VIRGINIA WATER RESOURCES RESEARCH CENTER

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

2015 Report of the Academic Advisory Committee for Virginia Department of Environmental Quality

Virginia WATER RESOURCES Research Center

SPECIAL REPORT

VirginiaTech Invent the Future

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Virginia Water Resources Research Center (MC 0444)
210 Cheatham Hall, Virginia Tech
310 West Campus Drive
Blacksburg, VA 24061
(540) 231-5624
FAX: (540) 231-6673
E-mail: water@vt.edu

Stephen Schoenholtz, Director

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

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by:

Andrew L. Garey
Member, Academic Advisory Committee
Deputy Director, VCU Rice Rivers Center
Virginia Commonwealth University

Greg C. Garman
Member, Academic Advisory Committee
Associate Professor, Department of Biology
Director, Center for Environmental Studies
Virginia Commonwealth University

Leonard A. Smock
Member, Academic Advisory Committee
Professor, Department of Biology
Director, VCU Rice Rivers Center
Virginia Commonwealth University

Edited by:
Jane L. Walker

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Virginia Water Resources Research Center
210 Cheatham Hall, Virginia Tech
310 West Campus Drive
Blacksburg, VA 24061

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Members of the 2015 Academic Advisory Committee to the Virginia Department of Environmental Quality

Stephen H. Schoenholtz, Chair
Virginia Water Resources Research Center / Department of Forest Resources and Environmental Conservation
Virginia Tech

E. Fred Benfield
Department of Biology
Virginia Tech

Paul Bukaveckas
Department of Biology / Center for Environmental Studies / Rice Center for Environmental Life Sciences
Virginia Commonwealth University

Andrew L. Garey
Department of Biology / Rice Center for Environmental Life Sciences
Virginia Commonwealth University

Gregory C. Garman
Department of Biology / Center for Environmental Studies
Virginia Commonwealth University

Carl Hershner
Department of Biology / Center for Coastal Resources Management
Virginia Institute of Marine Science
College of William and Mary

Wu-Seng Lung
Department of Civil and Environmental Engineering
University of Virginia

Kevin J. McGuire
Virginia Water Resources Research Center / Department of Forest Resources and Environmental Conservation
Virginia Tech

Daniel McLaughlin
Virginia Water Resources Research Center / Department of Forest Resources and Environmental Conservation
Virginia Tech

Leonard A. Shabman
Resources for the Future

Eric P. Smith
Department of Statistics
Virginia Tech

Leonard A. Smock
Department of Biology / Rice Center for Environmental Life Sciences
Virginia Commonwealth University

Kurt Stephenson
Department of Agricultural and Applied Economics
Virginia Tech

Jane L. Walker
Virginia Water Resources Research Center
Virginia Tech

Gene Yagow
Department of Biological Systems Engineering
Virginia Tech

Carl E. Zipper
Department of Crop and Soil Environmental Sciences
Virginia Tech

Introduction

This report details the progress made in fiscal year (FY) 2015 by the Academic Advisory Committee (AAC) toward the development of a biological assessment index for Class VII swamp waters in Virginia. The overarching 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 2015 proposal and actions taken in support of these tasks are as follows:

1) Development of the BCI database. All data detailed in this report – including biological, physicochemical water quality, habitat, and land cover data at an additional 150 candidate study sites – have been organized within a relational database system so the data can be rapidly queried and accessed.

2) Data acquisition. The focus of this task was primarily on reviewing existing fish and geospatial data that were collected for Virginia's Healthy Waters initiative, a multi-organizational effort developed and managed by the Virginia Department of Conservation and Recreation (DCR) and the Center for Environmental Studies at Virginia Commonwealth University (VCU) in coordination with the DEQ, the Virginia Department of Game and Inland Fisheries (DGIF), and the Virginia Coastal Zone Management Program. A portion of this dataset associated with probable Class VII in the Chowan River Basin of Virginia was used in the analyses conducted for this phase of the project. The candidate study sites in the existing database are currently being analyzed, and additional field investigations and geospatial analyses are ongoing for the next phase of the project.

3) Preliminary BCI development. The primary analytical focus of the FY 2015 investigation was on the development of the fish-based BCI. Macroinvertebrate-based index development, comparison of the macroinvertebrate index to the fish BCI, and further development of the Blackwater Habitat Protocol (BHP) will be included in the next phase of the project.

Methods

Study Sites

The criteria for selection of study sites were changed from those discussed in the FY 2014 report of the AAC (Garey *et al*. 2014) to make the most efficient use of newly available data and to best address the objectives of the current phase of the project. Data from 34 study sites were used in the FY 2015 analyses (Fig.1, Table 1). Thirty-two sites included in Virginia's Healthy Waters initiative and identified by field personnel as probable Class VII blackwater systems were used. Two sites from the BCI database were identified as having high percentages of forest land cover in their watersheds so were also included in the FY 2015 analyses. Fish and land cover data were obtained for all of the sites. In addition, water chemistry and BHP data have also been collected at ten of the 34 sites (Table 1). Collection of these data at the remaining 24 sites, as well as other Coastal Plain sites in the region, is ongoing. These data will be included in future phases of this project.

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 m 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 Virginia Department of Conservation and Recreation for the Healthy Waters initiative.

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 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 investigators' field notes on site conditions. Upon completion of the BCI, data from study sites will include all or most of the reference-filter parameters listed in the FY 2014 report of the AAC (Garey *et al*. 2014; Table 1). For this report, sites were preliminarily classified as *reference* if their surrounding watersheds consisted of >70% forest, as *intermediate* if their watersheds consisted of 50-70% forest, and as *altered* if their watersheds consisted of <50% forest. Although additional field investigations are needed for confirmation at all sites, none of the water chemistry surveys conducted for ten of the sites produced values outside the swamp reference-filter criteria proposed in the FY 2014 report. BHP scores for these ten sites (range of scores: 12-21) exceeded those typically observed at non-Class-VII sites in previous years (mean non-Class VII BHP scores: < 12), indicating that these sites would be classified as blackwater swamps according to the current BHP.

Multivariate Analysis

A non-metric multidimensional scaling (NMS) ordination was conducted on a Sorenson distance matrix created using the relative abundances of all fish species observed. The percentages of developed, agricultural, and forest land cover, as well as the relative abundances of each fish species were regressed against the ordination axes in order to 1) determine the strength of relationships between fish assemblage structure and land cover gradients, and 2) determine which species most strongly influenced variations in fish assemblage structure among sites.

BCI Development

A total of 28 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 (Table 2). Ecological information was derived from Jenkins and Burkhead (1993) when available, although final decisions regarding the traits of each species, as well as the expected responses of each metric to impairment (i.e., increase or decrease) were the best professional judgments of VCU fish biologists (Dr. Steve McIninch and Dr. Greg Garman).

After calculation of the raw metric values, percentile ranks of each metric value were calculated and used as final metric scores such that all metrics could be compared and aggregated on a common scale. Ranks for metrics expected to increase with stress were reversed, such that all metric scores increased with increasing biotic integrity. Metric scores ranged from 2.9%, corresponding to the lowest of the 34 metric values, to 100%, corresponding to the highest value. All possible pairwise comparisons of the scores for each metric (n=84 per metric) were made between *a priori*-defined altered and reference sites. The percentage of comparisons where reference sites scored higher than altered sites was considered as a measure of the accuracy of each metric (referred to hereafter as accuracy percentage). The average difference in percentile ranks between stressed and reference sites was also calculated; however, these values were strongly correlated with the accuracy percentages (r^2=0.82), and therefore only the accuracy percentages are discussed further.

The best-performing metrics were selected and aggregated by taking the mean of the percentile ranks to produce an overall preliminary BCI score, ranging on a theoretical scale from 2.9 – 100 and decreasing with predicted watershed alteration. The accuracy percentage of the overall BCI score was calculated in the same manner as for the individual metrics. The correlation of the BCI scores with the percentages of forest land cover was determined for all 34 study sites.

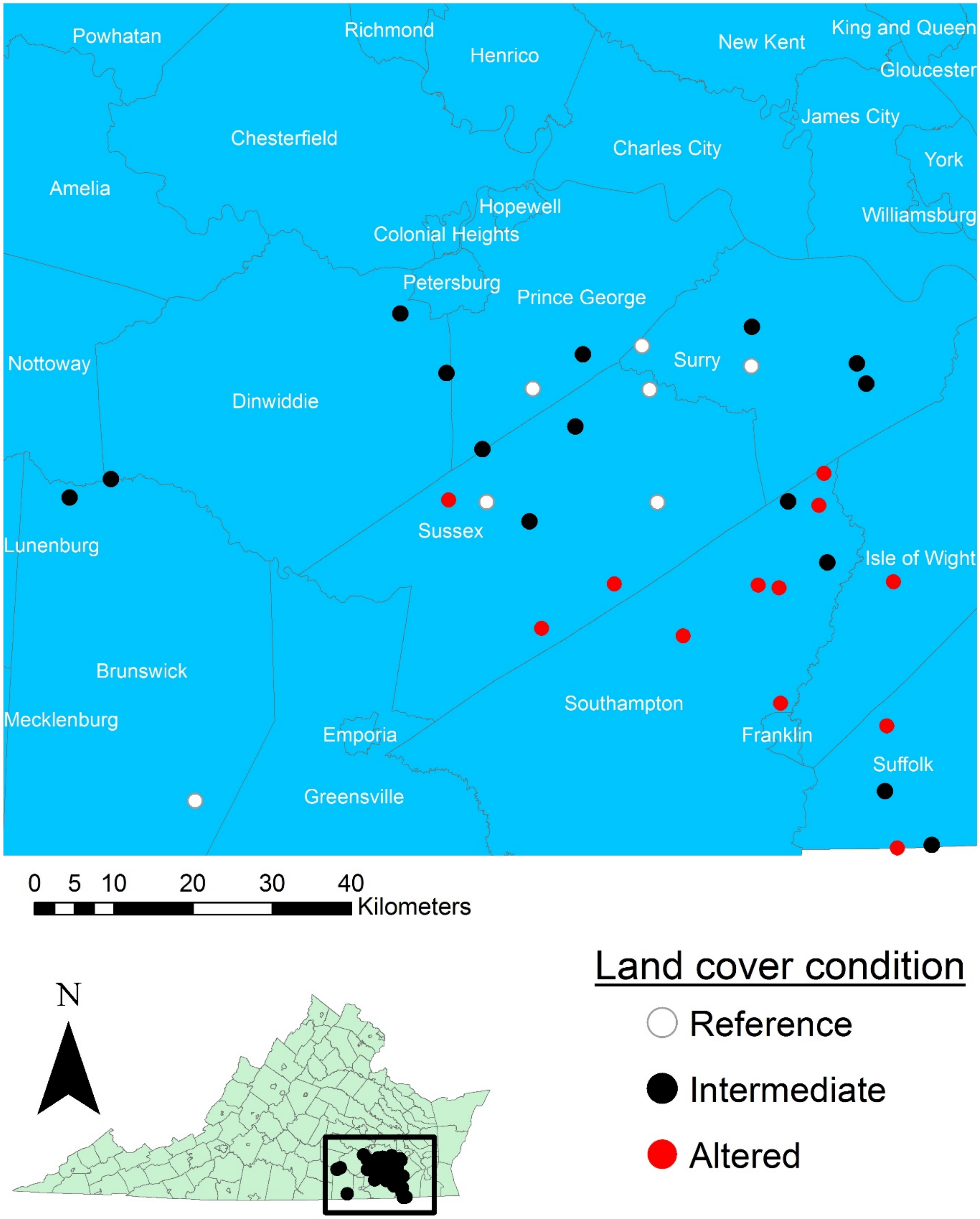

Figure 1: Locations and land cover condition ratings for the 34 study sites.

Table 1: Thirty-four study sites included in development of the Blackwater Condition Index.

Site Name	Fish Sampling Date	Lat.	Long.	Developed LC (%)	Agricultural LC (%)	Forest LC (%)	Blackwater habitat score	pH	Dissolved oxygen (mg/L)	Specific Conductance (µS cm^{-1})	Temperature (°C)
Wildcat Creek	7/19/2013	36.97	-77.94	0	11	69					
White Oak Creek*	10/3/2013	36.62	-77.77	1	11	71					
UNT Seacock Swamp	6/10/2005	36.95	-76.92	0	21	66					
UNT Nottoway River	7/30/2012	36.99	-77.88	0	11	66					
UNT Mill Run+	5/25/2012	36.80	-77.08	0	39	43					
UNT Joseph Swamp*	8/7/2012	37.08	-77.28	0	4	87	18	4.95	3.55	27.00	29.08
UNT Johnchecohunk Swamp*	3/25/2005	37.10	-76.97	0	1	73	12	4.25	8.93	31.00	14.70
UNT Blackwater River+	6/5/2007	36.72	-76.94	1	22	38					
Tucker Swamp	5/17/2005	36.88	-76.87	0	13	59					
Terrapin Swamp+	5/17/2005	36.98	-76.87	0	51	34	17	6.24	9.50	126.00	13.95
Summerton Creek Tributary	5/31/2005	36.55	-76.73	0	17	51					
Round Hill Swamp+	6/10/2005	36.85	-76.97	0	30	45					
Round Hill Swamp+	6/10/2005	36.85	-76.94	0	26	44	14	5.66	5.68	100.00	21.82
Raccoon Creek+	3/29/2012	36.81	-77.28	0	16	42	18	6.32	1.31	60.00	24.82
Parker Run+	5/25/2012	36.86	-77.17	0	39	30					
Parker Branch*	3/22/2012	36.95	-77.11	0	11	81	16	5.59	7.98	37.00	15.70
Otterdam*	10/15/2003	37.13	-77.12	0	0	71	21	3.76	3.81	39.77	10.84
Mush Pond Swamp*	7/30/2012	36.96	-77.35	0	8	77					
Mill Swamp+	5/31/2005	36.55	-76.78	1	11	44					

Site Name	Fish Sampling Date	Lat.	Long.	Developed LC (%)	Agricultural LC (%)	Forest LC (%)	Blackwater habitat score	pH	Dissolved oxygen (mg/L)	Specific Conductance (µS cm^{-1})	Temperature (°C)
UNT Mill Swamp	5/13/2005	37.08	-76.81	0	22	59	19	6.12	6.75	56.00	15.34
Kingsale Swamp+	9/29/2006	36.69	-76.79	5	39	40					
Joseph Swamp	8/1/2012	37.04	-77.22	0	10	56					
Jones Hole Swamp	8/22/2007	37.10	-77.40	0	19	57					
UNT Blackwater R.	8/11/2008	37.12	-77.21	1	13	63					
Hickaneck Swamp+	5/17/2005	36.94	-76.88	0	25	49					
Gosee Swamp	8/1/2012	37.02	-77.35	2	14	56					
Golden Hill Swamp	3/31/2005	37.10	-76.82	0	25	52					
Galley Swamp+	5/4/2005	36.96	-77.40	2	56	35					
Cypress	10/10/2003	37.15	-76.97	0	5	59	19	4.32	7.62	30.93	12.45
UNT Chapel Swamp	5/27/2005	36.62	-76.80	0	34	50					
Burnt Mills Swamp+	5/18/2005	36.85	-76.78	1	36	33	18	6.69	11.60	122.00	15.75
Blackwater River Tributary*	12/8/2004	37.08	-77.11	2	7	83					
Arthur Swamp	5/5/2005	37.17	-77.47	9	23	55					
Anderson Branch	3/29/2012	36.93	-77.29	0	5	63					
		Mean	(range):	1 (0-9)	19 (0-56)	56 (30-87)					

* denotes sites designated as reference based on land cover conditions; + denotes sites designated as altered. All remaining sites were designated as intermediate. Lat. = latitude; Long. = longitude; LC = land cover; UNT = unnamed tributary.

Table 2: Candidate metrics calculated for Blackwater Condition Index development.

Metric	Percent correct	Explanation	Expected response to stress
Opportunist species (%)*	86	Opportunist guild species (Garman *et al*. 2013)	Increase
Opportunist species (n)*	86	Opportunist guild species (Garman *et al*. 2013)	Increase
Blackwater species (%)*	80	Blackwater guild species (Garman *et al*. 2013)	Decrease
Tolerant species (n)*	79	Pollution-tolerant species (Garman *et al*. 2013)	Increase
Shannon diversity*	75	Shannon Diversity Index	Increase
Tolerant species (%)*	75	Pollution-tolerant species (Garman *et al*. 2013)	Increase
Structure-oriented species (%)*	69	Species that closely associate with structure	Decrease
Simpson diversity*	69	Simpson Diversity Index	Increase
Vegetative specialist species (%)*	68	Species that closely associate with vegetation	Decrease
CPUE*	68	Catch per unit effort (individuals min^{-1})	Increase
Introduced species (%)	65	Species not native to Chowan Basin	Increase
Native piscivore species (%)	65	Native piscivores	Decrease
Nester species (%)	64	Species that construct nests or spawn on nest of other species	Decrease
Omnivore species (%)	64	Feeding generalist species	Increase
Piscivore species (%)	64	All piscivore species	Decrease

Metric	Percent correct	Explanation	Expected response to stress
Vegetation spawning species (%)	58	Species that spawn on vegetation	Decrease
Introduced species (n)	45	Species not native to Chowan Basin	Increase
Pielou evenness	45	Shannon Diversity Index divided by richness	Increase
Simpson evenness	43	Simpson Diversity Index divided by richness	Increase
Blackwater species (n)	39	Blackwater guild species (Garman *et al*. 2013)	Decrease
Sensitive species (%)	39	Pollution-sensitive species	Decrease
Sensitive species (n)	32	Pollution-sensitive species	Decrease
Structure-oriented species (n)	30	Species that closely associate with structure	Decrease
Vegetation spawning species (n)	27	Species that spawn on vegetation	Decrease
Piscivore species (n)	26	All piscivore species	Decrease
Vegetation specialists (n)	20	Species that closely associate with vegetation	Decrease
Nester species (n)	19	Species that construct nests or spawn on nest of other species	Decrease
Native species (%)	15	Species native to Chowan Basin	Decrease

%: indicates metrics calculated as the percentage of individuals that fit the criterion; n: indicates metrics calculated as the number of species that fit the criterion; *: indicates metrics included in the preliminary Blackwater Condition Index. CPUE = catch per unit effort.

Results

Fish Collections

A total of 51 species were collected including those identified as blackwater guild members, such as *Chologaster cornuta* (swampfish), *Centrarchus macropterus* (flier), and *Enneacanthus chaetodon* (Black-banded sunfish). They also included opportunist guild members, such as *Ameiurus nebulosus* (brown bullhead), *Lepomis macrochirus* (Bluegill), and *Micropterus salmoides* (largemouth bass) (Garman *et al*. 2013). Most species (46 of 51) have been documented as native to the Chowan River Basin (Jenkins and Burkhead 1994), although four introduced species were also prevalent at several study sites (including *L. macrochirus* and *M. salmoides*). Species collected exhibited a wide range of variation with respect to feeding, habitat use, spawning, and pollution tolerance (Table 3).

Watershed Land Cover

Most watersheds were dominated by forest, although land cover varied widely (range of forest cover: 30-87%, mean: 56%, Table 1). Agricultural land represented the second most common cover type (mean: 19%, range: 0-56%). In contrast, developed land was uncommon in the study region, reflecting the prevalence of farms and pastures, relative to urbanized and residential areas (mean developed cover: 1%, range: 0-9%). Based on the land cover classification and analysis, seven sites were assigned to the reference class (forest>70%); 12 sites were identified as altered (forest <50%); and the remaining 15 sites were designated as intermediate (forest: 50-70%, Fig. 1, Fig. 2, Table 1).

Multivariate Analysis

The multivariate analysis indicated weak but consistent relationships between fish assemblage composition and the land cover gradient. Reference and stress sites were weakly separated along axis 1 of the ordination, with most reference sites grouped toward the positive end of axis 1 and most altered sites grouped toward the negative end (Fig. 3; but see single reference site with extreme negative score). Correlations of land cover with the axis were consistent with this result. Forest cover was positively correlated with axis 1 (r: 0.24), whereas agricultural and developed land cover were negatively correlated with the axis (r: -0.35 and -0.10, respectively). Four species showed relatively strong, negative correlations with axis 1, including the pollution tolerant *Gambusia holbrooki* (eastern mosquitofish, r: -0.68), the facultative air breathers *Amia calva* and *Umbra pygmaea* (Bowfin, r: 0.68 and eastern mudminnow, r: -0.57, respectively), and *Enneacanthus obesus* (banded sunfish, r: -0.56, Fig. 3). Forest cover was also positively correlated with axis 2 (r: 0.14) and negatively correlated with agricultural land cover (r: -0.10). *Centrarchus macropterus*, a blackwater guild species, was negatively correlated with axis 2 (r: -0.55), and a large group of species more typical of non-Class VII waters was positively correlated with axis 2 (e.g., *Etheostoma flabellare* [fantail darter], *Notropis procne* [swallowtail shiner], *Clinostomus funduloides* [rosyside dace]; r: 0.49). The NMS ordination explained 65% of the variation in the original distance matrix (r^2: 0.27 and 0.39 for axes 1 and 2, respectively).

BCI Development

The ten best performing metrics, which were 69-86% accurate in separating *a priori*-defined altered sites from reference sites (Table 2), were aggregated into a preliminary BCI. The accuracy percentage for the overall BCI index was 90%. The index scores produced a strong separation between altered sites (<50% forest) and reference sites (>70% forest), with the interquartile ranges of the distributions of the reference and altered sites not overlapping (Fig. 4). This index included some theoretical redundancy; for example, opportunist percentage and opportunist richness were both included. The index also exhibited potential statistical redundancy; for example, tolerant species richness and opportunist richness were strongly correlated (r^2: 0.77). However, initial attempts to include fewer, non-redundant metrics did not improve accuracy. The preliminary BCI showed a moderate correlation to the land cover gradient that was in the expected direction: that is, the index was positively correlated with the percentage of forest cover in each watershed (r: 0.54, $p<0.01$, Fig. 5). Although the BCI scores produced a relatively strong fit with the land cover gradient, the relationship was especially weakened by two sites that occurred in highly forested watersheds but received low BCI scores: Blackwater River Tributary (BCI score: 53, forest: 83%) and Mush Pond Swamp (BCI score: 40, forest: 77%, Table 1).

Table 3: List of 51 fish species observed at the 34 study sites.

Genus	Species	Omnivore	Veg. spawner	Veg. specialist	Nester	Invertivore	Chowan Native	Piscivore	Bw. guild	Opp. guild	Structural specialist	Tol.	Sen.	Intro.
Acantharchus	*pomotis*				XXX	XXX	XXX							
Ameiurus	*natalis*	XXX			XXX	XXX	XXX				XXX			
Ameiurus	*nebulosus*	XXX			XXX		XXX			XXX		XXX		
Amia	*calva*		XXX		XXX		XXX	XXX	XXX					
Anguilla	*rostrata*	XXX				XXX	XXX							
Aphredoderus	*sayanus*					XXX	XXX				XXX	XXX		
Catostomus	*commersonii*					XXX	XXX					XXX		
Centrarchus	*macropterus*				XXX	XXX	XXX		XXX					
Chologaster	*cornuta*					XXX	XXX		XXX		XXX			
Clinostomus	*funduloides*				XXX	XXX	XXX							
Cyprinella	*analostana*					XXX	XXX				XXX			
Enneacanthus	*chaetodon*		XXX	XXX	XXX	XXX	XXX		XXX				XXX	
Enneacanthus	*gloriosus*				XXX	XXX	XXX			XXX				
Enneacanthus	*obesus*			XXX	XXX	XXX	XXX		XXX					
Erimyzon	*oblongus*					XXX	XXX			XXX		XXX		
Erimyzon	*sucetta*		XXX			XXX	XXX						XXX	
Esox	*americanus*		XXX	XXX			XXX	XXX	XXX					
Esox	*niger*		XXX	XXX			XXX	XXX			XXX			
Etheostoma	*flabellare*					XXX	XXX				XXX			
Etheostoma	*fusiforme*		XXX	XXX		XXX	XXX		XXX				XXX	
Etheostoma	*nigrum*				XXX	XXX					XXX			XXX
Etheostoma	*olmstedi*				XXX	XXX	XXX				XXX	XXX		
Etheostoma	*serrifer*			XXX		XXX	XXX		XXX				XXX	
Etheostoma	*vitreum*					XXX	XXX						XXX	
Fundulus	*lineolatus*	XXX		XXX			XXX		XXX				XXX	
Gambusia	*holbrooki*					XXX	XXX			XXX				
Lampetra	*appendix*					XXX	XXX						XXX	

Genus	Species	Omnivore	Veg. spawner	Veg. specialist	Nester	Invertivore	Chowan Native	Piscivore	Bw. guild	Opp. guild	Structural specialist	Tol.	Sen.	Intro.
Lepomis	*auritus*				XXX	XXX	XXX							
Lepomis	*cyanellus*				XXX	XXX		XXX		XXX		XXX		XXX
Lepomis	*gibbosus*				XXX	XXX	XXX							
Lepomis	*gulosus*			XXX		XXX	XXX	XXX			XXX			
Lepomis	*macrochirus*				XXX	XXX				XXX		XXX		XXX
Lepomis	*microlophus*				XXX	XXX				XXX		XXX		XXX
Luxilus	*albeolus*				XXX	XXX	XXX						XXX	
Luxilus	*cerasinus*				XXX	XXX	XXX						XXX	
Micropterus	*salmoides*				XXX			XXX		XXX		XXX		XXX
Moxostoma	*cervinum*					XXX	XXX						XXX	
Nocomis	*leptocephalus*	XXX			XXX		XXX							
Nocomis	*raneyi*				XXX		XXX							
Notemigonus	*crysoleucas*						XXX			XXX		XXX		
Notropis	*bifrenatus*		XXX			XXX	XXX		XXX				XXX	
Notropis	*chalybaeus*					XXX	XXX		XXX				XXX	
Notropis	*procne*				XXX	XXX	XXX							
Noturus	*gyrinus*				XXX	XXX	XXX		XXX		XXX		XXX	
Noturus	*insignis*				XXX	XXX	XXX				XXX			
Percina	*peltata/neviscens*					XXX	XXX						XXX	
Percina	*roanoka*					XXX	XXX							
Pomoxis	*nigromaculatus*				XXX		XXX	XXX			XXX	XXX		
Rhinichthys	*atratulus*					XXX	XXX					XXX		
Semotilus	*atromaculatus*	XXX			XXX	XXX	XXX					XXX		
Umbra	*pygmaea*				XXX	XXX	XXX			XXX	XXX	XXX		

XXX indicates that a species exhibits a given ecological trait. Ecological information is described in Table 1. Veg.: vegetation, Bw.: blackwater, Opp.: opportunist, Tol.: tolerant, Sen.: sensitive, Intro.: introduced.

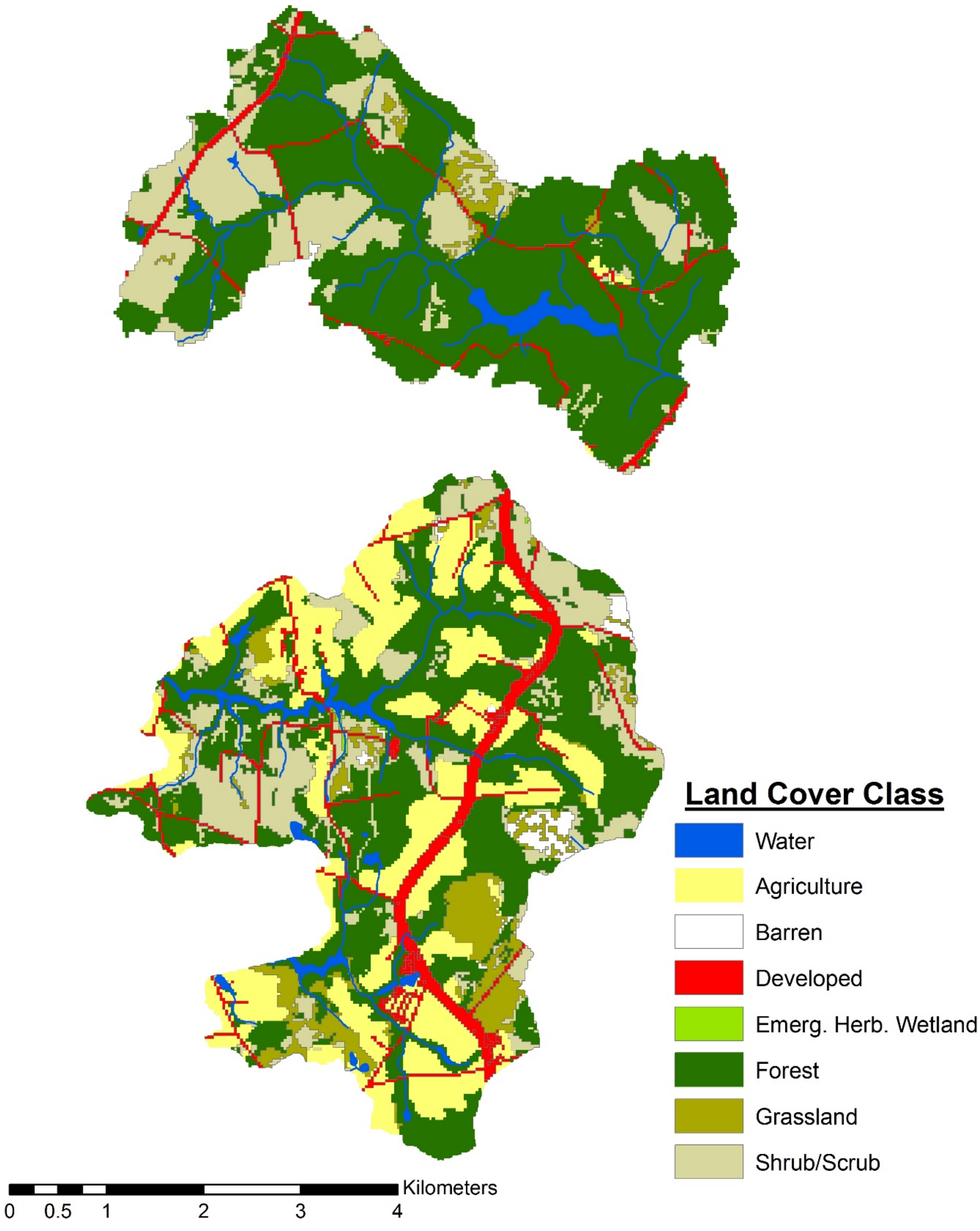

Figure 2: Example watershed land cover layouts for Otterdam Swamp (above: reference site, 71% forest) and Burnt Mills Swamp (below: altered site, 33% Forest).

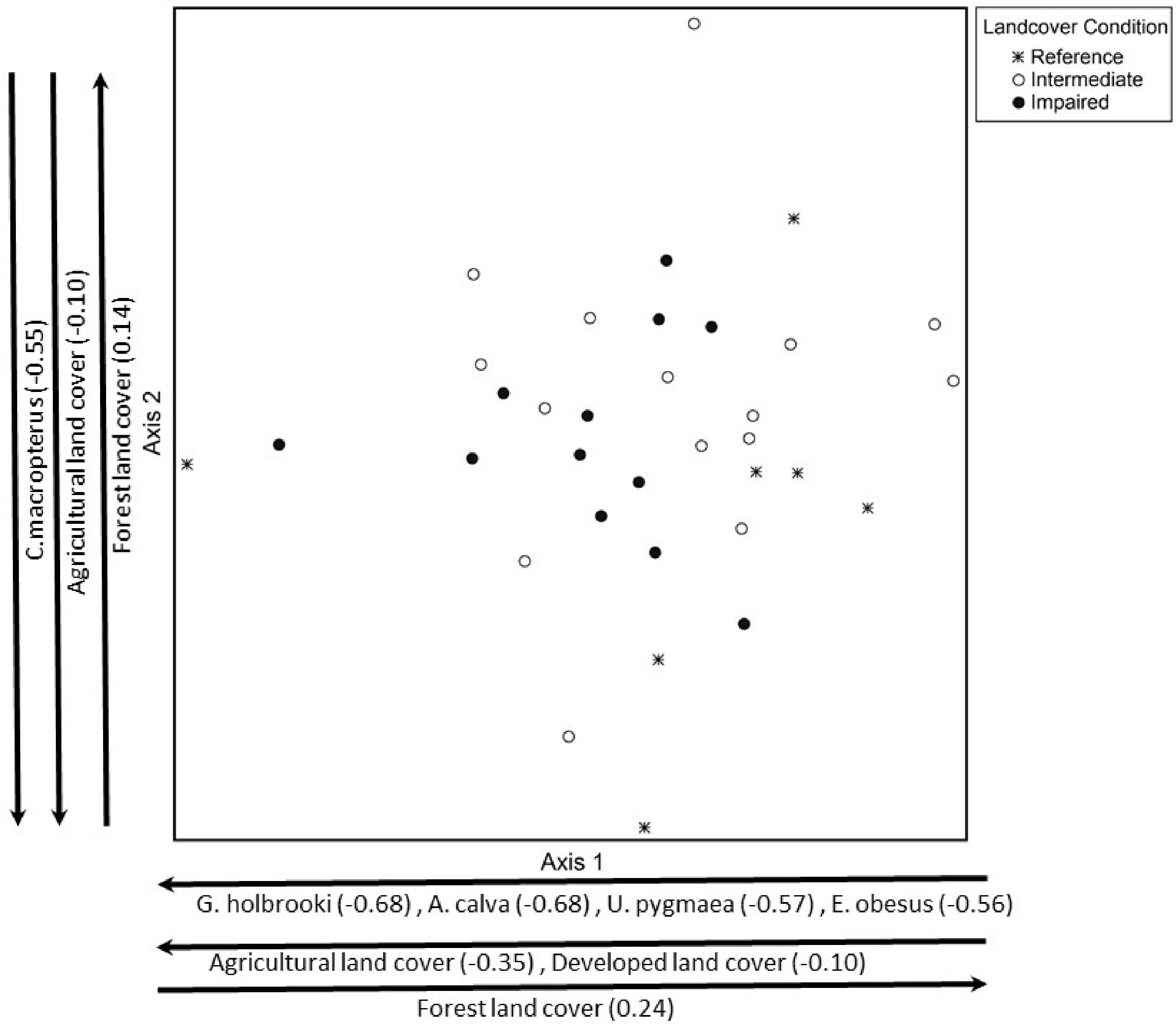

Figure 3: Non-metric multidimensional scaling ordination of 34 study sites in species relative abundance space. Arrows denote directions of correlations and values in parentheses denote r-values for linear correlations.

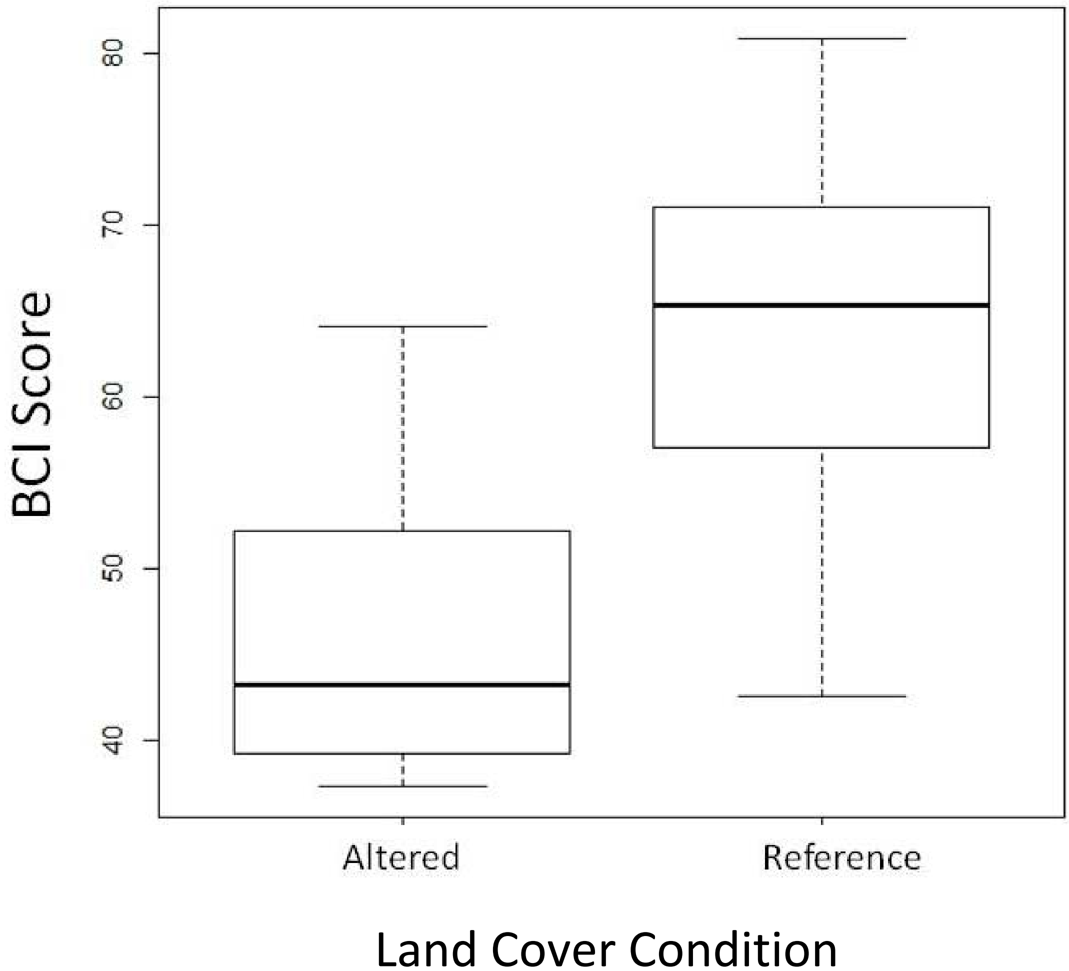

Figure 4: Distribution of Blackwater Condition Index (BCI) scores for altered and reference sites. Boxes indicate interquartile ranges; heavy lines indicate medians; and whiskers indicate minimum and maximum values.

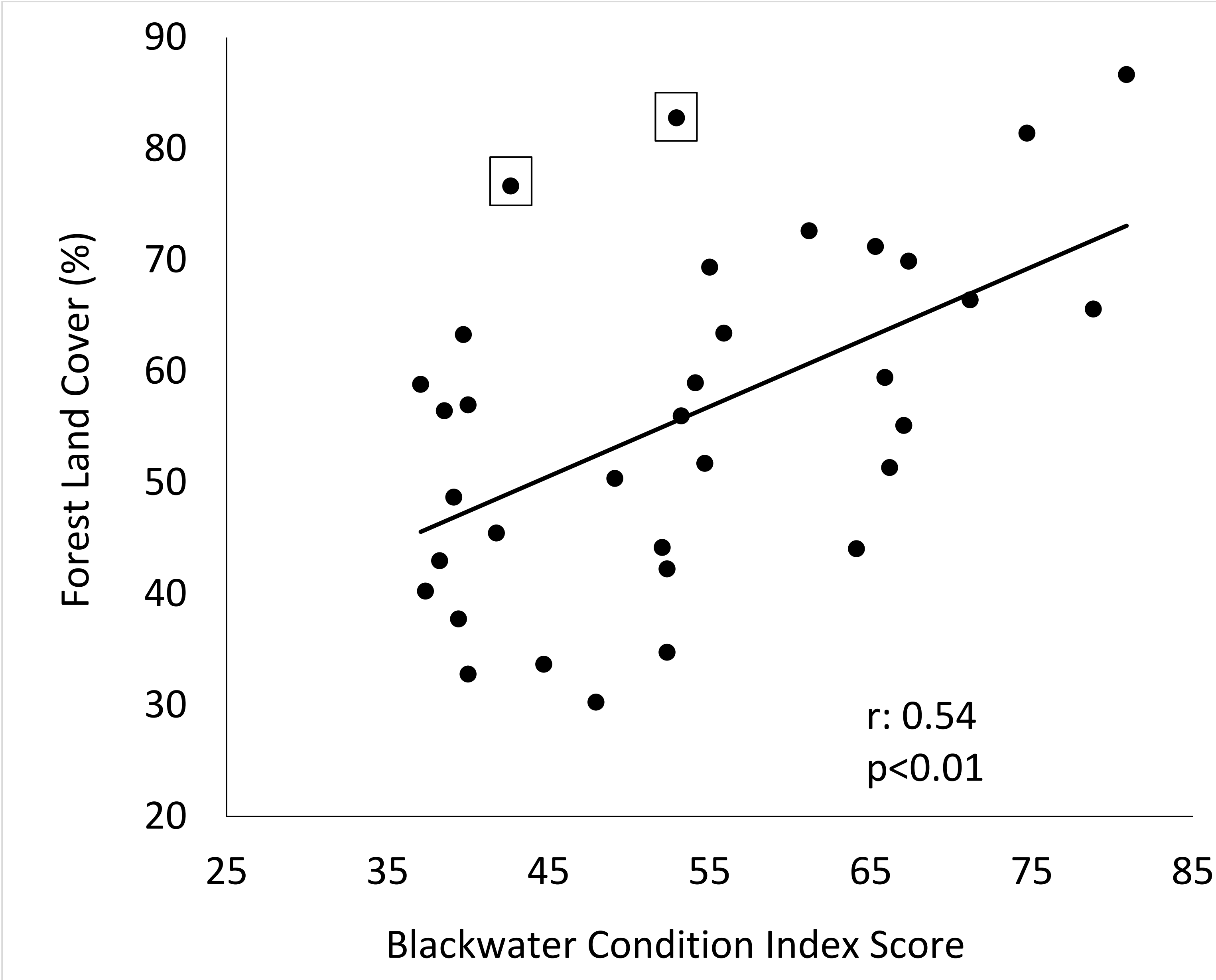

Figure 5: Correlation of preliminary BCI scores with forested land cover. Boxes indicate sites that were designated as reference based on land cover but received unexpectedly low BCI scores.

Conclusions and Recommendations

The preliminary BCI developed in this phase of the project provided strong separation between altered and reference sites, as designated by the land cover filters of >70% forest for inclusion in the reference dataset and <50% forest for inclusion in the altered dataset. The interquartile ranges of the distributions of the BCI scores for reference and altered sites did not overlap, indicating a high discriminatory power (*sensu* Barbour *et al.* 1996). In addition, the index accurately predicted reference and altered sites in 90% of pairwise comparisons. The correlation between the BCI scores and land cover gradient for all sites indicates a relatively strong overall relationship; however, some error occurred that may result in misclassification without further refinement of the index. Most notably, two study sites received low BCI scores despite being associated with heavily forested watersheds. The cause of this result is not known and requires additional field investigation. The low scores may have been the result of actual degradation; for example, if some of the developed or agricultural land cover was located in erosional areas close to the water, this highly erodible land could contribute disproportionally to the overall site conditions. Alternatively, the systems could have been impacted by natural conditions, such as hydrology changes caused by recent beaver activity or flooding events, or by past land use practices, such as farming in now-forested areas.

The preliminary BCI included redundant metrics, as indicated by their theoretical similarity and, in some cases, high pairwise correlations. Reduction of redundancy by examining individual pairwise correlations, however, has been shown to be ineffective for improving an index (Schoolmaster *et al.* 2012, van Sickle 2010). Therefore further analysis in the upcoming phase of this project will focus on redundancy reduction following the methods of Cao *et al.* (2007) and Schoolmaster *et al.* (2013).

The multivariate analysis provided evidence of the effects of land cover gradient on fish assemblage composition. However, the analysis also provided evidence of a natural environmental gradient. This result was most evident along axis 2 of the NMS ordination, where high scores were associated with stream-oriented species and low scores were associated with swamp-oriented species. Additional field evaluation is also needed to determine the cause of this trend, and most importantly, to confirm that all sites included here are appropriately designated as Class VII waters.

Further refinement of the BCI will include 1) a thorough evaluation of metric redundancy and continued analysis to improve accuracy, and 2) a determination of the degree to which metrics are correlated with, and therefore potentially confounded by, natural environmental variability. The first objective will require collection of additional land cover data, water-chemistry surveys, and evaluation of on-site conditions related to degradation of watersheds and aquatic systems.

In addition to further development of the fish-based BCI, the collaboration between VCU and DEQ biologists continues, which is focused on further development of the BHP. This work should facilitate the evaluation of natural environmental gradients that might affect biota in the systems. Finally, collection of benthic macroinvertebrates at the study sites is ongoing. These data will allow for a comprehensive evaluation of the potential for macroinvertebrate-based metrics for bioassessment of Class VII waters, as well as a comparison of the effectiveness of fish and macroinvertebrate indices.

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