industry, navigation, or as source water for drinking water facilities. Ambient water quality criteria become enforceable water quality standards when adopted by State, Territorial, Tribal, and local governments and approved by EPA.

For bacterial pollution in ambient water designated for recreational use, EPA has developed water quality criteria for *E. coli* in freshwater and enterococci in both freshwater and marine waters (51 FR 8012, March 7, 1986). On January 15, 2003, new bacteria standards in the VDEQs Quality Standards Section 9 VAC 25.260.170.A became effective. This formally directed *E. coli* and enterococci standards to be used in Virginia for water quality monitoring purposes (VDEQ, 2003). It should be noted that the EPA dictates that only one indicator should be used at a time, with *E. coli* being the suggested indicator for freshwater and enterococci for saltwater (EPA, 2002). Therefore, the focus of our bacteria monitoring program in Fairfax County will be on *E. coli*.

E. coli are a specific species of the coliform bacteria group that is part of the normal intestinal flora of humans and animals and are direct indicators of fecal contamination from these sources in water. Although *E. coli* is generally not harmful itself, the occurrence indicates the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoans which are correlated with swimming-associated gastroenteritis. There are a number of zoonotic diseases of concern to humans (diseases transferred from animals to humans) if ambient waters are contaminated with fecal material from non-human animal species (EPA, 2003). Of more concern is the potential of human fecal contamination because of the human specific pathogens that are typically found in human sewage (USGS, 2006).

The Fairfax County bacteria monitoring program was initiated in 1969 by the Department of Health’s Division of Environmental Health to generate a bacteria baseline for the waterways of the county. This bacteria baseline allowed the Health Department to monitor the water quality of the streams by establishing a “normal” level of bacteria for different sections of our waterways. By establishing a baseline, it enabled the Health Department to determine when a spike in the bacteria concentration occurred for a particular waterway and facilitated staff to locate pollution sources and to initiate corrective action or refer to the appropriate agency for corrective action (HD, 2002).

In 2003, the bacteria monitoring program was transferred from the Health Department to the Stormwater Planning Division, though all laboratory work is still completed by the Health Department. Past Health Department Stream Water Quality Reports and data can be found at: http://www.fairfaxcounty.gov/service/hd/strannualrpt.htm#data.

In 2005, Stormwater Planning Division discontinued sampling the original Health Department site locations and incorporated the bacterial sampling into its probabilistic monitoring (stratified random approach) program. Each year new sites are randomly selected to be sampled throughout the year for biological, bacterial, and water quality parameters. This site selection methodology is discussed further in Section D. The original 80 Health Department sites were based on ease of access and supervisor district representation and not scientifically founded. By adopting the new site selection method we are able to obtain a better understanding of the county's overall water quality status and trends. The 2005 sampling year included 39 sites in 14 watersheds. Each of the 39 sites was visited twice a season starting in the spring, for a total of six visits. In 2006 the number of randomly selected monitoring sites increased to 45. Each of these sites is sampled twice per quarter, or eight times annually.

As recommended by the EPA and VDEQ, Fairfax County completed its transition in 2005 to using *E. coli,* versus fecal coliform, as the primary indicator of possible fecal contamination. The basis behind this change stems from the 1986 EPA findings and the VDEQs 2003 memorandum that *E. coli* exhibits a stronger correlation to swimming borne illnesses for humans than fecal coliform. Thus by changing indicators, we are able to make better recommendations regarding the safety of our water for recreational uses. Additionally, in 2005 the Health Department updated its procedure to determine the concentration of *E. coli* in our waterways. Further discussion of recommended laboratory procedures are discussed in detail in a subsequent section in this document.

2. Virginia Department of Environmental Quality (DEQ) Standards

The Water Quality Standards which became effective on January 15, 2003, included the new bacteria standards in 9 VAC 25-260-170.A (VDEQ, 2003). The standards replaced the existing fecal coliform standard. The current bacteria standards in 9 VAC 25-260-170.A.2 are shown in Table C1:

Table C1: VDEQ water quality standards for E. coli and Enterococci

Name	Geometric Mean	Single Sample Maximum
Fresh Water E. coli (N/100mL)	126	235
Saltwater Enterococci (N/100 mL)	35	104

The suggested geometric mean correlates to the 1986 EPA recommended level of one-half of the density at which a health risk occurred. Again, EPA decrees that only one

indicator should be used at a time, therefore we focus on *E. coli* in Fairfax County. The geometric mean criteria in the water quality standards are for two or more samples taken during any calendar month. VDEQ interprets the bacteria standards as follows:

- Where effluent sampling is performed more then once per moth, the geometric mean applies.
- Where effluent sampling is performed once per month or less, the single sample maximum applies.

3. Collection Overview

The data used to calculate the geometric mean indicator densities corresponding to the accepted gastrointestinal illness rates are for "steady state" dry weather conditions. Henceforth, samples should be collected during dry weather periods to establish so-called "steady state" conditions (EPA, 1986).

The following field equipment is used for the bacteria/water parameter sampling:

- Whirl-pak® bags (Figure C1). Factory-sealed and sterilized. (Check to ensure factory seal has not been removed)
- High Density Polyethylene 16 oz. sample bottles (250 mL)
- Small cooler
- Ice or re-freezable "ice packs"
- Meters (YSI 85 and Handheld pH meter or YSI 556)
- Data sheets and site locations
- Writing implements (permanent marker & pen/pencil)

Bacteria sampling involves using whirl packs to take grab samples from the stream to determine the concentration of *E. coli* in the water. In addition to the assessment of bacteria, sterile bottles are used to collect samples to assess nitrate and total phosphorous as secondary tests for possible human inputs. Finally, chemical parameters, such as pH, water temperature, dissolved oxygen, and specific conductance are taken at time of bacteria sampling using a combination of YSI 85, YSI 556, and handheld pH meters (Figure C2).

In general, sample away from the stream bank, facing upstream in the main current. Never sample stagnant water. The outside curve of the stream is often a good place to sample, since the main current tends to hug this bank. In shallow stretches, carefully wade into the center current to collect the sample. Do not allow disturbed substrate, particulates, or suspended sediments to contaminate sample.

4. Collecting the Bacteria Samples: Whirl-pak® Bags

The following steps are followed to collect the stream samples for laboratory bacterial analysis.

1. Label the bag with the site number using a permanent marker.
2. Tear off the top of the bag along the perforation above the wire tab just prior to sampling (Figure C1). Avoid touching the inside of the bag. If you accidentally touch the inside of the bag, you must use another one.
3. *Wading.* Try to disturb as little bottom sediment as possible. In any case, be careful not to collect water that contains bottom sediment. Stand facing upstream. Collect the water sample in front of you.
4. Hold the two white pull tabs in each hand and lower the bag into the water on your upstream side with the opening facing upstream. Open the bag midway between the surface and the bottom by pulling the white pull tabs. The bag should begin to fill with water. You may need to "scoop" water into the bag by drawing it through the water upstream and away from you. Fill the bag no more than 3/4 full!
5. Lift the bag out of the water. Pour out excess water. Pull on the wire tabs to close the bag. Continue holding the wire tabs and flip the bag over at least 4-5 times quickly to seal the bag. Don't try to squeeze the air out of the top of the bag. Fold the ends of the wire tabs together at the top of the bag, being careful not to puncture the bag. Twist them together, forming a loop.
6. Fill in the bag number and/or site number on the appropriate field data sheet. This is important! It is the only way the lab coordinator knows which bag goes with which site.
7. Place the sample in the cooler with ice or cold packs. Take all samples to the lab.

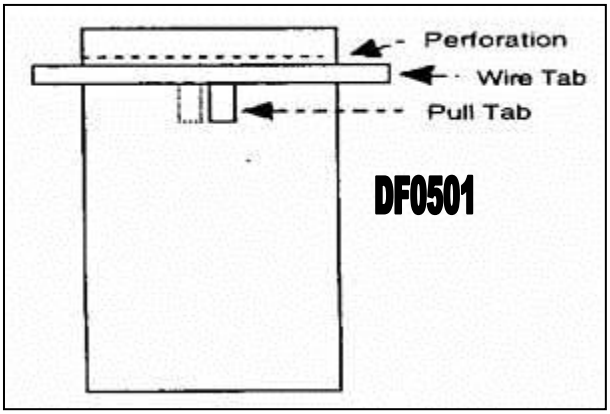

Figure C1: Sketch of a Whirl-pak® bag (Taken from EPA 2004 Monitoring and Assessing Water Quality, Chapter 5 Water Quality conditions)

5. Nitrogen and Phosphorus Sample Collection: Screw-cap HDPE Bottles

The following steps* are followed to collect the stream samples for laboratory chemical analysis.

*Taken from *EPA 2004 Monitoring and Assessing Water Quality, Chapter 5 Water Quality conditions*

1. Label the bottle with the site number (both on the cap and on the bottle).
2. Remove the cap from the bottle just before sampling. Avoid touching the inside of the bottle or the cap. If you accidentally touch the inside of the bottle, use another one.
3. *Wading.* Try to disturb as little bottom sediment as possible. In any case, be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream. Collect the water sample on your upstream side, in front of you.
4. *Rinsing.* The bottles should be rinsed three times with the stream water before a sample is collected. Plunge the bottle below the water surface, taking only an inch or two of water, screw cap onto bottle, and shake the bottle. Dump out the stream water and repeat two more times.
5. *Sampling.* Hold the bottle near its base and plunge it (opening downward) below the water surface. Collect a water sample 8 to 12 inches beneath the surface or mid-way between the surface and the bottom if the stream reach is shallow.
6. Leave a 1-inch air space. Do not fill the bottle completely (so that the sample can be shaken just before analysis). Recap the bottle carefully, remembering not to touch the inside.
7. Fill in the site number on the appropriate field data sheet. This is important because it tells the lab coordinator which bottle goes with which site.
8. If the samples are to be analyzed in the lab, place them in the cooler for transport to the lab.

6. Chemical Parameters: Meters

Handheld meters (either YSI 85 & handheld pH meter, or YSI 556) are used to obtain a reading of the current condition of the stream. Parameters tested include temperature, pH, DO, and specific conductance. Make sure that meters are properly calibrated and record results in their appropriate place. Note anything unusual in the comments box and follow up if necessary.

Figure C2: Bacteria sampling materials (clockwise from top: 16 oz. HDPE sample bottle, Whirl-pak® bag, YSI 85 meter, and handheld pH meter)

7. Laboratory Procedures

All water samples are kept on ice and brought to the Health Department lab within six hours for analysis. The Stormwater Planning Division does not perform any laboratory analysis, but delivers all samples to be processed to the Health Department. In 2005, the Health Department updated its procedure to assess the concentration of *E. coli* from the Modified *E. coli* method, which is a membrane filter technique, to the Colilert® Quanti Tray/2000 by Idexx. The Colilert *E. coli* method was approved in the June 10, 1992 US EPA Federal Register (Idexx, 2006). Further information, including videos on the Colilert® system, can be found at http://www.idexx.com/water/colilert/index.jsp. This new testing method increases the precision of the results and reduces the amount of human based error. Though the new method is more accurate, the upper limit of detection has been reduced from 6000 c.f.u. (colony forming units) using the membrane filter technique, to 2420 c.f.u. Therefore, serial dilutions need to be carried out to determine the density of E. *coli* in samples with concentrations higher than 2420 c.f.u. The nitrate-nitrogen and total phosphorous levels are determined by Skalar San++.

Water Chemistry: Streams Bacteriological Study

Fairfax County Health Dept, Laboratory
10777 Main St., #301, Fairfax, VA 22030

Sampling Zone 1
Report Results to: Danielle Derwin; 703-324-5616

Date Collected: _____
Collected by: _____
Team # _____
Meter type: _____

FIELD RESULTS

Sample ID #	*Point of Collection*	*Address/ Location*	*Time of Sample*	*pH*	*Sample Temp (°C)*	*Dissolved Oxygen (mg/L)*	*Specific Conductance (µS/cm)*
5-15	stream-Hickory Run (Difficult Run)	Roos Trail off of Manning St.					
5-16	stream-Difficult Run	Georgetwon Pike, near Difficult Run Park					
4-1	stream-Mine Run Branch (Pond Branch)	River Bend Road					
4-2	stream-Clarks Branch (Pond Branch)	Club View Drive					
4-3	un-named stream going into the Potomac (Pond Branch)	Blackberry Lane					
3-3	stream-Nichols Run	Springvale Road					
3-4	stream-Nichols Run	Beach Mill Road					
2-3	stream-Sugarland Run	near Intersection of Rt. 7 and Dranesville Rd.					
2-2	stream-Folly Lick Branch (Sugarland Run)	Cliveden Street					
2-4	stream-Sugarland Run	Spring St., in Sunset Business Park					

COMMENTS

Figure C3: Example field data sheet for bacteria monitoring (front).

Sample ID #	Lab Assigned Sample ID #	Sample ID #	Lab Assigned Sample ID #
5-15		3-3	
5-16		3-4	
4-1		2-3	
4-2		2-2	
4-3		2-4	

Figure C4: Example field data sheet for bacteria monitoring (back).

8. References

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D. SITE SELECTION METHODOLOGY

Stream sampling sites are randomly selected using a probabilistic design approach so that inferences on countywide stream health may be made with a high degree of confidence. Random selection of sites occurs from a defined stratum within the sample set of all potential stream sections within the county's borders. All stream segments are stratified by assigned physiographic province and stream order based upon the methodology defined below. More detailed analysis may reveal other meaningful strata (i.e.: land use, impervious cover) to be further imposed upon the set of all candidate streams to be randomly chosen for sampling each year. This further stratification may be limited by time constraints due to necessary increases in minimum sample sizes for each strata.

1. Computation of Stratum and Overall Mean and Variances for 2005 Probabilistic (Random) Sampling

A Digital Elevation Model derived synthetic stream network, generated at a 50 acre threshold, was utilized as the sampling frame (Figure D1). The stream network was stratified by Strahler stream order (1st through 5th) and samples allocated according to the proportion of total stream length in each stratum.

Stratum weights were therefore calculated as

$$W_h = \frac{L_h}{L_T}$$

where W_h is the weight of stratum *h*, L_h is the total stream length in the stratum, and L_T is the total stream length for the strata under consideration,

$$L_T = \sum_{h=1}^{n_s} L_h$$

and where n_s is the number of strata of interest. The sum of all weights must equal unity as

$$\sum_{h=1}^{n_s} W_h = 1/L_T \sum_{h=1}^{n_s} L_h = 1.0$$

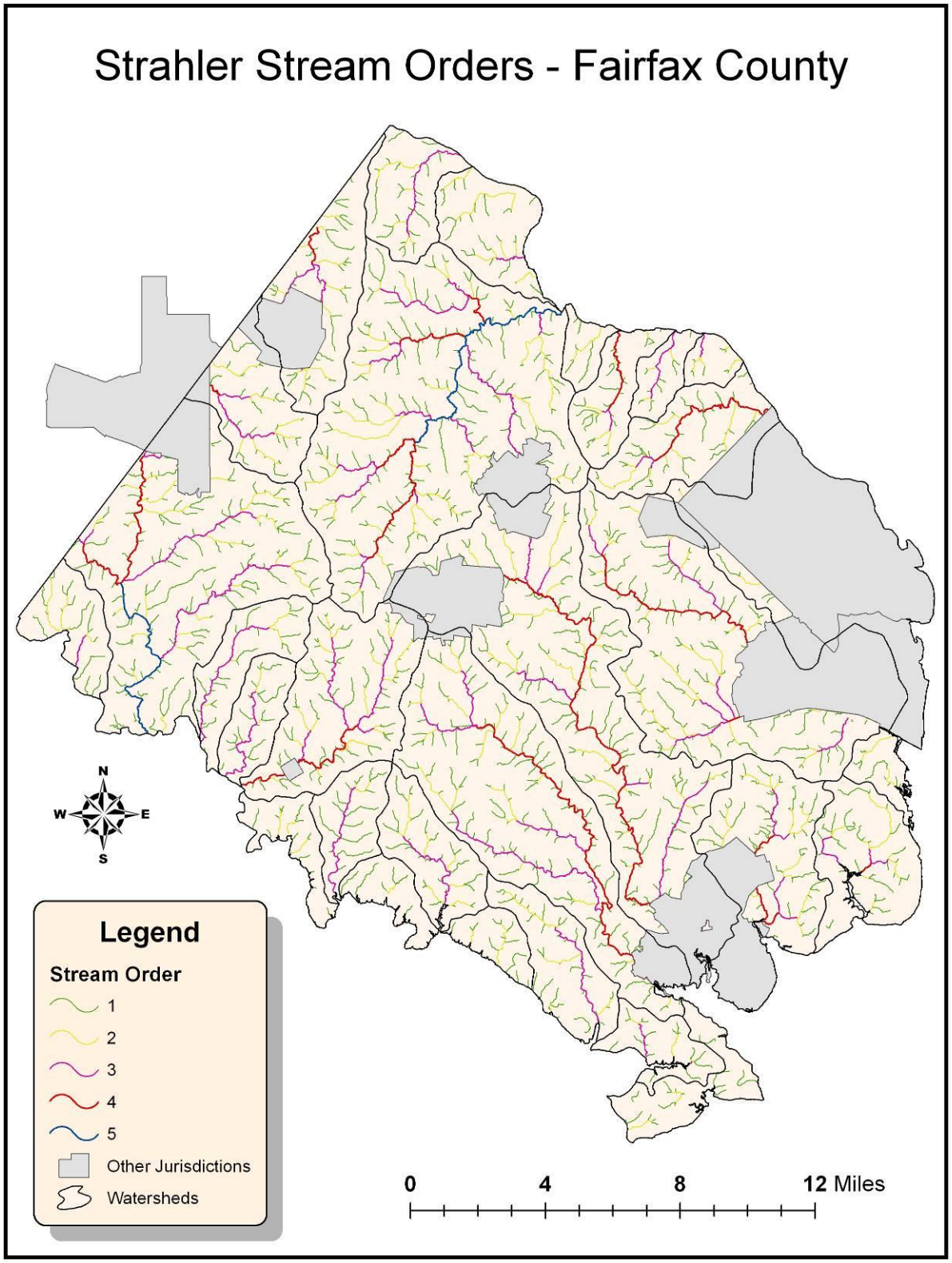

Figure D1: Digital elevation model–derived stream sampling frame.

A two-stage procedure was employed to determine sampling locations. Within each stratum, a stream segment was first selected at random. A sampling location was then randomly selected within this segment. The segment was then replaced, and the process repeated to obtain the required number of samples in each stratum.

Sample means and variances within each stratum were calculated based on computational procedures presented by Cochran (1977) and Gilbert (1987) for two-stage sampling when primary units are of unequal size and have the same chance of being selected. Stratum means were computed from

$$\bar{I}_h = \frac{\sum_{i=1}^{n_h} L_i I_i}{\sum_{i=1}^{n_h} L_i}$$

where $\bar{I}_h$ is the mean index in stratum *h*, I_i is the index value of the *ith* sample in the stratum, L_i is the length of the segment on which the *ith* sample was taken, and n_h is the number of samples taken in the stratum. If the total number of segments in each stratum is large compared to the number of segments sampled, then,

$$s_h^2 = \frac{1}{\bar{L}_h^2 (n_h - 1)} \sum_{i=1}^{n_h} L_i^2 (I_i - \bar{I}_h)^2$$

where s_h^2 is the index variance in stratum *h*, and is the mean segment length in the stratum.

The overall mean ($\bar{I}_o$) and variance (s_o^2) across two or more strata are obtained as

$$\bar{I}_o = \sum_{h=1}^{n_s} W_h \bar{I}_h$$

$$s_o^2 = \sum_{h=1}^{n_s} W_h^2 s_h^2$$

The variance of the overall mean, $s_o^2(\bar{I}_o)$, is computed as

$$s_o^2(\bar{I}_o) = \sum_{h=1}^{n_s} \frac{W_h^2 s_h^2}{n_h}$$

2. Final Field Selection of Sites

Once the list of candidate sampling locations has been generated, field investigations commence. Sampling locations that are difficult or impossible to access or sample are disqualified and removed from the list of candidate sites. Accepted sites are observed and flagged in the field (and then accurately mapped in the GIS). Field identification of sites continues in this fashion until the target number of sites (for each stratum) is reached (Table D1).

Disqualifying factors include:

- substantial inputs from tributary streams inside the 100 meter reach, or within 50-100 meters (depending on stream order) upstream or downstream of the candidate reach;
- the presence of hydraulic controls in the channel such as impoundments, off-line diversions, weirs, or large-scale channelization/stabilization structures (i.e.: concrete trapezoidal channels);
- channels (natural or manmade) greatly impacted by construction or industrial activities, (i.e. quarry sluices, landfill trenches, etc.);
- areas with limited or restricted access.

3. Modifications for 2006 sampling year

The 2005 procedure was slightly modified in 2006 to include another stratum – physiographic province (the two primary provinces in the County are the Piedmont and Coastal Plain). Additionally, the original DEM-derived sampling frame was adjusted to reflect the extent of perennially-flowing streams countywide. All perennial streams were mapped throughout the County during the 2002-2003 perennial streams mapping project (http://www.fairfaxcounty.gov/dpwes/watersheds/perennial.htm). This ensures all potential sample segments have sustained stream flow (year-round) and therefore should support aquatic life. This in effect removes all headwater ephemeral and intermittent channels from the sampling frame (set of all potential sample segments) See Figure D2 for the newly modified sample frame.

To ensure adequate sample sizes of strata, Coastal Plain streams of order 3, 4 and 5 were grouped together and Piedmont streams of order 4 and 5 were likewise grouped for reporting purposes. Table D1 shows the final number of sites within each stratum when the total number of sites chosen to be sampled is 45.

Table D1: Target number for samples within each stratum

Stratum 1: Physiographic Province	Stratum 2: Stream Order	Proportion of County	Number of Sites
Coastal Plain	1	12.53%	5
	2	5.93%	2
	3+	3.94%	2
Piedmont/Triassic Basin	1	39.39%	16
	2	18.42%	7
	3	13.49%	5
	4+	6.30%	3
	Total	100.00%	40

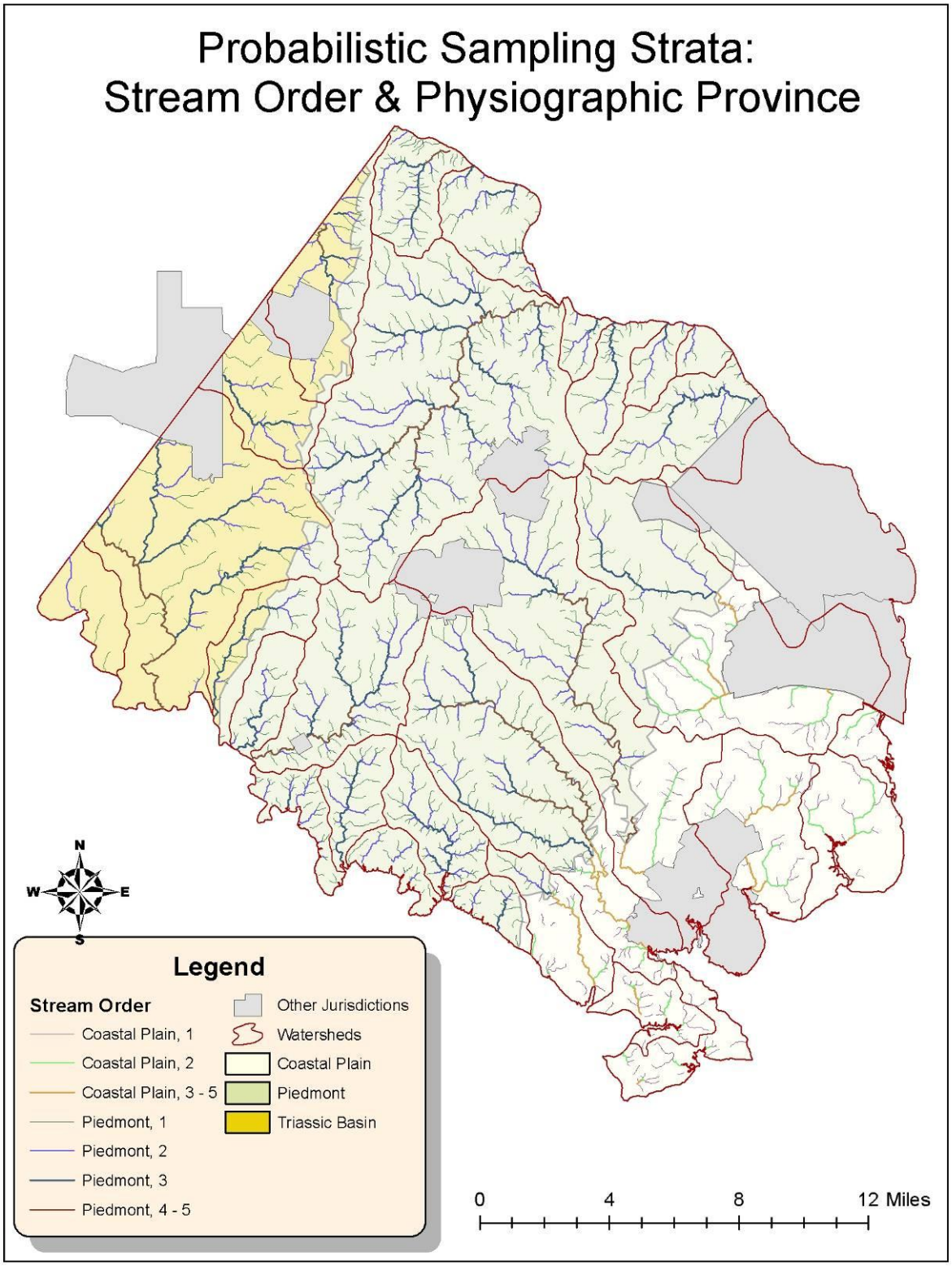

Figure D2: New Sample Framework showing strata groups.