VIRGINIA WATER RESOURCES RESEARCH CENTER

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

2016 Report of the Academic Advisory Committee for Virginia Department of Environmental Quality

SPECIAL REPORT

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DEVELOPMENT OF AQUATIC LIFE USE ASSESSMENT PROTOCOLS FOR CLASS VII WATERS IN VIRGINIA

2016 Report of the Academic Advisory Committee for Virginia Department of Environmental Quality

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Introduction

This report details the progress made in fiscal year (FY) 2016 by the Academic Advisory Committee (AAC) toward the development of a biological assessment index for Class VII swamp waters in the Chowan River Basin of Virginia (commonly referred to as blackwaters). The goal of this work is to provide a working Blackwater Condition Index (BCI) that can be used by the Virginia Department of Environmental Quality (DEQ) for monitoring associated with the agency's semi-annual 305(b)/303(d) report to the U.S. Environmental Protection Agency. Specific tasks outlined in the previously approved FY 2016 proposal and actions taken in support of these tasks are as follows:

1) Evaluation of BCI metric redundancy and continued analysis to improve its effectiveness. Task 1 will include both continued evaluation of the current BCI candidate metrics, as well as comparison of these metrics to additional fish and macroinvertebrate metrics.

2) Determination of the degree to which metrics are correlated with, and therefore potentially confounded by, natural environmental variability. Task 2 will involve analysis to determine how best to modify the BCI to reduce the confounding effects of natural environmental variability.

At the request of AAC members, supplementary data have been provided to aid in the interpretation of this report. These data include the following:

- Appendix 1: raw fish data for the 67 sites described herein;
- Appendix 2: names and latitude/longitude coordinates for each site; and
- Appendix 3: Figure 4 from the FY 2014 report showing the degree of agreement among investigators regarding the blackwater habitat scores at 11 study sites.

Methods

Study Sites and Dataset

Site selection for the FY 2016 investigation focused on field evaluations of 34 sites used previously in the FY 2015 investigation as well as 37 additional sites from the Healthy Waters database, which is maintained by Virginia Commonwealth University (VCU) with support from the Virginia Department of Conservation and Recreation (DCR) and DEQ. The FY 2015 sites were evaluated in the field between March 1, 2016 and March 31, 2016 using the Blackwater Habitat Assessment Protocol (BHP) in order to determine if they were appropriately classified as Class VII waters. In addition, dissolved oxygen, pH, temperature, specific conductance, and nitrogen and phosphorus concentrations were evaluated to ensure that the impairment status of each site was correctly classified as *altered*, *reference*, or *intermediate* based on previously selected reference-filter criteria. Based on field investigations, four of the 34 sites included in the 2015 investigation were excluded because they did not exhibit characteristics of Class VII Swamp waters. These sites included: an unnamed tributary of the Blackwater River, Hickaneck Swamp, White Oak Creek, and Wildcat Creek. Each of these sites exhibited a single, well-defined channel, moderate to fast flow, and relatively coarse substrate; these characteristics are typical of Coastal Plain streams that are appropriate for evaluation using the Coastal Plain

Macroinvertebrate Index. In addition, five sites could not be accessed in 2016 due to landowner postings but were included in the study based on past work and best professional judgment.

In addition to the FY 2015 data, 37 new sites were incorporated into the dataset for FY 2016. Sites were selected to include those in southeastern Virginia and northeastern North Carolina, within the Chowan River basin. Evaluation of each site location within a Geographic Information System (GIS) indicated that the watershed topography of each site exhibited undefined or braided-channel morphologies typical of swamp systems. Blackwater Habitat Protocol data had been collected at six of the 37 sites added for FY 2016.

At all sites, fish and (if available) benthic macroinvertebrate data were downloaded from the Healthy Waters database. In addition, a GIS was used to delineate the watersheds of each study site, and land cover was quantified within each watershed as detailed below. Therefore, the dataset used for this report includes fish assemblage and watershed land cover data from a total of 67 Chowan Basin sites. In addition, BHP data are available for 31 of these 67 sites.

Analysis of benthic macroinvertebrate data is ongoing and will not be detailed in this report. The benthic macroinvertebrate data include eight new collections made in 2016 and two previously existing collections. Collection sites were those classified as either reference or altered based on land cover analysis in order to best evaluate the effectiveness of macroinvertebrate metrics for evaluating Class VII sites and to compare this effectiveness to that of the existing fish-based BCI.

Fish Collections

VCU biologists collected fish at each site by single-pass electrofishing using a Smith-Root Model LR-24 direct-current backpack electrofisher (Smith-Root, Inc., Vancouver, WA). The sampling area at each site encompassed 100 meters along the main channel of each system, as well as several sweeps in backwater habitat adjacent to the channel. All fish collections included here were made between 2003 and 2013. Fish community sampling was conducted for, and funded by, the DCR for the Healthy Waters initiative.

Water Physicochemistry

At the 29 sites from the FY 2015 dataset that were visited for this investigation, pH, specific conductance, dissolved oxygen, and temperature were recorded in the field using a YSI multimeter (YSI/Xylem, Inc. Yellow Springs, OH). All sites met the filter criteria for pH and specific conductance (less than 6.4 and less than 200 µS/cm, respectively); therefore, these data did not affect the classification of impairment status and are not discussed further. Analysis of water samples from each site for nitrogen and phosphorus concentrations are ongoing.

Watershed Land Cover

All geospatial analyses were conducted using ARCMAP, Version 10.2 (ESRI, Inc., Redlands, CA). Watersheds were delineated using 3-m and 10-m digital elevation models downloaded from the United States Geological Survey National Elevation Dataset (http://ned.usgs.gov/; 3-m data were used when available). Watershed land cover data were downloaded from the 2011 National Land Cover Database (NLCD; http://www.mrlc.gov/nlcd2011.php) and clipped to the watershed boundaries. The original 16-category classification employed by the NLCD was simplified as follows: the low-, medium-, and high-intensity and open developed classes were

aggregated in the *developed class;* the cultivated crops and pasture/hay classes were aggregated in the *agricultural class;* and the evergreen, deciduous, mixed, and wooded wetland classes were aggregated in the *forest class*. After the reclassification, the percentages of the total land cover area within each watershed comprised of developed, agricultural, and forest areas were calculated.

Classification of study sites as reference (best-available), intermediate, or altered for this analysis was conducted using the NLCD data in conjunction with a review of field notes by the investigators on site conditions. Each site was classified as *reference* if its watershed consisted of >70% forest, *intermediate* if its watershed consisted of 50-70% forest, or *altered* if its watershed consisted of <50% forest. Based on this classification of watershed land cover, 41 sites were classified as altered, 18 sites were intermediate, and 8 were classified as reference (Figure 1).

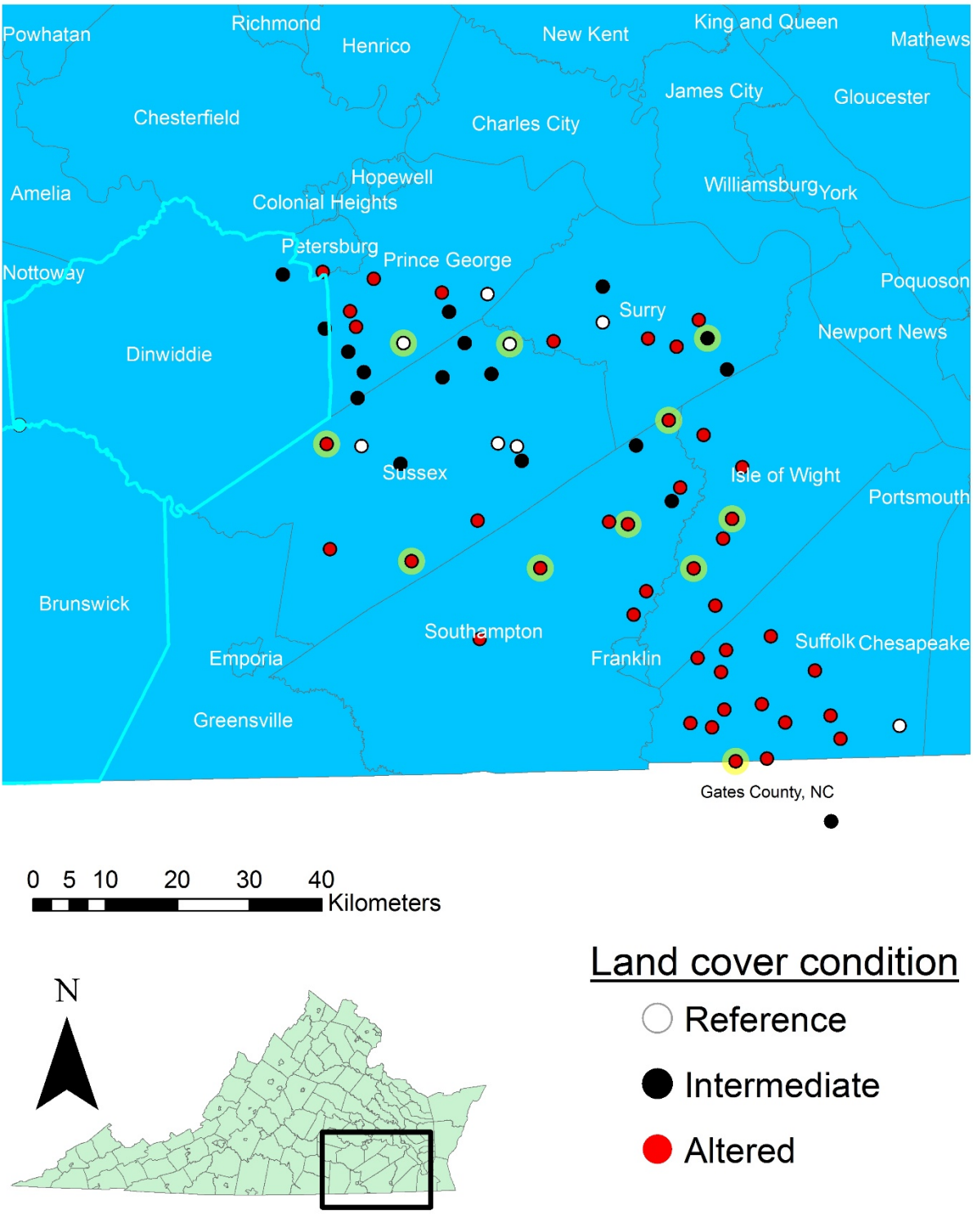

Figure 1: Locations and land cover conditions of the 67 study sites. Highlighted sites are those for which macroinvertebrate data are available.

Blackwater Condition Index Development

A total of 41 candidate metrics were calculated for the fish-based BCI, including the four original metrics proposed in the FY 2013 report of the AAC (Garman *et al*. 2013). Metrics were selected to include fish assemblage abundance, evenness, richness, and diversity, as well as ecological traits of the fish species observed that were associated with feeding, habitat use, spawning, and pollution tolerance. Ecological information was derived from Jenkins and Burkhead (1994) and other published sources. Final decisions regarding the traits of each species were the best professional judgments of VCU fish biologists (Dr. Steve McIninch and Dr. Greg Garman).

After calculation of the raw metric values, metrics exhibiting 0 values at 50 percent or more study sites were eliminated from the selection process. An inflation of zeros can yield misleadingly low or high correlation values with stressors based on limited numerical variability.

Based on simple linear correlations between percent forest land cover and raw metric values, the response of each metric to stress was determined (increase or decrease in metric values with increasing watershed disturbance). Metrics were then scaled as described by Blocksom (2003), such that maximum and minimum values for each metric were set at the 97.5 and 2.5 percentiles, respectively, and the percent comparability of each raw metric value to these endpoints was used as the final metric score. All metrics exhibited scores between 0 and 100, with higher BCI scores indicating greater ecological integrity (see Blocksom 2003 for further details).

The 67 study sites were randomly divided into a model set, used for construction of the BCI, and a test set, used for model validation. The model set consisted of approximately 60 percent of sites in each class (25, 11 and 5 sites from the altered, intermediate, and reference classes, respectively). The final BCI model was constructed by selecting the metric score combination (arithmetic mean of metric scores) that yielded the highest correlation with the percentage of forest cover within each watershed. To effectively achieve this result, a code script was developed using R, version 3.1 (R Core Team 2014), following the algorithm presented by Schoolmaster *et al*. (2013). Briefly, this algorithm allows for the selection of the most effective subset from a set of n metrics without evaluating all possible combinations, which is prohibitively inefficient.

Blackwater Condition Index Evaluation

In addition to the analysis conducted for the model dataset, the correlation between the BCI scores and the percentage of watershed forest land cover for the test dataset was also calculated, providing an independent validation of the index at 16 altered sites, seven intermediate sites, and three reference sites. For both the model and test datasets, all possible pairwise comparisons of index scores were made between altered and reference sites (125 and 48 comparisons for the model and test datasets, respectively). The percentage of comparisons where reference sites scored higher than altered sites was taken as the accuracy percentage for the BCI.

To determine whether the BCI was potentially biased by natural environmental variation, simple linear correlations between the BCI scores with total watershed area and with the six continuous BHP metrics were calculated (i.e., between the BCI and benthic organic matter percentage, percent canopy cover, channel score, flow score, wetland width, and riparian elevation). For the

categorical BHP metrics forest score and submerged and emergent vegetation, Analysis of Variance (ANOVA) was conducted to determine if BCI scores were significantly different among sites that scored differently with respect to the BHP metrics.

Results

The most effective index selected by the algorithm (i.e., that showing the strongest correlation with forest watershed land cover) consisted of eight metrics (Table 1). These eight metrics represent several distinct aspects of community composition that are commonly expected to respond to anthropogenic stress, including the degree of habitat specialization (percentage and number of opportunistic species, blackwater guild species, and vegetation specialist species), feeding (percentage of omnivore species), pollution tolerance (number of tolerant species), and taxonomic evenness (Pielou and Simpson evenness indices). The eight-metric BCI showed strong, and statistically significant linear correlations with the percentage of forest land cover in each watershed for both the model and test datasets (r: 0.73 and 0.40, respectively; $p < 0.05$; Figure 2). The percentage of correct assignments among pairwise comparisons was 96.8 % (121 of 125 comparisons) and 87.5 % (42 of 48 comparisons) for the model and test datasets respectively, and both datasets showed clear distinctions among the distributions of altered and reference sites (Figure 3).

The BCI score was not strongly related to any of the natural environmental variables. Correlations between continuous natural environmental variables (BHP metrics and watershed area) and BCI scores were weak to moderate ($r < 0.01$ to 0.30, maximum correlation between BCI and benthic organic matter), and none were significant at $p \leq 0.05$. The relationships between the BCI scores with forest score and with submerged and emergent vegetation were also not significant (ANOVA, $p > 0.15$).

Table 1: Eight metrics selected for the Blackwater Condition Index.

Metric[*]	Explanation	Response to Stress[†]
Opportunist species (%)	Proportion of opportunist guild species (Garman *et al*. 2013)	Increase
Opportunist species (n)	Number of opportunist guild species (Garman *et al*. 2013)	Increase
Tolerant species (n)	Number of pollution-tolerant species (Garman *et al*. 2013)	Increase
Omnivore species (%)	Number of feeding generalist species	Increase
Pielou evenness	Shannon Diversity Index divided by richness (all species)	Increase
Simpson evenness (natives)	Simpson Diversity Index divided by richness (native species only)	Increase
Blackwater species (n)	Number of blackwater guild species (Garman *et al*. 2013)	Decrease
Vegetation specialists (n)	Number of species that closely associate with vegetation	Increase

[*] % = proportion or percentage of individuals; n = number of species

[†] Increase / Decrease = response of raw metric values to increasing watershed land cover disturbance.

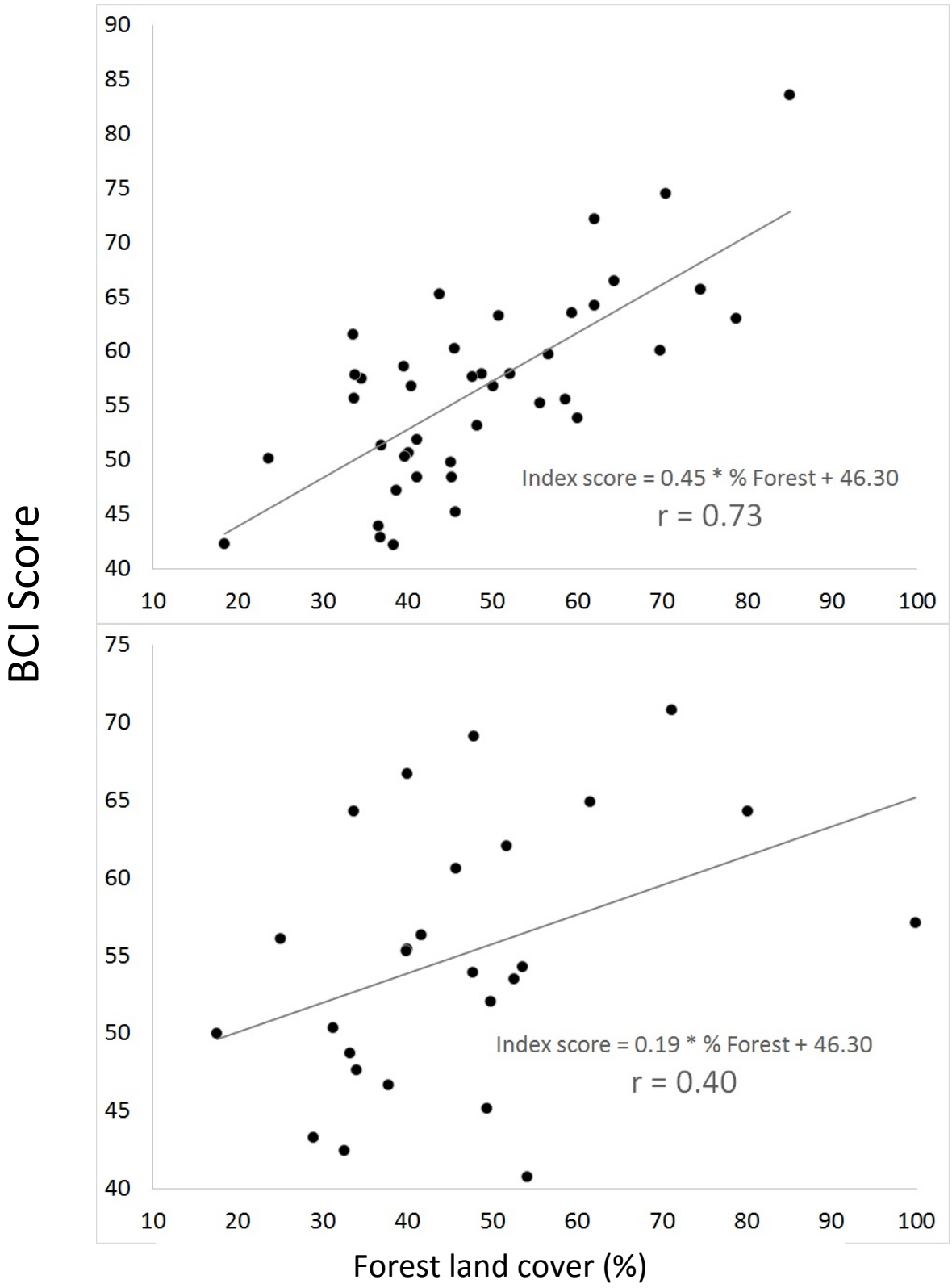

Figure 2: Correlations between BCI scores and percentage of watershed forest land cover for model sites (top panel) and test sites (bottom panel).

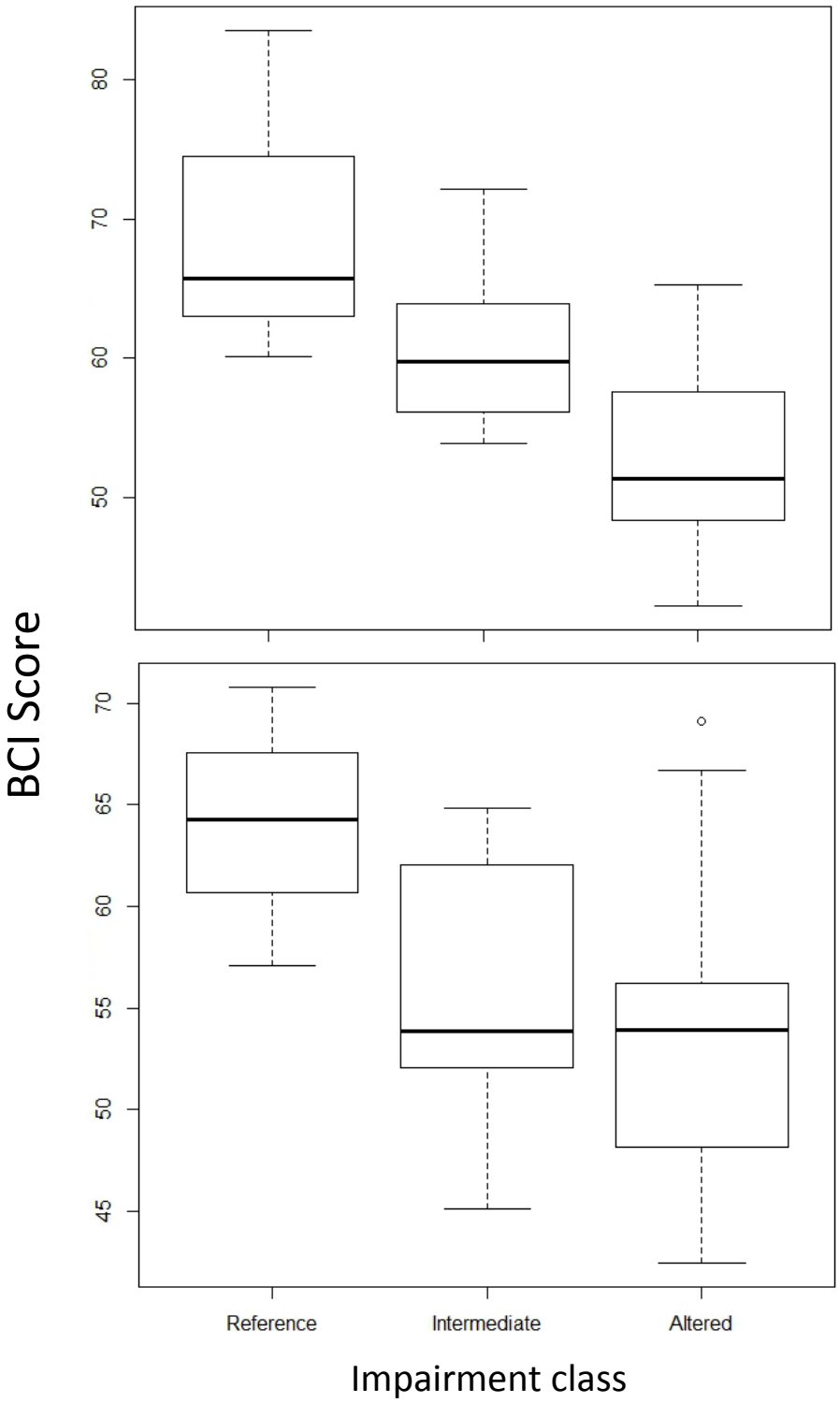

Figure 3: Discrimination among impairment classes for model sites (top panel) and test sites (bottom panel). Boxes indicate interquartile ranges (IQR's); dark bars indicate medians; whiskers indicate most extreme non-outlier values; and points represent outliers (> 1.5 x IQR).

Conclusions and Recommendations

Task 1) Evaluation of BCI metric redundancy and expanded analyses to improve effectiveness.

This analysis produced a fish community-based bioassessment index that effectively discriminates between altered and reference-quality swamp sites in the Chowan River Basin. The index was successfully validated with independent data not used in model creation. Laboratory analysis of macroinvertebrates and nutrient concentrations at the study sites are ongoing. These data will be provided as an addendum to this report in July 2016. Although these data are not expected to substantially modify the recommendations that follow, that addendum will include any additional recommendations based on the nutrient and macroinvertebrate data.

Based on the results observed in this investigation, the current version of the BCI is adequate for providing preliminary assessments of the ecological integrity of Class VII waters in the Chowan River Basin of Virginia. Further validation, with data from additional study sites and sampling dates should improve the effectiveness of the BCI for evaluating anthropogenic impairment in swamp waters. This additional validation should be conducted by DEQ biologists to ensure that they can effectively use the BCI for regulatory assessments.

Task 2) Determination of the degree to which metrics are correlated with, and therefore potentially confounded by, natural environmental variability.

No strong or statistically significant relationships were observed between natural environmental variables and the BCI scores, suggesting that the confounding effects of natural variability are minimal. Future studies should continue to evaluate such natural factors, however, in order to account for their potential effects on index performance. Further index refinement may include an evaluation of the effects of residualizing metric values to remove the effects of potentially confounding natural environmental factors (see Schoolmaster *et al*. 2013 and discussion of whole-set residulization). Based on the limited analysis presented here, however, we expect the BCI to be robust to natural environmental variability, and effective at identifying impairment across the gradient of natural environmental variability that occurs in the Chowan River Basin in southeastern Virginia.

Future study should be focused on the effects of temporal variability on the effectiveness of the BCI. It is essential that the index is robust to such variability in order for it to reliably and consistently indicate the degree of anthropogenic alteration of Class VII Swamp waters in the region. Additional evaluations of the effects of spatial variability on index effectiveness, such as those presented here, should also be conducted.

We have developed a sound analytical framework and a working dataset for development of a biological assessment tool for Class VII swamp waters in the Chowan River Basin. The next phase of this study will focus on a recommended plan for thorough validation of this methodology by DEQ scientists. This plan should allow DEQ to develop a realistic timeline for implementation of the BCI for regulatory assessment of Class VII waters in the Chowan Basin of Virginia.

Based on consensus among AAC members to focus on Chowan River basin sites for FY2016, this index has not been evaluated for Class VII waters in other river basins. Therefore, additional future research by the AAC should focus on adaptation of the BCI for Class VII swamp waters in other river basins.

References

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Appendix 1: Raw fish data for 67 sampling sites (total individuals collected per. sample).

Species	*Amia calva*	*Ameiurus natalis*	*Ameiurus nebulosus*	*Acantharchus pomotis*	*Anguilla rostrata*	*Aphredoderus sayanus*	*Clinostomus funduloides*
Common name	bowfin	yellow bullhead	brown bullhead	mud sunfish	American eel	pirate perch	rosyside dace
K23002	0	0	0	5	0	4	0
K23003	2	0	0	0	0	2	0
K23004	1	0	0	10	0	0	0
K23007	0	0	0	3	0	3	0
K23008	0	0	0	3	1	1	0
K23010	4	0	0	4	0	2	0
K23012	0	3	5	12	1	35	0
K23013	0	0	0	2	0	19	0
K23015	0	0	0	2	0	1	0
K23017	0	0	0	11	2	0	0
K24002	0	0	0	0	0	3	0
K24005	0	1	0	0	3	5	0
K25001	0	2	0	0	1	3	0
K25002	1	0	0	1	0	1	0
K28001	0	0	0	1	0	2	0
K29001	0	0	0	0	0	1	0
K29007	0	1	0	0	0	2	0
K29008	0	0	0	0	0	0	0
K29009	0	0	0	5	0	18	0
K29011	0	0	0	1	0	1	0
K31002	0	0	1	0	0	0	0
K31003	0	0	1	0	0	1	0
K31004	0	0	0	0	1	1	0
K31005	0	0	0	0	2	5	0
K31009	0	0	0	0	0	0	0
K31010	0	1	0	0	0	1	0

Species *Common name*	*Amia calva* bowfin	*Ameiurus natalis* yellow bullhead	*Ameiurus nebulosus* brown bullhead	*Acantharchus pomotis* mud sunfish	*Anguilla rostrata* American eel	*Aphredoderus sayanus* pirate perch	*Clinostomus funduloides* rosyside dace
K31020	0	0	0	0	4	75	0
K32002	0	1	0	2	1	7	0
K32004	0	1	0	0	0	3	0
K32007	0	4	0	1	0	11	0
K32203	0	0	0	3	0	3	0
K32205	0	1	0	1	0	9	0
K32220	0	0	0	1	1	3	0
K33003	0	2	0	0	0	3	0
K33004	0	4	2	0	1	4	0
K33005	0	1	0	2	15	7	0
K33006	2	0	0	1	0	1	2
K33009	0	1	0	0	1	4	0
K33011	0	0	2	1	0	7	0
K34001	0	1	0	0	1	19	0
K34003	0	2	0	1	0	7	0
K34007	0	7	0	1	1	19	0
K34009	0	3	0	7	0	22	0
K34010	0	0	0	0	5	7	0
K35001	0	1	0	1	2	17	0
K35004	0	2	0	1	0	19	0
K35006	1	1	1	2	1	20	1
K36003	2	0	0	3	0	2	2
K36005	1	1	0	0	0	0	1
K36006	0	0	0	0	1	5	0
K36016	0	0	0	0	0	7	0
K36017	0	1	0	2	0	23	0
K36018	0	0	3	0	1	5	0

Species *Common name*	*Amia calva* bowfin	*Ameiurus natalis* yellow bullhead	*Ameiurus nebulosus* brown bullhead	*Acantharchus pomotis* mud sunfish	*Anguilla rostrata* American eel	*Aphredoderus sayanus* pirate perch	*Clinostomus funduloides* rosyside dace
K38001	1	0	0	0	1	2	1
K38002	1	0	0	0	5	0	1
K38004	0	0	1	0	0	1	0
K38006	0	3	0	0	1	13	0
K38007	0	3	0	6	1	9	0
K38008	0	0	1	1	1	4	0
K38009	0	2	0	2	7	5	0
K38010	0	0	0	0	0	0	0
K38011	0	0	0	0	0	3	0
K39006	0	1	0	11	0	8	0
K39008	0	0	0	0	0	5	0
K39009	0	0	0	0	0	0	0
K39010	0	0	0	0	0	1	0
K42021	0	0	0	0	0	1	0

Species *Common name*	*Chologaster cornuta* swampfish	*Centrarchus macropterus* flier	*Cyprinella analostana* satinfin shiner	*Esox americanus* redfin pickerel	*Enneacanthus obesus* bluespotted sunfish	*Enneacanthus chaetodon* blackbanded sunfish	*Etheostoma flabellare* fantail darter
K23002	0	2	0	2	0	0	0
K23003	0	1	0	0	0	0	0
K23004	0	1	0	4	4	0	0
K23007	0	0	0	4	3	0	0
K23008	0	0	0	2	0	0	0
K23010	0	7	0	2	23	0	0
K23012	0	17	0	7	7	0	0
K23013	0	7	0	6	1	0	0

Species *Common name*	*Chologaster cornuta* swampfish	*Centrarchus macropterus* flier	*Cyprinella analostana* satinfin shiner	*Esox americanus* redfin pickerel	*Enneacanthus obesus* bluespotted sunfish	*Enneacanthus chaetodon* blackbanded sunfish	*Etheostoma flabellare* fantail darter
K23015	0	62	0	1	0	3	0
K23017	0	12	0	1	0	0	0
K24002	0	1	0	6	2	0	0
K24005	0	4	0	5	0	0	0
K25001	0	1	0	0	1	0	0
K25002	0	1	0	1	1	0	0
K28001	0	0	0	1	2	0	0
K29001	0	3	0	2	2	0	0
K29007	0	6	0	8	0	0	0
K29008	0	0	0	0	0	0	0
K29009	0	0	0	2	0	0	0
K29011	1	1	0	0	0	0	0
K31002	0	0	0	0	0	0	0
K31003	0	3	0	1	0	0	0
K31004	0	0	0	0	0	0	0
K31005	0	4	0	3	0	0	0
K31009	0	1	0	1	0	0	0
K31010	0	1	0	1	0	0	0
K31020	0	7	0	3	0	0	0
K32002	2	6	0	3	0	0	0
K32004	0	0	0	0	0	0	0
K32007	0	6	0	2	0	0	0
K32203	0	0	0	1	0	0	0
K32205	0	0	0	8	0	0	0
K32220	0	0	1	1	3	0	0
K33003	2	0	0	0	3	0	0
K33004	4	2	0	1	4	0	0

Species *Common name*	*Chologaster cornuta* swampfish	*Centrarchus macropterus* flier	*Cyprinella analostana* satinfin shiner	*Esox americanus* redfin pickerel	*Enneacanthus obesus* bluespotted sunfish	*Enneacanthus chaetodon* blackbanded sunfish	*Etheostoma flabellare* fantail darter
K33005	1	0	2	15	7	0	0
K33006	0	0	1	0	1	0	0
K33009	1	0	0	1	4	0	0
K33011	0	2	1	0	7	0	0
K34001	1	0	0	1	19	0	0
K34003	2	0	1	0	7	0	0
K34007	7	0	1	1	19	0	0
K34009	3	0	7	0	22	0	0
K34010	0	0	0	5	7	0	0
K35001	1	0	1	2	17	0	0
K35004	2	0	1	0	19	0	0
K35006	1	1	2	1	20	0	7
K36003	0	0	3	0	2	0	0
K36005	1	0	0	0	0	0	0
K36006	0	0	0	1	5	0	0
K36016	0	0	0	0	7	0	0
K36017	1	0	2	0	23	0	0
K36018	0	3	0	1	5	0	0
K38001	0	0	0	1	2	0	0
K38002	0	0	0	5	0	0	0
K38004	0	1	0	0	1	0	0
K38006	3	0	0	1	13	0	0
K38007	3	0	6	1	9	0	0
K38008	0	1	1	1	4	0	0
K38009	2	0	2	7	5	0	0
K38010	0	0	0	0	0	0	0
K38011	0	0	0	0	3	0	0

Species *Common name*	*Chologaster cornuta* swampfish	*Centrarchus macropterus* flier	*Cyprinella analostana* satinfin shiner	*Esox americanus* redfin pickerel	*Enneacanthus obesus* bluespotted sunfish	*Enneacanthus chaetodon* blackbanded sunfish	*Etheostoma flabellare* fantail darter
K39006	1	0	11	0	8	0	0
K39008	0	0	0	0	5	0	0
K39009	0	0	0	0	0	0	0
K39010	0	0	0	0	1	0	0
K42021	0	0	0	0	1	0	3

Species Common name	*Etheostoma fusiforme* swamp darter	*Enneacanthus gloriosus* banded sunfish	*Esox niger* chain pickerel	*Erimyzon oblongus* creek chubsucker	*Etheostoma olmstedi* tessellated darter	*Etheostoma serrifer* sawcheek darter	*Etheostoma vitreum* glassy darter
K23002	0	1	0	2	0	0	0
K23003	0	0	0	2	0	0	0
K23004	0	1	0	0	0	0	0
K23007	0	3	4	6	0	0	0
K23008	0	3	1	4	1	0	0
K23010	0	0	0	0	0	0	0
K23012	0	0	0	33	0	0	0
K23013	0	0	2	0	0	0	0
K23015	0	4	0	1	0	0	0
K23017	0	0	0	0	0	0	0
K24002	3	1	0	4	0	0	0
K24005	0	3	0	0	0	0	0
K25001	0	0	0	3	0	0	0
K25002	0	3	1	5	0	0	0
K28001	0	0	0	0	0	0	0
K29001	0	0	0	0	0	0	0
K29007	0	1	0	2	0	0	0

Species Common name	*Etheostoma fusiforme* swamp darter	*Enneacanthus gloriosus* banded sunfish	*Esox niger* chain pickerel	*Erimyzon oblongus* creek chubsucker	*Etheostoma olmstedi* tessellated darter	*Etheostoma serrifer* sawcheek darter	*Etheostoma vitreum* glassy darter
K29008	0	0	0	0	0	0	0
K29009	0	0	0	0	0	0	0
K29011	0	0	0	2	0	0	0
K31002	0	0	0	0	0	0	0
K31003	0	2	0	1	0	0	0
K31004	3	8	1	0	0	0	0
K31005	0	0	0	0	0	0	0
K31009	0	1	0	0	0	0	0
K31010	0	2	1	0	0	0	0
K31020	0	53	0	1	0	0	0
K32002	0	1	0	1	0	0	0
K32004	0	1	0	1	3	0	0
K32007	0	5	0	0	0	0	0
K32203	0	0	0	2	0	0	0
K32205	0	0	0	0	0	0	0
K32220	0	0	4	0	0	0	0
K33003	4	0	4	0	0	0	2
K33004	0	0	4	0	0	0	0
K33005	1	0	4	0	0	0	0
K33006	0	0	0	0	0	0	0
K33009	0	0	0	0	0	0	0
K33011	4	0	0	0	0	0	0
K34001	0	0	0	0	0	0	0
K34003	0	0	3	0	0	0	0
K34007	13	0	1	0	0	0	0
K34009	5	0	1	0	0	0	0
K34010	0	0	5	0	0	0	0

Species Common name	*Etheostoma fusiforme* swamp darter	*Enneacanthus gloriosus* banded sunfish	*Esox niger* chain pickerel	*Erimyzon oblongus* creek chubsucker	*Etheostoma olmstedi* tessellated darter	*Etheostoma serrifer* sawcheek darter	*Etheostoma vitreum* glassy darter
K35001	0	0	3	0	0	0	0
K35004	1	0	1	0	0	0	0
K35006	4	0	7	0	0	0	0
K36003	7	0	3	0	0	0	1
K36005	4	0	7	2	0	0	0
K36006	0	0	2	0	0	0	0
K36016	0	0	3	0	0	0	0
K36017	0	0	9	0	0	0	0
K36018	16	0	2	0	0	0	0
K38001	3	0	14	1	0	0	0
K38002	0	0	5	0	0	0	0
K38004	0	0	3	0	0	0	0
K38006	0	0	9	0	0	0	0
K38007	0	0	19	1	0	0	0
K38008	1	0	9	2	0	0	0
K38009	3	0	8	1	0	0	0
K38010	8	0	3	0	0	0	0
K38011	0	0	0	0	0	0	0
K39006	0	0	44	120	0	0	0
K39008	10	0	12	7	0	0	0
K39009	0	0	0	0	0	0	0
K39010	0	0	1	6	0	0	0
K42021	0	0	1	0	0	0	0

Species Common name	*Gambusia holbrooki* mosquitofish	*Lepomis auritus* redbreast sunfish	*Lepomis cyanellus* green sunfish	*Lepomis gibbosus* pumpkinseed	*Lepomis gulosus* warmouth	*Lepomis macrochirus* bluegill	*Micropterus salmoides* largemouth bass
K23002	0	0	0	0	1	0	0
K23003	1	0	0	0	1	2	0
K23004	43	0	0	0	0	1	0
K23007	9	0	0	0	1	6	0
K23008	13	0	0	0	0	34	0
K23010	30	0	0	0	0	1	0
K23012	0	5	0	0	0	15	0
K23013	1	0	0	0	2	20	0
K23015	0	0	0	0	0	0	0
K23017	0	0	0	1	0	2	0
K24002	0	0	0	0	0	0	0
K24005	3	11	0	0	0	14	0
K25001	0	0	0	0	0	0	0
K25002	0	0	0	0	0	3	0
K28001	11	0	1	0	0	0	0
K29001	0	0	0	4	0	0	0
K29007	0	0	0	1	0	22	2
K29008	0	0	0	0	0	0	0
K29009	6	0	0	0	0	1	0
K29011	0	0	0	0	0	2	0
K31002	0	0	0	1	0	0	0
K31003	0	0	0	0	0	6	0
K31004	1	0	0	0	0	10	2
K31005	0	0	0	0	0	0	0
K31009	0	0	0	0	0	2	0
K31010	0	0	0	1	0	2	0
K31020	49	0	0	10	6	0	0

Species Common name	*Gambusia holbrooki* mosquitofish	*Lepomis auritus* redbreast sunfish	*Lepomis cyanellus* green sunfish	*Lepomis gibbosus* pumpkinseed	*Lepomis gulosus* warmouth	*Lepomis macrochirus* bluegill	*Micropterus salmoides* largemouth bass
K32002	0	0	0	0	0	0	0
K32004	0	0	0	0	0	0	0
K32007	0	0	0	12	3	2	1
K32203	0	0	0	0	0	0	0
K32205	0	0	0	0	0	0	0
K32220	0	0	1	0	0	0	0
K33003	64	1	14	0	0	0	23
K33004	0	0	0	0	0	0	11
K33005	1	1	4	6	2	0	0
K33006	2	4	1	0	0	0	2
K33009	0	0	19	6	0	0	0
K33011	7	0	2	0	0	0	4
K34001	3	1	1	0	1	0	0
K34003	0	0	0	0	0	0	0
K34007	1	0	8	0	0	0	0
K34009	16	0	4	0	0	0	2
K34010	2	0	9	0	0	0	0
K35001	11	0	4	0	0	0	0
K35004	0	0	0	0	0	0	0
K35006	48	0	4	0	0	0	0
K36003	100	3	16	0	0	0	105
K36005	0	0	1	0	0	0	0
K36006	6	0	1	4	0	0	2
K36016	19	0	5	0	0	0	2
K36017	5	0	0	0	4	0	3
K36018	0	0	6	0	1	0	18
K38001	0	0	1	0	0	0	2

Species Common name	*Gambusia holbrooki* mosquitofish	*Lepomis auritus* redbreast sunfish	*Lepomis cyanellus* green sunfish	*Lepomis gibbosus* pumpkinseed	*Lepomis gulosus* warmouth	*Lepomis macrochirus* bluegill	*Micropterus salmoides* largemouth bass
K38002	0	0	2	0	0	0	0
K38004	0	0	0	0	0	0	1
K38006	4	0	2	0	0	0	0
K38007	24	0	3	0	0	0	7
K38008	15	0	11	0	0	0	38
K38009	6	0	0	0	0	0	29
K38010	1	0	0	0	0	0	23
K38011	0	0	0	0	0	0	1
K39006	0	0	0	0	0	0	1
K39008	28	0	0	0	0	0	7
K39009	0	0	0	0	0	0	0
K39010	2	0	0	0	0	0	0
K42021	1	0	0	0	0	0	11

Species Common name	*Notropis chalybaeus* ironcolor shiner	*Notemigonus crysoleucas* golden shiner	*Noturus gyrinus* tadpole madtom	*Noturus insignis* margined madtom	*Nocomis leptocephalus* bluehead chub	*Notropis procne* swallowtail shiner	*Pomoxis nigromaculatus* black crappie
K23002	0	0	0	0	0	0	0
K23003	0	0	0	0	0	0	0
K23004	0	0	0	0	0	0	0
K23007	0	1	0	0	0	0	0
K23008	0	1	0	0	0	0	0
K23010	0	7	0	0	0	0	0
K23012	0	4	0	0	0	0	0
K23013	0	0	0	0	0	0	1
K23015	0	0	0	0	0	0	0

Species Common name	*Notropis chalybaeus* ironcolor shiner	*Notemigonus crysoleucas* golden shiner	*Noturus gyrinus* tadpole madtom	*Noturus insignis* margined madtom	*Nocomis leptocephalus* bluehead chub	*Notropis procne* swallowtail shiner	*Pomoxis nigromaculatus* black crappie
K23017	0	0	0	0	0	0	0
K24002	0	0	0	0	0	0	0
K24005	0	0	0	0	0	0	0
K25001	0	0	0	0	0	0	0
K25002	0	3	0	0	0	0	0
K28001	0	0	0	0	0	0	0
K29001	0	0	0	0	0	0	0
K29007	0	18	0	0	0	0	0
K29008	0	0	0	0	0	0	0
K29009	0	0	0	0	0	0	0
K29011	0	0	0	0	0	0	0
K31002	0	0	0	0	0	0	0
K31003	0	1	0	0	0	0	0
K31004	0	0	0	0	0	0	0
K31005	0	0	1	0	0	0	0
K31009	0	10	0	0	0	0	0
K31010	0	0	0	0	0	0	0
K31020	0	8	0	0	0	0	0
K32002	0	0	0	0	0	0	0
K32004	2	0	1	0	0	0	0
K32007	0	0	1	0	0	0	0
K32203	0	0	0	0	0	0	0
K32205	0	0	0	0	0	0	0
K32220	0	0	10	0	0	0	0
K33003	0	0	4	2	0	0	0
K33004	0	0	5	0	9	0	0
K33005	0	0	0	0	0	0	0

Species Common name	*Notropis chalybaeus* ironcolor shiner	*Notemigonus crysoleucas* golden shiner	*Noturus gyrinus* tadpole madtom	*Noturus insignis* margined madtom	*Nocomis leptocephalus* bluehead chub	*Notropis procne* swallowtail shiner	*Pomoxis nigromaculatus* black crappie
K33006	0	0	0	0	0	1	0
K33009	0	0	1	0	0	0	0
K33011	0	0	1	0	3	0	0
K34001	0	0	0	0	0	0	0
K34003	0	0	0	0	0	0	0
K34007	0	0	0	1	0	0	0
K34009	0	0	2	0	0	0	0
K34010	0	0	8	1	1	0	0
K35001	0	0	0	1	1	0	0
K35004	0	0	0	4	0	0	0
K35006	0	0	0	2	0	0	0
K36003	0	0	1	2	1	0	0
K36005	0	0	0	0	0	0	0
K36006	0	0	0	1	1	0	0
K36016	0	0	0	0	10	0	0
K36017	0	0	0	0	0	0	0
K36018	0	0	0	1	2	0	0
K38001	0	0	0	0	0	0	0
K38002	0	0	0	0	0	0	0
K38004	0	0	0	0	1	0	0
K38006	0	0	0	0	0	0	0
K38007	0	0	0	1	0	0	0
K38008	0	0	0	0	0	0	0
K38009	0	0	0	0	0	0	0
K38010	0	0	0	0	0	0	0
K38011	0	0	0	0	0	0	0
K39006	0	0	5	0	23	5	0

Species Common name	*Notropis chalybaeus* ironcolor shiner	*Notemigonus crysoleucas* golden shiner	*Noturus gyrinus* tadpole madtom	*Noturus insignis* margined madtom	*Nocomis leptocephalus* bluehead chub	*Notropis procne* swallowtail shiner	*Pomoxis nigromaculatus* black crappie
K39008	0	0	1	3	12	0	0
K39009	0	0	0	0	1	0	0
K39010	0	0	1	0	0	0	0
K42021	0	0	0	0	0	0	0

Species Common name	*Semotilus atromaculatus* creek chubsucker	*Umbra pygmaea* mudminnow
K23002	0	0
K23003	0	0
K23004	0	35
K23007	0	1
K23008	0	0
K23010	0	256
K23012	0	3
K23013	0	4
K23015	0	0
K23017	0	3
K24002	0	0
K24005	0	2
K25001	0	0
K25002	0	0
K28001	0	0
K29001	0	2
K29007	0	1
K29008	0	18
K29009	0	49
K29011	0	0

Species	*Semotilus atromaculatus*	*Umbra pygmaea*
Common name	creek chubsucker	mudminnow
K31002	0	12
K31003	0	0
K31004	0	0
K31005	0	0
K31009	0	0
K31010	0	0
K31020	0	18
K32002	0	0
K32004	0	0
K32007	0	0
K32203	0	8
K32205	0	0
K32220	3	0
K33003	3	0
K33004	0	0
K33005	8	2
K33006	3	0
K33009	4	0
K33011	3	0
K34001	0	0
K34003	0	0
K34007	5	0
K34009	0	0
K34010	0	0
K35001	1	1
K35004	0	0
K35006	10	0
K36003	9	0
K36005	0	0

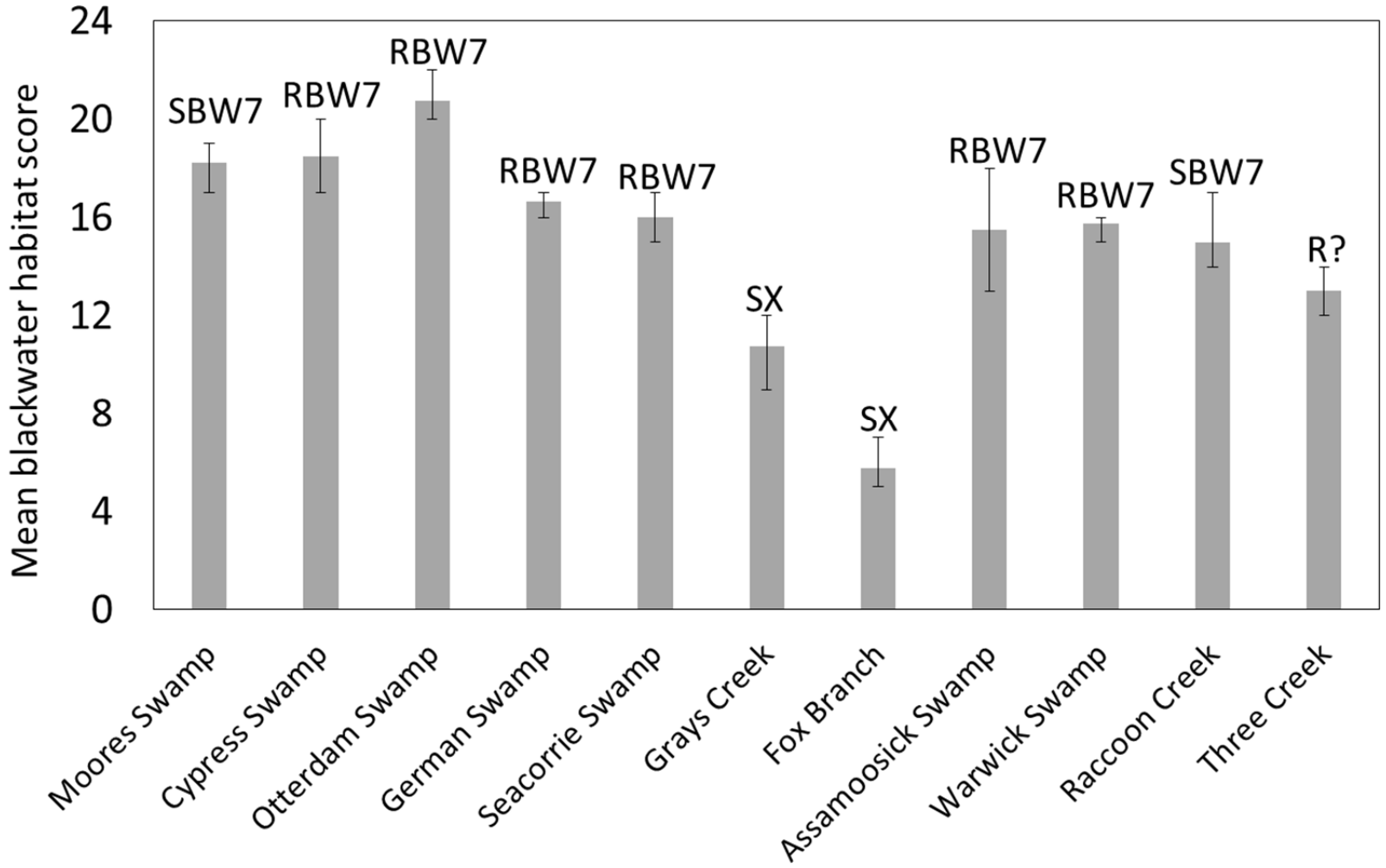
Species	*Semotilus atromaculatus*	*Umbra pygmaea*
Common name	creek chubsucker	mudminnow
K36006	1	0
K36016	3	0
K36017	0	0
K36018	48	0
K38001	0	0
K38002	1	0
K38004	0	0
K38006	4	0
K38007	2	0
K38008	3	0
K38009	4	0
K38010	3	0
K38011	0	0
K39006	1	0
K39008	44	0
K39009	0	0
K39010	0	0
K42021	1	0

Appendix 2: Site names and coordinates

Site Code	Name	Longitude	Latitude
K23002	Arthur Swamp	-77.46598655480	37.17241919190
K23003	Jones Hole Swamp	-77.36664226830	37.07420909980
K23004	Galley Swamp	-77.40333944300	36.95964340030
K23007	Jones Hole Swamp 2	-77.40216583290	37.10381602480
K23008	Jones Hole Swamp 3	-77.34291378120	37.04847176240
K23010	Mush Pond Swamp	-77.34941000000	36.95611999960
K23012	Gosee Swamp	-77.35377500000	37.01642778030
K23013	Joseph Swamp	-77.22075833000	37.03943611040
K23015	UNT Joseph Swamp	-77.28001389000	37.08343333000
K23017	UNT Nottoway River	-77.88113000000	36.99104000010
K24002	Anderson Branch	-77.28943611000	36.93295556030
K24005	Parker Run	-77.17152222000	36.85971388990
K25001	UNT Spring Creek	-77.40216378480	36.82841201590
K25002	Raccoon Creek	-77.27569167000	36.81113333030
K28001	Buckhorn Swamp	-77.17316398790	36.71213247420
K29001	Parker Branch	-77.10733000000	36.95106000020
K29007	UNT Mill Run	-77.07598333000	36.79862221980
K29008	UNT Assamoosick Swamp	-77.13664521310	36.95543618490
K29009	Black Swamp	-77.14421069770	37.04217837580
K29011	Seacorie Swamp	-77.10041552940	36.93291946820
K31002	Seconds Swamp	-77.40324396300	37.17443785680
K31003	UNT Warwick Swamp	-77.36236731640	37.12484437850
K31004	Seconds Swamp	-77.32425632920	37.16450210810
K31005	Warwick Swamp	-77.18466976910	37.08173346720
K31009	Blackwater Swamp	-77.21813353270	37.14520822520
K31010	Warwick Swamp 2	-77.35351994790	37.10499668130
K31020	UNT Blackwater Swamp	-77.20787585880	37.12121292510
K32002	UNT Johnchecohunk Swamp	-76.96894359040	37.10286554500
K32004	Cypress Swamp	-77.04636000230	37.08088999780
K32007	UNT Blackwater River	-77.11446969240	37.07910131270
K32203	Hazel Swamp	-76.89889000550	37.08096999600
K32205	Cypress Swamp 2	-76.96727999510	37.14755999990
K32220	Otterdam Swamp	-77.14730433000	37.14208060030
K33003	Tucker Swamp	-76.86903851910	36.87752302360
K33004	Vellines Swamp	-76.75793222650	36.91743056910
K33005	Terrapin Swamp	-76.87024989880	36.97866485640
K33006	Antioch Swamp	-76.79123167110	36.82889556510
K33009	Pig Swamp	-76.85553452240	36.89407681050
K33011	Burnt Mills Swamp	-76.77634004120	36.85328814330

Site Code	Name	Longitude	Latitude
K34001	Passenger Swamp	-76.77702692490	37.03963181160
K34003	UNT Rattlesnake Swamp	-76.81661753510	36.95872081530
K34007	Golden Hill Swamp	-76.81914683510	37.10266277600
K34009	UNT Mill Swamp	-76.80639866080	37.07934346390
K34010	Moores Swamp	-76.85493147810	37.06991196670
K35001	Round Hill Swamp	-76.93823230570	36.85009729790
K35004	UNT Seacock Swamp	-76.92204663890	36.94810255190
K35006	Round Hill Swamp 2	-76.96762541760	36.85389491660
K36003	UNT Kingsdale Swamp	-76.83598388390	36.68100878670
K36005	Corrowaugh Swamp	-76.80635530080	36.74583491020
K36006	Cypress Swamp 2	-76.93317949230	36.73721510940
K36016	Ducks Swamp	-76.83819078250	36.79296780830
K36017	Cattail Swamp	-76.91257138050	36.76609367990
K36018	Kingsale Swamp	-76.79138031830	36.68990588470
K38001	UNT Summerton Creek	-76.73327153140	36.55314928610
K38002	Spivey Swamp	-76.70321401900	36.59715330110
K38004	Chapel Swamp	-76.80016379990	36.66278213100
K38006	Chapel Swamp 2	-76.81657353830	36.59393389280
K38007	UNT Jones Swamp	-76.73862438290	36.62105359000
K38008	UNT Chapel Swamp	-76.79677227280	36.61586460020
K38009	Mill Swamp	-76.78156396950	36.55099269020
K38010	March Swamp	-76.85009379480	36.60017420680
K38011	Quake Swamp	-76.72131460210	36.70536421730
K39006	Adams Swamp	-76.61858592710	36.57480827730
K39008	Dragon Swamp	-76.63262093460	36.60410726760
K39009	Moss Swamp	-76.52643453470	36.58894371140
K39010	Cypress Swamp 3	-76.65458373160	36.66084388120
K42021	Duke Swamp	-76.63665003230	36.47229432360

Appendix 3: Figure 4 from FY 2014 report.



Bars show Mean Blackwater Habitat Protocol scores at 11 test sites. Error bars indicate range of scores among investigators. Designation classes: SBW7 = Stressed, Class VII, Blackwater; RBW7 = Reference, Class VII, Blackwater; SX = Stressed, Not Class VII; R? indicates that investigators disagreed as to the correct typology classification.