Development of
Aquatic Life Use Assessment Protocols
for
Class VII Waters in Virginia

Report of the Academic Advisory Committee
for
Virginia Department of Environmental Quality

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FOR
CLASS VII WATERS IN VIRGINIA

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Contents

Photo by John Bunnell

Background

The Commonwealth of Virginia currently recognizes a distinct category of streams, termed Class VII-Swamp Waters. Because of natural conditions, these are waters that may be exceeding the pH and dissolved oxygen (DO) criteria designed to protect aquatic life in Class III waters. Swamp waters, often called blackwaters, typically are those occurring in low-gradient areas such as in the Coastal Plain physiographic province. They constitute about 5,000 stream miles in Virginia, or about 10% of the total stream miles in the Commonwealth. The low gradient of the channel, along with a typically fine-grained, sandy or peaty sediment, results in slow flow with few to no riffles. Inputs of organic matter from the surrounding riparian vegetation can be substantial, and due to the low flow, this organic matter can be retained in the streams/swamps for long periods rather than be flushed downstream.

Naturally low pH (<6.0) and DO (< 4.0 mg/L) in these streams can result from a combination of factors. Microbial decomposition of organic matter releases humic and fulvic acids that lower the pH of the water. Furthermore, microbes that decompose organic matter consume DO and can substantially reduce DO concentrations in the water column. The high levels of dissolved organic matter stain the water a deep dark color that reduces light penetration. Because of the darkly colored water and shading by riparian vegetation, DO is not adequately replenished from photosynthesis by periphyton. In addition, DO levels are reduced because of slow flow with no riffles that reduce natural reaearation from the atmosphere.

Biological organisms that are endemic to blackwater habitats are physiologically and behaviorally adapted to natural conditions of low pH, low oxygen saturation, and high temperature. For example, many of the fishes native to Class VII waters are facultative air-breathers or possess other adaptations to the unusual natural conditions. In contrast, taxa that have a more cosmopolitan (i.e., geographically broad) distribution lack specific adaptations to blackwater conditions and are uncommon in or absent from blackwater systems. As a consequence, it might be possible to use the taxonomic and functional composition of biotic assemblages (e.g., fishes, macroinvertebrates) collected from coastal freshwaters of Virginia to accurately classify Class VII waters and to evaluate the level of ecological health—or conversely, impairment—using biological (cp. chemical) criteria.

The application of physicochemical criteria (e.g., pH and DO) to Class VII streams, including blackwater systems, has at least two important implications. First, streams that are ecologically healthy, or even relatively pristine, would be listed as 'impaired' under Section 303d, increasing—unnecessarily—the number of Virginia stream miles requiring some form of restoration or mitigation. Second, the tactics that might be used to 'restore' blackwater streams and other swamp waters would, in fact, degrade the ecological integrity of the systems by facilitating the establishment of nonindigenous and potentially invasive aquatic species and cause the loss of endemic blackwater species, several of which (e.g., blackbanded sunfish, *Enneacanthus cheatodon*) are protected.

The Virginia Department of Environmental Quality (DEQ) uses a "Natural Conditions Study" to categorize streams that are suspected of having water quality problems based on natural stressors.   This process entails having personnel evaluate a stream based on the following and having the stream meet all of these criteria:

1. Does the stream have low flow or low gradient (<0.5%) and is it associated with fringing wetlands?

2. Does the stream have naturally low nutrient levels? This is defined as being less than the USGS determined national background averages from undeveloped areas for all of the following: nitrates (NO_3-N) <0.6 mg/L; total nitrogen (TN) <1.0 mg/L; total phosphorus (TP) <0.1 mg/L.

3. Do DO levels show a natural seasonal fluctuation as occurs because of the DO-temperature relationship? (If the winter DO does not increase substantially over summer levels, then there is the possibility of human inputs causing a higher than normal biochemical oxygen demand (BOD) and hence depressed DO.)

4. Are there any permitted or observed inputs of pollutants that could affect DO, to include both point and non-point sources?

If the stream meets all of the above criteria, it is categorized by DEQ as a Class VII Swamp Water. A stream placed into Class VII because of naturally low pH then must meet a pH criterion of 3.7-8.0 or be considered in violation of water quality standards. However, there is no criterion for DO for Class VII waters.

Given the constraints of not using a DO criterion, what is an appropriate means to assess the ability of Class VII waters to meet their designated uses (e.g., the support of aquatic life)? Some have proposed the possibility of developing a surrogate for a DO criterion or a procedure to determine a site-specific DO criterion in these waters.

BOD has the potential to be a surrogate for DO criteria in that it is in fact the direct stressor that causes unnaturally low DO. Higher than normal BOD would suggest anthropogenic organic matter inputs, and the decomposition of this matter would lower DO below its natural concentration. For BOD to be used as a surrogate, DEQ would first need to determine the natural background BOD of swamp waters. Also required would be an understanding of the natural seasonal variation of BOD, which would be affected by seasonal inputs of leaf litter and changes in temperature. These issues provide confounding factors that make BOD an unduly complicated surrogate for DO criteria in Class VII waters. In addition, DEQ would have to add BOD testing and its not inconsequential cost to its standard monitoring program.

Using nutrients as a surrogate for DO criteria in Class VII waters also has been posed. Nutrient levels, however, do not directly affect DO, as does BOD, but rather increases the rate of organic matter decomposition and hence the BOD. To use nutrients as a surrogate would require development of a dose-response curve that shows the effect of increased nutrients on DO. The only clear reason for using nutrients as a surrogate is that their concentrations are being determined as part of the DEQ monitoring program and thus would not add much cost. However, the indirect nature of the relationship between nutrients and the need for an understanding of the dose-response relationship makes the use of nutrients as a surrogate for DO criteria in Class VII waters very tenuous.

Development of appropriate site-specific criteria, which has also been raised as a possibility for Class VII waters, has many problems. The criterion cannot be based on data from the stream itself unless it was clearly shown that there were no known anthropogenic stressors, in which case the present conditions would set the standard for future monitoring. For streams that are known or suspected of having anthropogenic stressors, a criterion would need to be developed based on information from a stream or streams known to be unaffected by human stressors (or at least as being "least disturbed). Also, any site-specific criterion would need to incorporate seasonal fluctuations of DO based on temperature and the variation in organic matter inputs. These problems make the use of site-specific criteria unfeasible in our opinion for Class VII waters.

Given the problems of the above approaches, the Academic Advisory Committee (AAC) was charged in 2011 with determining the feasibility of using community-level biological metrics and aquatic life use criteria as an alternative to the current assessment approach for a specific subset of Class VII waters, namely, blackwater streams. Smock and Garman (2011) determined that such an approach, based on fish community data, was feasible and that potential fish metrics were correlated to an independent model of stream ecological health.

Project Objectives for 2012

In 2012, the AAC was asked to expand the earlier feasibility analysis to include the following objectives:

Task 1   Develop and test a field protocol that uses non-biological data to consistently separate 'blackwater' systems from other coastal streams;

Task 2   Develop a pilot biological index that can be used to identify impairment in blackwater streams; and

Task 3   Test index performance against a known dataset of blackwater streams.

Summary of 2011 Findings

Using published and unpublished resources on the zoogeographic distributions of fishes and aquatic macroinvertebrates within the study region, the INSTAR (INteractive STream Assessment Resource) database, and related databases (e.g., DEQ ProbMon), Smock and Garman (2011) identified a diagnostic guild of fish taxa that were reliable indicators of healthy (i.e., non-impaired) blackwater streams. That group of taxa included: mud sunfish (*Acantharchus pomotis*), redfin pickerel *Esox americanus*), ironcolor shiner (*Notropis chalybaeus*), blackbanded sunfish, lined topminnow (*Fundulus lineolatus*), sawcheek darter (*Etheostoma serrifer*) and swampfish (*Chologaster cornuta*) (Figure 1). These taxa occur in Chesapeake Bay and/or Chowan Basin streams and swamps, are rarely present in non-blackwater coastal streams, and occur infrequently in assemblages dominated by habitat generalists, e.g., creek chubsucker (*Erimyzon oblongus*), or non-native species, e.g., bluegill (*Lepomis macrochirus*).

Figure 1. Locations of blackwater streams with one or more occurrences of endemic guild species.

A similarly extensive analysis of aquatic macroinvertebrate assemblages was unable to identify taxa that were reliably diagnostic for blackwater conditions. A similar effort by the Maryland Department of the Environment to assess blackwater streams using criteria based on aquatic macroinvertebrates has been largely abandoned for the same reason (S. Stranko, Maryland Department of Natural Resources, pers. comm.). As a result, all subsequent analyses for this project were limited to fish community data.

In 2011, we also evaluated several possible metrics of blackwater stream health, including counts of individuals and proportion of total individuals that represented diagnostic species, number of diagnostic species, and proportion of diagnostic species. The absence of non-native and cosmopolitan species (e.g., bluegill) was also a good predictor of blackwater ecological health (Smock and Garman 2011). Finally, the 2011 study evaluated a possible relationship between candidate biological metrics and an independent model of ecological integrity (health). Paired data for n=133 coastal streams in the INSTAR database were used for the analysis. Preliminary results suggested a relatively strong (r=0.72) and positive relationship between the INSTAR stream assessment score and the proportion of diagnostic fish species, providing some support for basing impairment decisions on one or more biological metrics for Class VII waters.

Current Findings for Task 1: Develop and Test a Field Protocol that Uses Non-biological Data to Consistently Separate 'Blackwater' Systems from Other Coastal Streams

Methods

We met with Alex Barron, David Whitehurst, Warren Smigo, Bill Shanabruch, Tony Silvia and Rick Browder from DEQ to clarify the study objectives and to discuss potential non-biological criteria for identifying blackwater systems. Based on these meetings, we developed a preliminary habitat assessment data sheet that was designed to aid in the rapid collection of a wide range of data pertaining to habitat characteristics and water physicochemical parameters for the identification of potential class VII swamp waters.

In follow-up correspondence with Shanabruch, Smigo and Silvia, we selected potential study sites to reflect a wide range of variation with respect to the chosen habitat parameters. Our goal was to include not only blackwater swamp sites in the investigation but also non-blackwater swamp sites and free-flowing, Coastal Plain streams. Whereas the major focus of this work is on blackwater sites, we included such a wide range of aquatic habitat types in order to place potential blackwater sites within the context of the overall gradients of variation in important habitat parameters that occur in the region. For example, a blackwater swamp site might be similar to non-blackwater swamp sites with respect to benthic organic matter and emergent vegetation, and similar to free-flowing streams with respect to water turbidity and sand substrate prevalence. We also prioritized sites base on the availability of previously-collected fish or macroinvertebrate data, so that these data may be used to guide the development and validation of biocriteria for the blackwater sites (Task 2).

Based on our remote selection criteria and field reconnaissance, we selected twelve sites for the investigation: six sites in the Chowan River Basin, five sites in the James River Basin, and one site in the York River Basin (Table 1). Field activities were coordinated with DEQ biologists. On April 13, 2012, we worked with Warren Smigo and Bill Shanabruch of the DEQ Piedmont Regional Office to evaluate three study sites (Cypress Swamp and Otterdam Swamp in the Chowan River Basin and Bailey's Branch in the James River Basin). On April 16, 2012, we coordinated with Tony Silvia of the DEQ Tidewater Regional Office to evaluate three additional sites (France Swamp in the York River Basin and Round Hill and Burnt Mill Swamps in the Chowan River Basin). These initial field outings allowed us to further refine the habitat evaluation protocol and to come to agreement with the DEQ biologists on the correct interpretation of each included habitat parameter (Appendix 1). On April 20, 2012, we worked independently to evaluate the six additional sites listed in Table 1.

Before extensively measuring or evaluating any site characteristics, we began each survey by subjectively classifying each site as either a blackwater swamp system, a non-blackwater swamp system, or a free-flowing Coastal Plain stream. We then measured pH, DO, temperature and conductivity using YSI and Hydrolab multimeters (respectively, YSI Inc., Yellow Springs, OH;

Hach Inc., Loveland, CO). We evaluated water color at each site by collecting a 2-dram vial of stream water and comparing it to a Munsell color chart typically used for soil color evaluation. Because the hue and value metrics were prohibitively difficult to evaluate, we only evaluated the chroma of each water sample (all were evaluated at hue 2.5YR). We then visually estimated a series of in-stream and watershed habit characteristics, which, generally, included:

- Channel formation and flow characteristics: this category included metrics associated with the number of clearly-defined channel systems along the study reach, flow velocity and directionality, and benthic substrate.
- Riparian zone characteristics: this category includes evaluations of riparian zone vegetation type and prevalence, the presence of riparian wetlands, and topography.
- Stream and watershed disturbance: data in this category were not used to classify sites, but, rather, were used to identify potential sources of anthropogenic disturbance that might serve to confound the determination of the correct system classification based on the observed habitat metrics.

Data analysis

All of the observed habitat characteristics contain useful information for further refinement of this protocol; however, to provide a concise, quantitative analysis for this report, we focused on habitat assessment metrics and water quality parameters that 1) were most likely to vary between swamps and stream systems and between blackwater and non-blackwater systems due to natural conditions, and 2) were easily quantifiable such that variations among system types could be objectively evaluated.

Parameters for which measurements or estimations yielded continuous data were simply scored using the raw data (e.g., meters of riparian wetland, mg/L of dissolved oxygen), while those indicating categorical attributes (e.g., single, multiple or undefined channel) were given numerical scores based on *a-priori* hypotheses regarding the potential for each categorical condition to occur in Class VII waters. The habitat parameters used in the below-described analysis, as well as an explanation of each parameter, and the quantitative scoring system used for each, are listed in Table 2.

To visualize relationships among the study sites, with respect to all of the observed habitat metrics, we conducted Principal Components Analysis (PCA) ordination, a linear multivariate analysis technique. On ordination plots, sites are represented by points and the distances among points represent the degree of the overall differences among sites in terms of overall habitat structure. The axes on the ordination plots are unitless, and simply represent linear composites of the observed habitat metrics. To eliminate the effects of variations in numerical scaling among metrics on the PCA analysis results, the percentile rank of each metric score was obtained, and these data were used in the analysis.

Table 1: Site location information and investigation dates for Task 1.

Site	Location Description	Date Investigated	Latitude	Longitude	River Basin
Baileys Branch	Downstream of rte 10	13 April 2012	37.18002	-77.01246	James River
Otterdam Creek	Above rte 602	13 April 2012	37.12923	-77.12337	Chowan River
Cypress Swamp	Upstream of rte 647	13 April 2012	37.14749	-76.96672	Chowan River
France Swamp	Downstream of rte 606	16 April 2012	37.42151	-76.78423	York River
Burnt Mill Swamp	Adjacent to rte 638	16 April 2012	36.84514	-76.81329	Chowan River
Wards Creek	Upstream of rte. 10	20 April 2012	37.21577	-77.08106	James River
College Run	Above rte 10	20 April 2012	37.12224	-76.80166	James River
Grays Creek	Upstream of rte 626	20 April 2012	37.16511	-76.86927	James River
Dark Swamp	Upstream of rte 626	20 April 2012	37.16196	-76.84925	James River
Moore's Swamp	Upstream of rte 622	20 April 2012	37.06905	-76.85122	Chowan River
Passenger Swamp	Upstream of rte 626	20 April 2012	37.0369	-76.77941	Chowan River
Round Hill Swamp	Downstream of rte 614	20 April 2012	36.85745	-76.92093	Chowan River

rte = route

Table 2: Explanation of habitat metrics and water chemistry parameters used in site classification for Task 1.

Data parameter or category name	Description	Units	Score	Final Metric*
Single Channel	Proportion of reach composed of a single, defined channel	Percent of 100 m reach	1	
Multiple Channels	Proportion of reach composed of multiple channels	Percent of 100 m reach	2	1) Channel form
Undefined Channel pattern	Proportion of reach with no defined channel	Percent of 100 m reach	3	
No Flow	No perceptible flow	Percent of 100 m reach	1	
Slow flow	Sluggish, but apparent flow	Percent of 100 m reach	2	
Moderate flow	Moderate, laminar flow	Percent of 100 m reach	3	2) Flow Velocity
Rapid, laminar flow	Rapid, laminar flow	Percent of 100 m reach	4	
Turbulent flow	Rapid, turbulent flow	Percent of 100 m reach	5	
One flow vector	One clearly-defined direction of flow	Percent of 100 m reach	1	
Multiple flow vectors	Multiple, clearly-defined flow directions	Percent of 100 m reach	2	3) Flow Direction
No flow vectors	Undefined flow direction (no discernible flow)	Percent of 100 m reach	3	
Silt	Frequency of silt substrate	Percent of 100 m reach	1- mineral substrate size; 2- organic matter content	
Sand	Frequency of sand substrate	Percent of 100 m reach	2- mineral substrate size; 1- organic matter content	
Clay hardpan	Frequency of hard-pan clay substrate	Percent of 100 m reach	3- mineral substrate size; 1- organic matter content	4) Mineral substrate size

Rock	Frequency of gravel and cobble substrate	Percent of 100 m reach	3- mineral substrate size; 1- organic matter content	
Fine particulate organics	Frequency of substrate composed of fine particulate organic matter	Percent of 100 m reach	0- mineral substrate size; 3- organic matter content	5) Organic matter content
Coarse particulate organics	Frequency of substrate composed of coarse particulate organic matter	Percent of 100 m reach	0- mineral substrate size; 3- organic matter content	
Trees in channel	Frequency of trees within wetted area	0-absent, 1-rare/sparse, 2-common, 3- abundant	0-3	6) Submerged or emerged vegetation
Other vegetation in channel	Frequency of emergent and/or submerged aquatic vegetation	0-absent, 1-rare/sparse, 2-common, 3- abundant	0-3	
pH	pH	Standard units	rank of values	7) pH
Dissolved Oxygen	Dissolved Oxygen	mg/L	rank of values	8) Dissolved oxygen
Conductivity	Conductivity	µS/cm	rank of values	9) Conductivity
Chroma	Chroma from Munsell color chart	Levels: 1-8	rank of values	10) Water color
Turbidity	Water turbidity- estimated	Levels: 0-clear, 1-slightly turbid, 2-turbid, 3-highly turbid	rank of values	11) Turbidity
Bank Erosion	Frequency of eroded areas along banks	Percent of 100 m reach (both sides)	rank of values	12) Bank erosion
Pools	Frequency of pools along reach	Percent of 100 m reach	rank of values	13) Pools
Depth variation	Depth variation, average to maximum	Difference in meters	rank of values	14) Depth variation
Wetland width	Width of apparent wetland area- total cumulative, contiguous wetland width from center point of wetted area	Width in meters	rank of values	15) Riparian wetland width

* Final score values for metrics 1 through 5 were calculated by multiplying each category score value by the percentage of the study reach occupied by that category, summing these products, and dividing by 100. The final score for metric 6 was calculated by summing the scores for trees and other vegetation.

Results

Of the 12 sites investigated, we originally classified five as blackwater swamps, four as non-blackwater swamps, and three as non-swamp streams (Fig. 1, Table 3). In most instances, our initial, subjective classifications corresponded with the habitat metric scores and water chemistry measurements.

The most apparent differences between sites subjectively classified as swamps (either as blackwater or non-blackwater) and those classified as non-swamp streams were that non-swamp streams exhibited more clearly-defined channels with apparent beds and banks, greater flow velocity and more defined flow directionality. In addition, non-swamp systems exhibited a greater proportion of large mineral substrate, less benthic organic matter, less channel vegetation, more pool areas and greater overall depth variation than blackwater and non-blackwater swamps. Riparian wetland areas were also much narrower adjacent to non-swamp systems, effectively consisting of only areas within the incised channels (Table 3).

In general, sites originally classified as blackwater swamps had more darkly-colored, but less turbid, water than those classified as non-blackwater swamps (Table 3; compare Fig 1a to 1b). Blackwater swamps also exhibited lower DO concentrations, pH, conductivity, and less pool areas than non-blackwater swamps (Table 3).

The PCA ordination of all 12 sites using both the water chemistry and habitat assessment parameters showed a strong separation among the study sites that generally corresponded to the subjective site classifications (Figure 2). One exception to this was Moore's Swamp (MSP), which was originally classified as a non-blackwater swamp site, but grouped more closely in the ordination to the other sites classified as blackwater swamps. It was noted on the field data sheet that this site was a "marginal blackwater, non-blackwater system." Whereas the aggregate of all the observed parameters indicated that the site was more similar to the blackwater sites, Moore's Swamp did exhibit elevated DO and pH, making it more similar to the non-blackwater swamp sites with respect to these characteristics. The PCA ordination of all 12 sites explained a total of 83% of the original distance matrix (Axis 1: 65%, Axis 2: 17%), indicating that the analysis provided an accurate representation of the overall variation in the habitat parameters among the sites.

The three site classes formed a continuum along axis one. Almost all of the observed habitat metrics were correlated with axis 1 (Table 4). The strongest correlations occurred between axis 1 and the frequency of pool areas, flow directionality and velocity, frequency of bank erosion areas, channel formation, depth variation and in-stream vegetation. These habitat metrics and water chemistry variables are generally effective at separating free-flowing streams from swamps (both blackwater and non-blackwater). Metrics were less-strongly correlated with axis 2, but those that showed relatively strong correlations with the axis included benthic organic matter content, mineral substrate size, water chroma, riparian wetland width, pH, conductivity

and turbidity (Table 4). This combination of metrics is effective at separating blackwater and non-blackwater sites, as these classes are completely separated along axis 2.

The PCA ordination of the swamp sites generally corresponded with the river basin in which the sites occurred. The Chowan River Basin sites were all grouped relatively closely together. The non-blackwater swamp sites from the James River and York River were also relatively closely grouped (Figure 3).

Three of the 12 study sites (Burnt Mill Swamp [BSP], Moore's Swamp [MSP] and College Run [CRN]; indicated by asterisks on Figures 2 and 3) were located near extensive watershed development (agriculture) and, therefore, these sites were excluded, from an additional PCA analysis to remove the potential confounding effect of anthropogenic impairment on the habitat classifications. In addition, the measured water chemistry data (DO, pH and conductivity) were excluded from this analysis to determine if the visually-estimated habitat parameters yielded the same site classifications as the original analyses. The PCA ordination of the remaining nine sites and excluding the water chemistry measurements showed a similar separation pattern to the previous ordination (Figure 4). This is an indication that the potential impairment at the three excluded sites did not affect the analysis. In addition, the exclusion of the water chemistry data showed the visually-assessed habitat parameters alone were effective at separating the three site classes.

The three subjective site classes also exhibited relatively distinct riparian forest types, though we did not conduct a sufficiently thorough survey of the riparian vegetation to use these data in the quantitative analyses discussed above (Table 5). In general, blackwater swamp sites were dominated by tupelo (*Nyssa*, undetermined species, either *N. aquatic* [water tupelo] or *N. biflora* [swamp tupelo]). Non-blackwater swamp sites were dominated by bald cypress (*Taxodium distichum*) and bottomland hardwood species such as sycamore (*Plantanus occidentalis*), red maple (*Acer rubrum*) and smooth alder (*Alnus serrulata*). Free-flowing stream sites were dominated by upland hardwood species, predominately white oak (*Quarcus alba*) and American beech (*Fagus grandifolia*).

Table 3: Habitat metric scores and water physicochemical parameters used to evaluate the 12 study sites for Task 1.

Site	Subjective system class	Channel form score	Flow score	Flow direct. score	Mineral substrate size score	Benthic organic matter score	Channel vegetation score	pH	Dissolved oxygen (mg/L)	Conduct. (µS/cm)	Color score	Turbidity score	% Erode banks	% Pool	Depth variability (m)	Wetland width (m)
Wards Creek	Non-swamp stream	1	2.15	1	2.81	1.04	1	5.41	8.45	76	1	1	100	35	0.75	8
College Run	Non-swamp stream	1	2.6	1	2.85	1.1	0	4.56	9.4	62	2	0	100	15	0.1	2.5
Baileys Branch	Non-swamp stream	1	2.2	1	2.4	1.2	1	7.62	10.81	247	1	1	80	30	0.75	9
Mean (± 1 std. error)		**1 (0)**	**2.3 (0.1)**	**1 (0)**	**2.7 (0.1)**	**1.1 (0.0)**	**0.7 (0.3)**	**5.9 (0.9)**	**9.6 (0.7)**	**128 (59)**	**1.3 (0.3)**	**0.7 (0.3)**	**93 (7)**	**27 (6)**	**0.5 (0.2)**	**6 (2)**
Grays Creek	Non-blackwater	1.5	2.2	1.5	0.4	2.6	5	7.09	8.6	233	1	2	0	15	0.5	300
Dark Swamp	Non-blackwater	1	2	1	0	3	2	7.21	8.1	247	1	3	5	20	0.5	400
Moore's Swamp	Non-blackwater	3	1	3	0.6	2.4	5	6.2	11.8	117	2	1	0	0	0	100
France Swamp	Non-blackwater	2	2.05	2	0	3	5	6.74	6	263	1	2	0	5	0.25	200
Mean (± 1 std. error)		**1.9 (0.5)**	**1.8 (0.3)**	**1.9 (0.5)**	**0.3 (0.2)**	**2.8 (0.2)**	**4.3 (0.9)**	**6.8 (0.3)**	**8.6 (1.4)**	**215 (38)**	**1.3 (0.3)**	**2.0 (0.5)**	**1 (1)**	**10 (5)**	**0.3 (0.1)**	**250 (75)**
Passenger Swamp	Blackwater	3	1	3	0	3	4	5.33	0.78	52	6	1	0	0	0	100
Otterdam Creek	Blackwater	3	1	3	0.1	2.9	4	3.76	4.26	30	4	1	0	5	0.25	100
Burnt Mill Swamp	Blackwater	2.5	1.7	2	0.3	2.7	5	5.85	6.75	155	1	1	0	5	0.2	50
Cypress Swamp	Blackwater	1	2.95	1.05	0	3	5	4.32	7.62	24	4	1	0	10	0.5	250
Round Hill Swamp	Blackwater	2.6	1.8	1.3	1.2	1.8	3	5.66	5.68	94	3	1	0	5	0.25	400
Mean (± 1 std. error)		**2.4 (0.5)**	**1.7 (0.5)**	**2.0 (0.5)**	**0.3 (0.3)**	**2.7 (0.3)**	**4.2 (0.5)**	**5.0 (0.5)**	**5.0 (1.5)**	**71 (31)**	**3.6 (1.0)**	**1 (0)**	**0 (0)**	**5 (2)**	**0.2 (0.1)**	**180 (83)**

Table 4: Correlations of habitat metric scores and water chemistry parameters with the Principal Components Analysis ordination of the 12 study sites in habitat space.

	Axis 1[*]		Axis 2[*]	
	r	r^2	r	r^2
% Pools	0.939	0.881	-0.035	0.001
Flow direction score	-0.936	0.875	-0.089	0.008
% Eroded banks	0.89	0.792	0.245	0.06
Channel form score	-0.881	0.777	-0.024	0.001
Depth variation (m)	0.738	0.545	-0.216	0.047
Channel vegetation score	-0.719	0.518	-0.467	0.218
Flow velocity score	0.707	0.5	-0.027	0.001
Dissolved oxygen (mg/L)	0.67	0.448	0.039	0.001
Benthic organic matter score	-0.629	0.396	-0.54	0.292
Mineral substrate size score	0.519	0.269	0.554	0.306
Chroma	-0.644	0.415	0.583	0.34
Wetland width (m)	-0.267	0.071	-0.682	0.465
pH	0.41	0.168	-0.721	0.52
Conductivity (µS/cm)	0.331	0.11	-0.768	0.59
Turbidity score	0.136	0.018	-0.911	0.83

[*] Shaded cells indicate r^2 values greater than 0.25.

Table 5: Dominant riparian tree species of the 12 study sites.

Site	System class	Forest Type
Burnt Mill Swamp	Blackwater swamp	Bald cypress/tupelo (water tupelo, black gum)
Cypress Swamp	Blackwater swamp	Nyssa spp. /bald cypress, mixed wetland hardwoods
Passenger Swamp	Blackwater swamp	Tupelo
Moore's Swamp	Blackwater swamp	Tupelo
Otterdam Creek	Blackwater swamp	Tupelo
Round Hill Swamp	Blackwater swamp	Tupelo
Grays Creek	Non-blackwater swamp	Bald cypress, red maple
Dark Swamp	Non-blackwater swamp	Bald cypress
France Swamp	Non-blackwater swamp	Sycamore, red maple, smooth alder
Wards Creek	Free-flowing stream	Beech, white oak
College Run	Free-flowing stream	Beech, white oak
Baileys Branch	Free-flowing stream	Beech, white oak

Figure 1a: Dark swamp, a non-blackwater swamp in the James River watershed. Five gallon bucket contains water collected from the site.

Figure 1b: Passenger swamp, a blackwater swamp in the Chowan River watershed. Five gallon bucket contains water collected from the site.

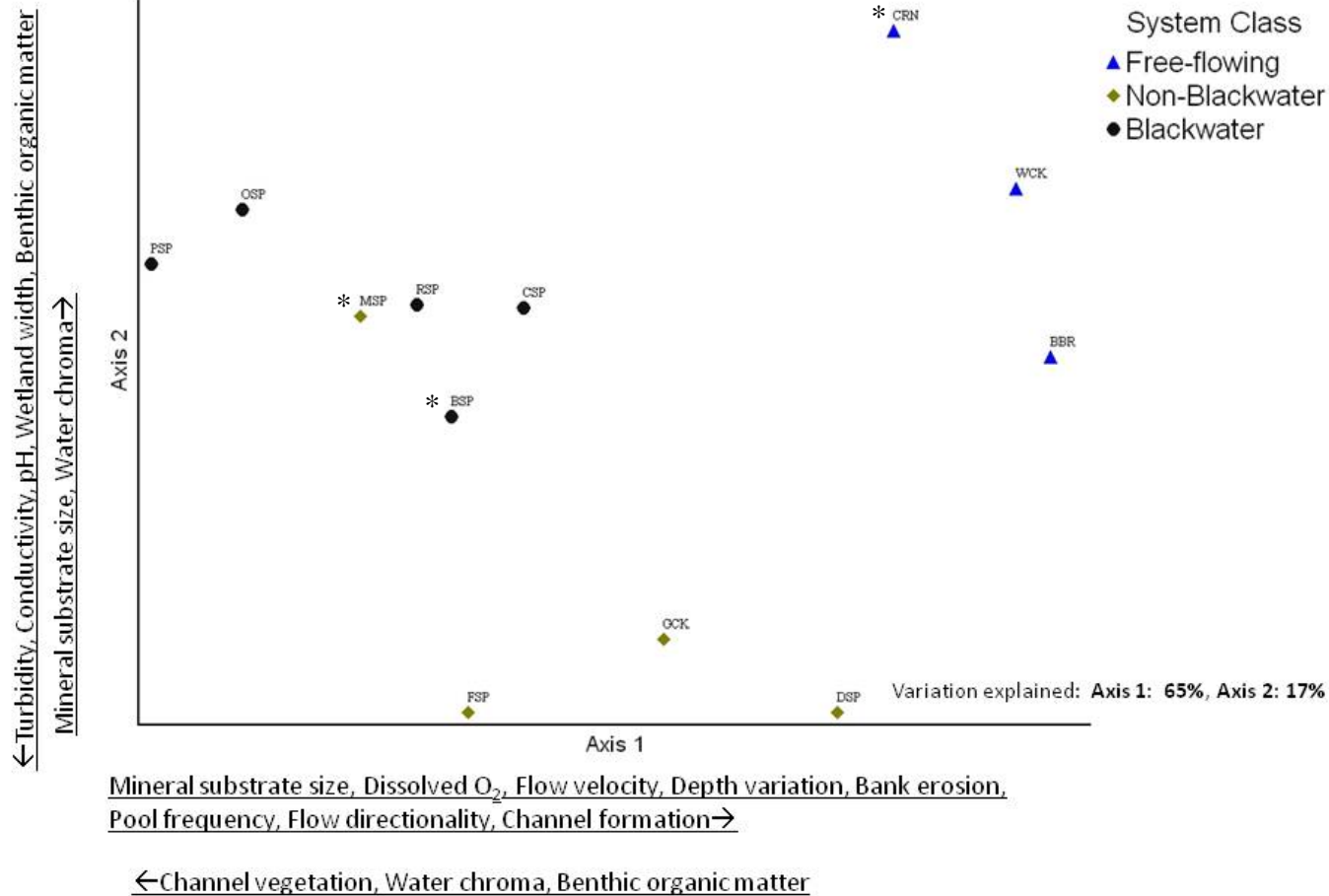Figure 1c: Bailey's Branch, a non-swamp stream in the James River watershed.

Figure 2: Principal components analysis plot of the 12 study sites ordinated using the selected water chemistry and visually-assessed habitat parameters. Sites are symbolized according to their subjective stream classifications. Sites denoted with asterisks exhibited substantial disturbance in the nearby watershed from agriculture that may have affected the habitat parameters.
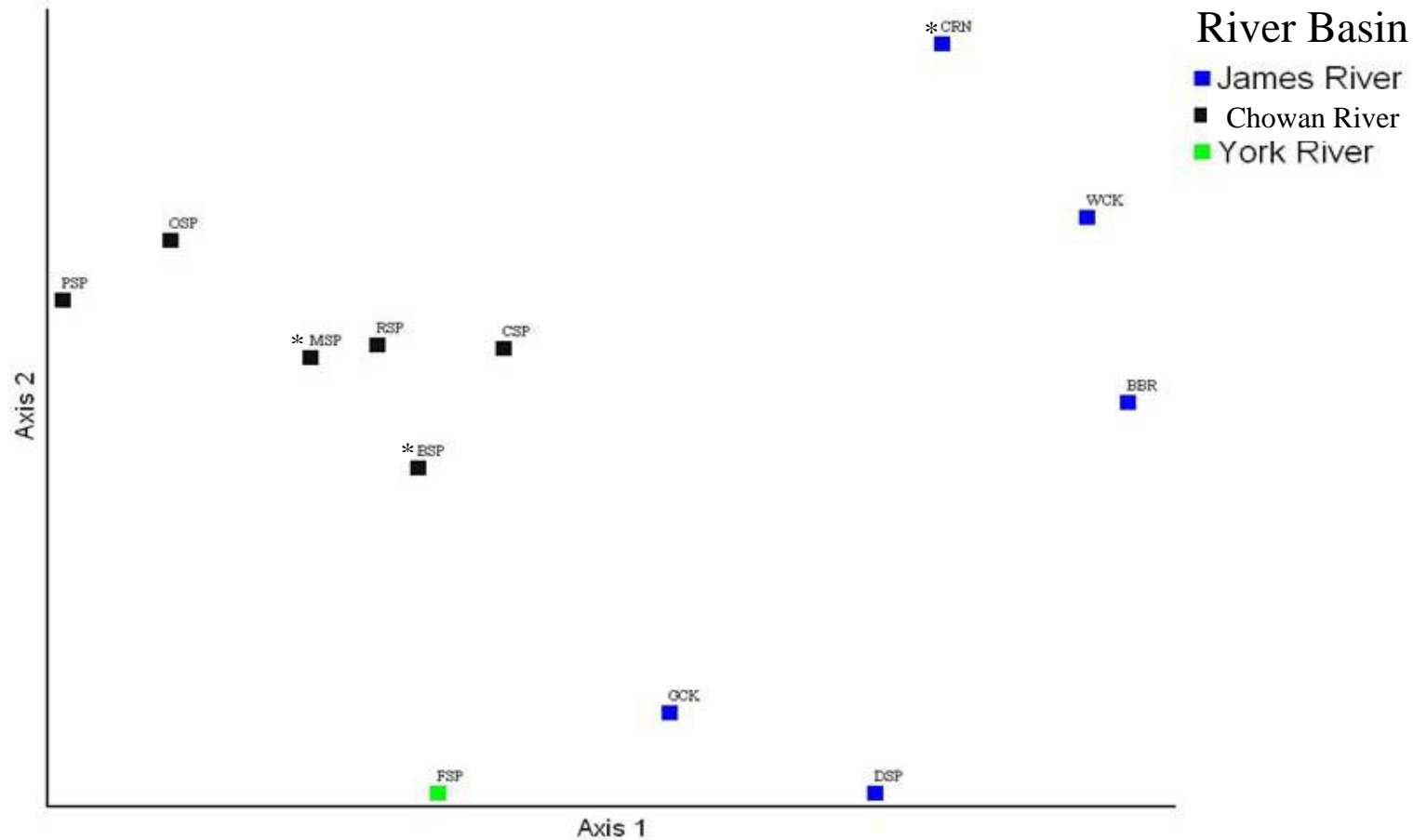
Figure 3: Principal components analysis plot of the 12 study sites ordinated using the selected water chemistry and visually-assessed habitat parameters. Sites are symbolized according to their watershed. Sites denoted with asterisks exhibited substantial disturbance in the nearby watershed from agriculture that may have affected the habitat classification.
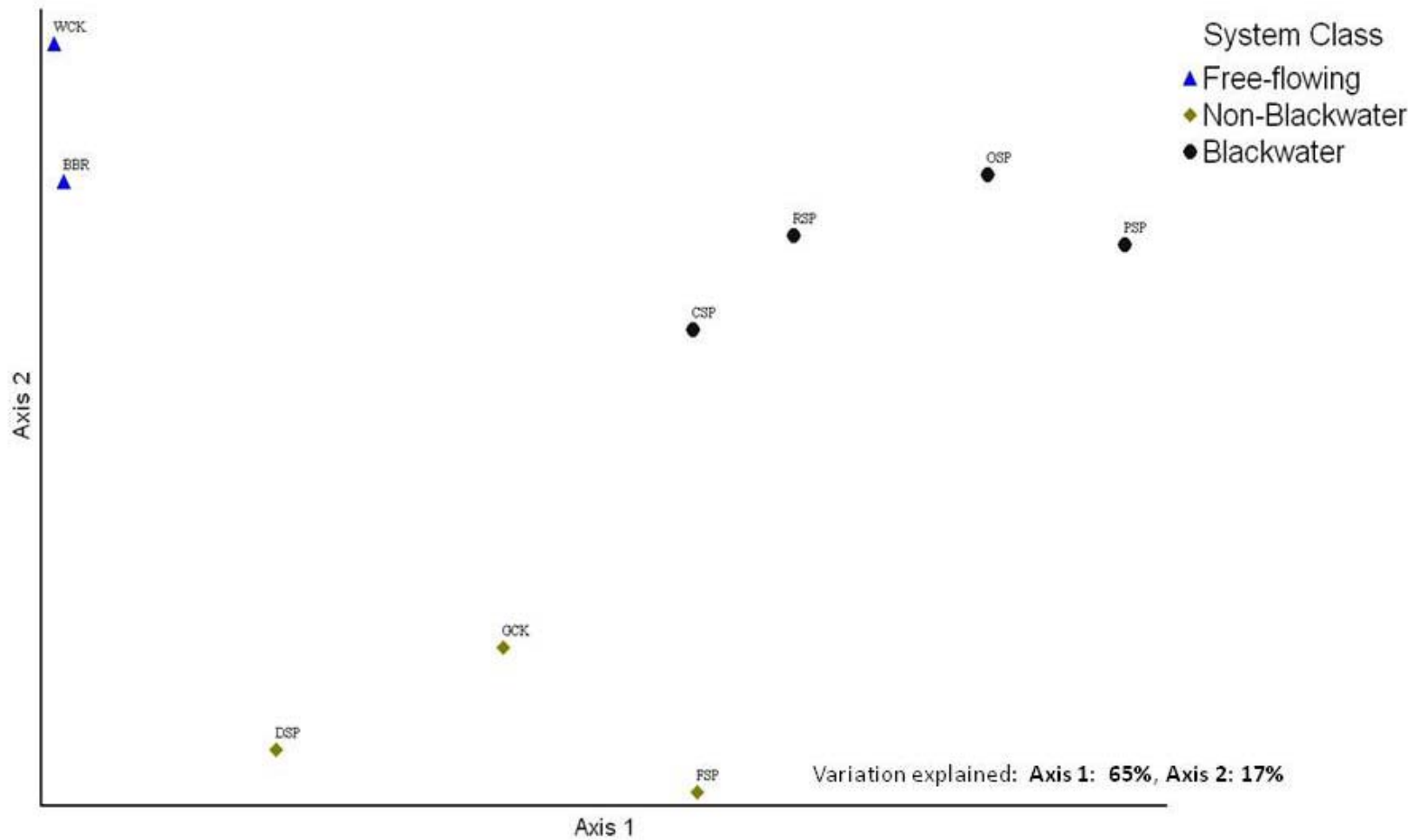
Figure 4: Principal components analysis plot of the nine study sites without apparent watershed disturbance ordinated using only the visually-assessed habitat parameters. Sites are symbolized according to their subjective classifications.

Conclusions and Recommendations, Task 1

Based on our field investigations and this preliminary analysis, we have revised the field habitat data sheet (Appendix 2). The revised data sheet emphasizes the core metrics that were analyzed here, and includes several changes and additions. For example, we revised the water color metric to include a qualitative assessment, rather than a quantitative determination of hue, value and chroma. Although the quantitative color evaluation conducted for this analysis provided useful data, comparison of water samples to the Munsell color charts was most difficult in shaded locations, and is not likely to yield consistent results among different studies and investigators. In addition, we have included a metric for evaluation of the type and frequency of in-stream vegetation. Although this metric was not included on the original data sheet, variations with in-stream vegetation were noted during the initial field investigation and subsequently noted on the comments section of the field data sheet for all sites included here.

Our results indicate that we have developed an effective protocol for assessing key habitat characteristics that separate free-flowing stream systems and swamp systems and distinguish between blackwater swamps and non-blackwater swamps. We estimate that, after appropriate training, DEQ biologists can execute the protocol outlined in Appendix 2 in approximately 30 minutes per site. This time is likely to be reduced if this assessment is conducted in conjunction with other field activities, such as collection of biological or additional non-biological data, as these activities generally require observation of many of the same aquatic ecosystem characteristics as described here.

It is important to note that we have evaluated the study sites included here and analyzed the resulting data by comparing the sites to each other, but we have not provided an absolute scoring system for classifying Coastal Plain systems. Within the scope of this initial phase, we analyzed data from only a limited number of sites and from a limited geographical area, and likely did not encompass the entire range of natural variation that occurs among Virginia Coastal Plain Stream Systems. In addition we have not yet empirically determined which of the habitat metrics included here are most important for shaping the natural variation in aquatic animal communities among these systems and, therefore, which metrics are truly most important for classification. In conjunction with the biocriteria development phases of this project (Tasks 2 and 3) as well as during later validation of the established protocols, the swamp stream classification protocol created in this phase should be executed in order to further refine the protocol and develop a more accurate and precise means of classification.

Current Findings for Tasks 2 and 3:
Develop a Pilot Biological Index that Can Be Used to Identify Impairment in Blackwater Streams, and Test Index Performance against a Known Dataset of Blackwater Streams

Methods and Results

We analyzed an existing quantitative database (INSTAR) of stream fish assemblages in the Virginia portion of the Chowan River Basin where streams in this watershed have a high probability of being classified as blackwater ecosystems (cp. coastal but non-blackwater streams). With this approach, we minimize the chance of incorrectly classifying an ecologically degraded blackwater stream as non-blackwater Class VII. In addition, we developed a complementary guild of *opportunistic* fish species, represented by habitat generalists and nonindigenous taxa, that was consistently associated (probability analysis) with degraded (i.e., INSTAR model class='compromised') blackwater streams in the Chowan River Basin. Taxa comprising this opportunistic guild included: creek chubsucker, golden shiner (*Notemigonus crysoleucas*), eastern mosquitofish (*Gambusia affinis*), eastern mudminnow (*Umbra pygmaea*), bluegill, largemouth bass (*Micropterus salmoides*), and warmouth (*L. gulosus*). Of several potential metrics evaluated, both percent composition and number of taxa of opportunistic and blackwater fish guilds (Smock and Garman 2011) appeared to be diagnostic of stream ecological integrity (Table 6), based on INSTAR model classes, and were considered appropriate candidate metrics—or response variables—for biocriteria in blackwater systems (Figure 5). For example 42% of species in compromised streams comprised the opportunistic guild, compared to only 8% of species represented by the blackwater guild. In contrast, over one-quarter of fish species in streams classified by INSTAR as 'healthy' or 'exceptional' were blackwater endemics, compared to 16% of fishes that were members of the opportunistic guild (Table 6). Results were comparable when taxonomic richness was used as the response variable (Table 6). Both fish guild metrics were used to develop a simple Index of Biotic Integrity (IBI) for blackwater streams (Table 7) that ranges between 2 (low integrity) and 10 (high integrity). Scoring criteria for the draft blackwater IBI were developed following an iterative analysis of data for 33 randomly selected Chowan River Basin streams (INSTAR database). An impairment threshold of IBI=5 (Figure 6) successfully classified (impaired *vs*. non-impaired) for 94 percent of 33 randomly selected streams in the Chowan River Basin.

Conclusions and Recommendations

The preliminary analyses presented in this report suggest that a simple Index of Biotic Integrity (IBI), based on analysis of fish community guilds may be able to determine impairment of blackwater (Class VII) streams and swamps in Virginia. Furthermore, a rapid field protocol, based on non-biological measures, was successful in separating blackwater and non-blackwater streams within the Virginia Coastal Zone. We believe that a combination of both analyses (Figure 7) should be refined and tested as a potential assessment protocol for Class VII waters,

and blackwater streams specifically. We believe that such an approach has several advantages, including the ability to leverage an extensive and expanding biological database (INSTAR, DEQ ProbMon) for the region and the ability to conduct *real-time* stream assessments by appropriately trained DEQ personnel. It is essential that all phases of this project be conducted in cooperation with DEQ scientists who will ultimately be responsible for execution of the newly-developed protocols. This classification protocol should be used in a similar manner to narrative criteria for water quality assessment (such as biocriteria), that is, rather than a rigid scoring system for classification, the protocol should serve to inform best professional judgment regarding the most appropriate means of assessing a given stream system. Therefore, the input of DEQ personnel who conduct assessments in Virginia coastal systems is essential for the success of this project. Additional recommendations include: 1.) validation of the draft blackwater IBI on an independent and larger blackwater dataset that is not limited to the Chowan River Basin; 2.) in concert with DEQ Biologists, conduct field testing of the blackwater field protocols and consider the inclusion of remotely sensed data (e.g., soils layer) and GIS methods as screening tools for separating blackwater and non-blackwater aquatic systems; 3.) refine IBI metrics, scoring criteria and impairment threshold, as appropriate, for other coastal zone basins and for a range of stream orders; 4.) reach out to Maryland and North Carolina, both of which have a significant number of Class VII and blackwater stream systems.

Table 6. Relationship among candidate Index of Biotic Integrity (IBI) metrics and INSTAR[*] stream health model classes for 30 randomly selected streams in the Chowan River Basin, Va.[†]

INSTAR Stream Integrity Category	Opportunistic Guild (% taxa)	Blackwater Guild (% taxa)	Opportunistic Guild (# taxa)	Blackwater Guild (# taxa)
Compromised	42.0	7.7	4	>1
Restoration Potential	28.2	23.2	3	3
Healthy/Exceptional	16.0	25.4	2	4

[*] INteractive STream Assessment Resource
[†] Data source: http://instar.vcu.edu

Table 7. Draft Index of Biotic Integrity (IBI), including metrics and scoring criteria, for blackwater streams in Virginia.

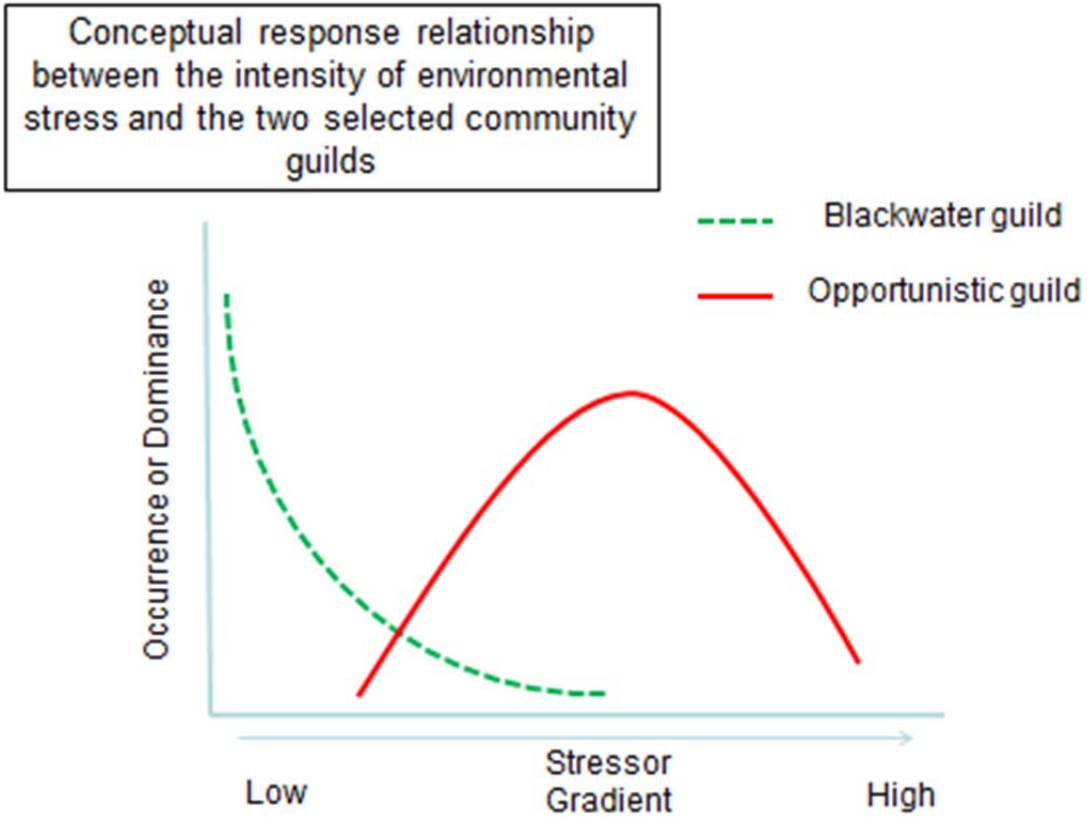| Metrics	1	3	5
Opportunistic guild (*proportion of taxa*)	>50%	20-50%	<20%
Blackwater guild (*proportion of taxa*)	<20%	20-50%	>50%
Opportunistic guild (*number of taxa*)	>4	2-4	<2
Blackwater guild (*number of taxa*)	<2	2-4	>4

Figure 5. Conceptual relationship between candidate metrics for a Blackwater Index of Biotic Integrity (IBI) and a generalized stressor gradient. The Y axis could be any measure of relative abundance or numerical dominance.
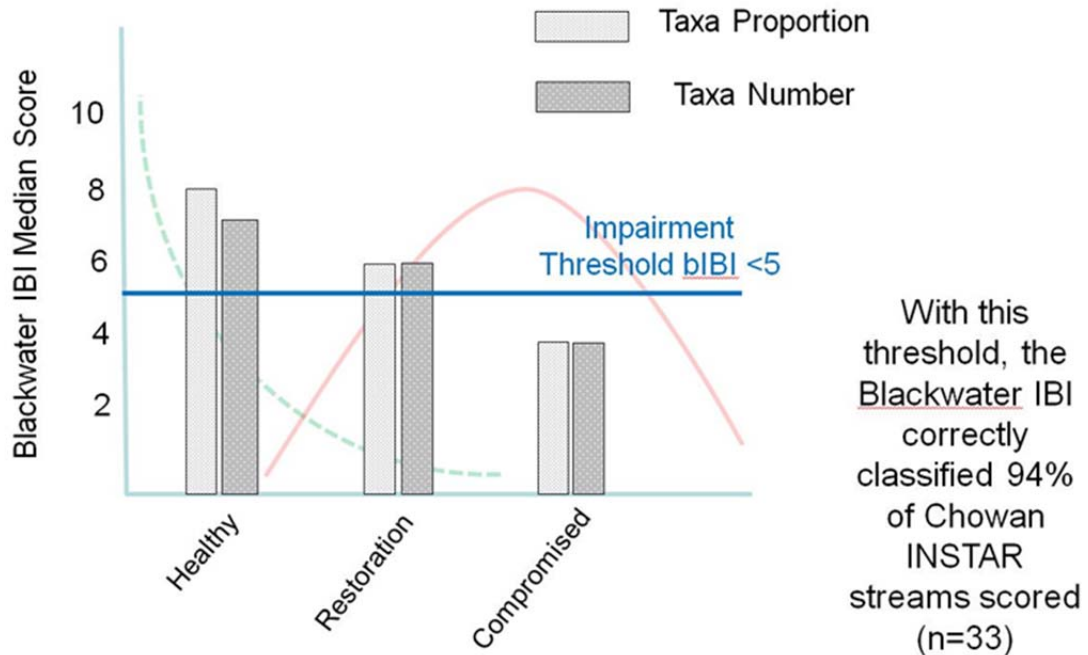
Figure 6. Draft Blackwater Index of Biotic Integrity (IBI) scores, based on either proportion or taxonomic richness, for three INSTAR model classes: healthy, restoration potential, and compromised. A proposed impairment threshold of IBI=5 correctly classified a random sample of 33 Chowan streams.

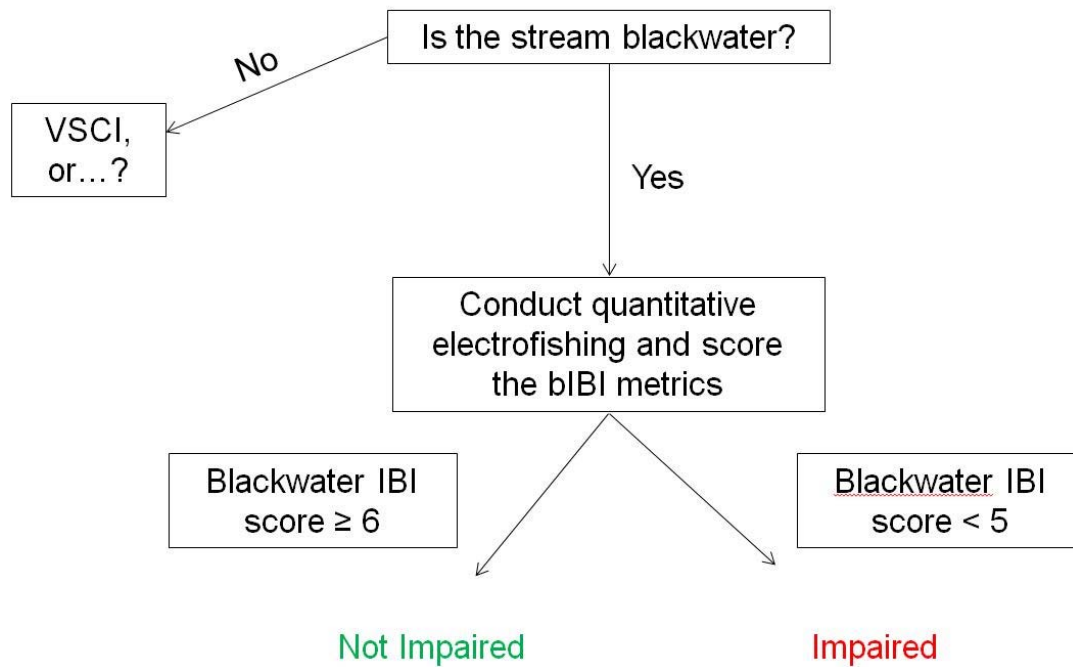# Blackwater Field Assessment Protocol

Figure 7. Proposed blackwater field assessment protocol for use in Virginia's coastal streams. VSCI = Virginia Stream Condition Index; bIBI = Blackwater Index of Biotic Integrity

Appendix 1: Field Protocol for Evaluation of Habitat and Water Quality Parameters Associated with Swamp Waters--Version 1.0

Site name/description:

Date: Time on site: Time off site:

Investigators:

Lat: Long: Datum:

Has rain occurred recently? y/n   Comments on recent rain events:

Air Temperature:

Notes on weather conditions:

Subjective classification of system:

Water Level: below normal/ normal / above normal

(assessments conducted during high water levels may be less reliable)

Section 1) Water Physicochemistry (indicate units where appropriate):

pH:_____   DO_____   Cond/Spc. Cond_____

Temp_____   Others (indicate parameters and units):

Water color_____

        Hue_____   Value_____   Chroma_____

Water clarity (clear, slightly turbid, very turbid)

Section 2) Channel and flow characteristics

2a Study reach length_____ (m)

2b Channel morphology: Indicate the percentage of the study reach occupied by:

Single, defined channel with clearly-defined banks and streambed _____%

Multiple, defined channels (i.e., braided system) _____%

        If multiple channels present, indicate approximate number of channels or range, if highly variable

Undefined bed and banks _____% (if undefined banks predominate, metrics 2c, 2d may be difficult to evaluate)

2c Bank stability

Percent of bank area with signs of erosion: Left_____%   Right_____%

2d Sinuosity Ratio (stream channel length/straight-line distance):_____

2e Flow velocity:

No perceptible flow_____ % of reach

Sluggish flow_____ % of reach

Moderate, laminar flow_____ % of reach

Rapid, laminar flow_____ % of reach

Rapid, turbulent flow_____ % of reach

Other (explain)_____ % of reach

2f Flow direction and definition

One, clearly-defined flow vector/area where flow predominates_____ % of reach

Several, clearly-defined flow vectors/areas where flow predominates_____ % of reach

No clearly defined areas where flow predominates _____

2g Canopy cover (percent cover above wetted area): _____

2h Bottom Substrate types

Type_____ Percent_____

Type_____ Percent_____

Type_____ Percent_____

Type_____ Percent_____

2i Percentage of wetted area comprised of pools:

2j Pool depth/ variability: (max, min, commonness of deep pools, number of pool size classes present- to be revised based on field obs.):

Section 3) Riparian Zone Characteristics

3a Riparian vegetation type (note major veg types and widths from stream channel)

Left bank (descending)   Right bank (descending)

3b Riparian vegetation protection (note percentages of natural land cover and widths from channel)

Left bank (descending)   Right bank (descending)

Riparian zone topography (approx. elevation above stream bank -- if variable, a diagram may be appropriate)

Left bank (descending)   Right bank (descending)

Section 4) Stream/watershed disturbance

4a Channel alteration (if present, note type)

4b Riparian land use (if multiple, note percentages)

4c Other indicators of anthropogenic disturbance:

Section 5) Stream cross section

5a. Draw a typical stream cross section and/or indicate: **normal flow depth/width, normal high flow depth and width, and extreme high flow depth/width** (i.e., dimensions of total stream incisement- see example figure)
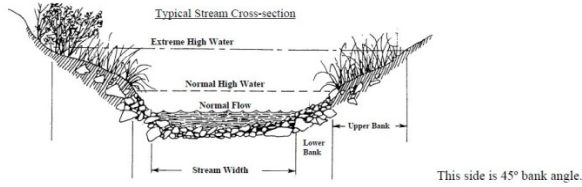

Diagram from North Carolina Department of Environment and Natural Resources standard operating procedures. see:

http://portal.ncdenr.org/c/document_library/get_file?uuid=c2fdf380-aa8a-481e-8388-a6e6596c6a96&groupId=38364

5b. Notes Regarding site conditions and other data collected:

5c. Remotely-derived data (not collected on-site)

Watershed land use/land cover (note types and percentages):

Moisture indices at time of investigation: PDSI_____ CMI_____

USGS Gauge Data: Nearby Stream_____ Stream flow (percentile)_____

Notes on comparability of gage data_____

Appendix 2: Field Protocol for Evaluation of Habitat and Water Quality Parameters Associated with Swamp Waters--Version 1.1

Site name/description:

Date: Time on site: Time off site:

Investigators:

Lat: Long: Datum:

Has rain occurred recently? y/n Comments on recent rain events:

Air Temperature:

Notes on weather conditions:

Subjective classification of system:

Water Level: below normal/ normal / above normal

(assessments conducted during high water levels may be less reliable)

Section 1) Water Physicochemistry (indicate units where appropriate):

pH:_____   DO_____   Cond/Spc. Cond_____

Temp_____   Others (indicate parameters and units):

Water color

(evaluate by observing water in a clean, white, 5-gallon bucket filled with stream water):

Clear, no color_____   Pale Yellow _____   Brown _____

Dark Brown_____   Other_____

Turbidity: Clear_____ Slightly turbid_____ Turbid_____ Highly turbid_____

***Note: future versions of this form may include representative photo graphs of each color and turbidity category.

Section 2) Channel and flow characteristics

2a Study reach length_____ (m)

2b Channel morphology: Indicate the percentage of the study reach occupied by:

Single, defined channel with clearly-defined banks and streambed _____%

Multiple, defined channels (i.e., braided system) _____%

If multiple channels present, indicate approximate number of channels or range, if highly variable

Undefined bed and banks _____% (if undefined banks predominate, metrics 2c, 2d may be difficult to evaluate)

2c Bank stability

Percent of bank area with signs of erosion: Left_____%   Right_____%

2d. Flow velocity:

No perceptible flow_____ % of reach

Sluggish flow_____ % of reach

Moderate, laminar flow_____ % of reach

Rapid, laminar flow_____ % of reach

Rapid, turbulent flow_____ % of reach

Other (explain)_____ % of reach

2e Flow direction and definition

One, clearly-defined flow vector/area where flow predominates_____ % of reach

Several, clearly-defined flow vectors/areas where flow predominates_____ % of reach

No clearly defined areas where flow predominates _____

<u>2f Bottom Substrate types</u>

Type_____ Percent_____

Type_____ Percent_____

Type_____ Percent_____

Type_____ Percent_____

<u>2g Percentage of wetted area comprised of pools</u>:

<u>2h Pool depth/ variability</u>: (max, min, commonness of deep pools, number of pool size classes present- to be revised based on field obs.):

Section 3) Riparian Zone Characteristics

<u>3a Canopy cover </u>(percent cover above wetted area): _____

<u>3b Riparian vegetation type</u> (note major veg types and widths from stream channel)

Left bank (descending)   Right bank (descending)

<u>3c Riparian vegetation protection</u> (note percent cover of natural land cover and widths from channel)

Left bank (descending)   Right bank (descending)

<u>3d Riparian zone topography</u> (approx. elevation above stream bank   if variable, a diagram may be appropriate)

Left bank (descending)   Right bank (descending)

<u>3e Riparian zone wetlands</u> – note presence and lateral width of riparian wetlands from center of wetted area

Left bank (descending)   Right bank (descending)

<u>3f Submerged/emergent aquatic vegetation</u>: absent_____   rare_____   common_____ abundant_____

3g Tress in surface water body (also indicate presence of cypress knees) :

absent_____ rare_____ common_____ abundant_____

**Indicate types of trees/vegetation noted indicated in metrics 3e and 3f

Section 4) Stream/watershed disturbance

4a Channel alteration (if present, note type)

4b Riparian land use (if multiple, note percentages)

4c Other indicators of anthropogenic disturbance:

Section 5) Stream cross section

5a Draw a typical stream cross section and/or indicate: **normal flow depth/width, normal high flow depth and width, and extreme high flow depth/width** (i.e., dimensions of total stream incisement- see example figure). In swamp systems, constructing a detailed cross section may not be feasible. In these cases, include the maximum and average depths and width of the wetted area.
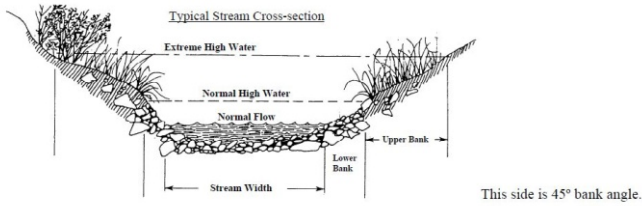

Diagram from North Carolina Department of Environment and Natural Resources standard operating procedures. see:

http://portal.ncdenr.org/c/document_library/get_file?uuid=c2fdf380-aa8a-481e-8388-a6e6596c6a96&groupId=38364

5b Notes Regarding site conditions and other data collected:

5c Remotely-derived data (not collected on-site)- as needed to aid in classification

Watershed land use/land cover (note types and percentages):

Moisture indices at time of investigation: PDSI_____ CMI_____

USGS Gauge Data: Nearby Stream Name_____

Stream flow (percentile)_____

Notes on comparability of gauge data: