

West Virginia Fish IBI

In 2012, WV Legislature passed SB562 which mandated that DEP develop rules to assess the biologic component of our narrative water quality standards. SB562 requires evaluation of the holistic health of the aquatic ecosystem and a determination that the stream:

- (i) Supports a balanced aquatic community that is diverse in species composition;
- (ii) contains appropriate trophic levels of fish, in streams that have flows sufficient to support fish populations; and
- (iii) the aquatic community is composed of benthic invertebrate assemblages sufficient to perform the biological functions necessary to support fish communities within the assessed reach, or, if the assessed reach has insufficient flows to support a fish community, in those downstream reaches where fish are present.

In order to comply with the second criterion, WVDEP contracted with WVU to develop methods to determine “appropriate trophic levels of fish”. It was determined that the most logical way to meet this criteria would be to develop a multi-metric IBI for fish in WV. Additionally, metrics that included some measure of trophic level would be prioritized for inclusion if they met other selection criteria. The process is described in the attached Fish Based Index of Biotic Integrity for Wadeable Warm Water Streams in WV, Anderson and Petty, 2015.

The Potomac Basin does not have enough existing data to develop an IBI at this time. The Fish IBIs for the Upper Kanawha (Kanawha River drainage sites upstream of Kanawha Falls) and the Ohio Drainage / Central Appalachian regions were not finalized at the time DEP filed its legislative rules outlining how DEP will assess the biologic component of our narrative water quality standards.

Coldwater streams are those that should naturally support fish species typically associated with colder waters. These streams naturally have fewer species and cannot be assessed using the IBIs developed for warmwater streams. Coldwater streams will be assessed based on the IBI scores of the benthic macroinvertebrate community.

FISH BASED INDEX OF BIOTIC INTEGRITY FOR WADEABLE WARM WATER STREAMS IN WEST VIRGINIA

Prepared for:

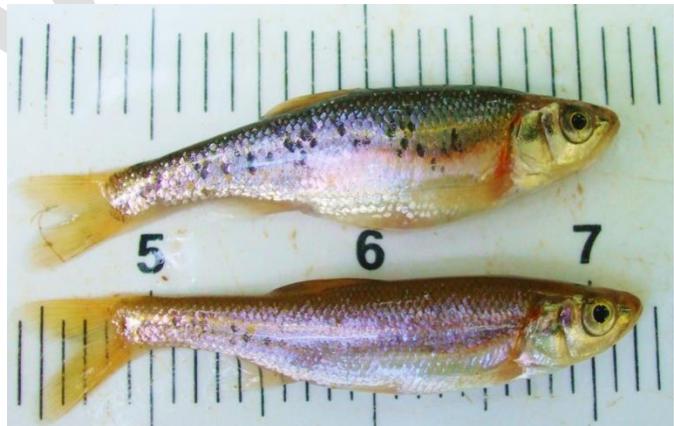
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EXECUTIVE SUMMARY

The addition of biocriteria to traditional water quality monitoring programs has allowed state agencies to determine the health of aquatic ecosystems, ensuring the designated uses of streams and rivers are met. The designated uses of streams and rivers determine the criteria thresholds, both biological and chemical, used to determine the extent of stream impairment. However, given the vast efforts in protecting designated uses, approximately 41% of streams and rivers in West Virginia are considered impaired and have been placed on West Virginia's 303(d) impaired streams list (WV DEP 2012). Of the streams assessed, increased fecal coliforms, increased iron, and degraded biological condition based on the West Virginia Stream Condition Index (WVSCI) scores, are the top three leading causes of impairment for West Virginia streams. Even though the benthic macroinvertebrate based WVSCI scores have proven useful in determining stream impairment due to its high correlation with aquatic stressors and contaminants, it may not represent the entire stream ecosystem.

Fish species, and communities, have been shown to respond differently to environmental stress than benthic macroinvertebrates. Fishes, due to their relatively high mobility and long life, are thought to represent watershed scale stressors while benthic macroinvertebrates represent local degradation. Fish based multimetric indices, or an Index of Biotic Integrity (IBI), have been used to determine stream condition based on fish community structure and composition. In addition, a fish based bioassessment tool maybe better suited in determining impairment of streams based on specific designated uses such as Warm Water Fishery, Troutwater, and Contact Recreation. Additionally, a fish based bioassessment tool in conjunction with a benthic stream condition index would provide a holistic assessment of overall stream condition.

Due to the lack of a cohesive fish based IBI in West Virginia, the overall objective of this research was to construct a fish based Index of Biotic Integrity for wadeable warm water West Virginia streams using a reference condition approach. In order to meet this objective the following tasks were established: 1) compile a comprehensive traits table for fish species in the state; 2) identify biomonitoring regions; 3)

identify reference, or least impacted, sites; and 4) select metrics that are responsive to anthropogenic stressors in order to construct and validate a fish based index of biotic integrity for wadeable West Virginia streams.

Information gathered from a thorough literature review and from local experts was used to classify all fish species found within West Virginia based on their reproduction strategy, feeding behavior, tolerance, and native status, as well as other life history traits. This information was used to quantify, or summarize, fish community assemblage structure into richness and proportion metrics. Based on fish distributions and community metrics within previously identified reference sites, 4 biomonitoring regions were delineated in which an IBI was constructed for warm water streams within each region. Final biomonitoring regions were determined by using distinct watershed boundaries and included: Monongahela Central Appalachians and Ridge-Valley (Mon CARV); Ohio Central Appalachians (Ohio CA); Ohio and Monongahela Western Allegheny Plateau (Ohio Mon WAP); and Upper Kanawha (UK).

All fish community metrics were evaluated within each biomonitoring region for their: 1) overall range; 2) correlation with drainage area; 3) discrimination between reference and stressed; 4) correlation with land-use; and 5) redundancy with other metrics. Final lists consisting of 7 – 9 metrics were retained within each region for the inclusion into a final IBI. The development of Indices of Biotic Integrity for West Virginia warm water, wadeable streams followed common standardized techniques for selection fish community metrics so that the final index in most of the biomonitoring regions were sensitive and responsive to anthropogenic impacts. Attempts were made to select metrics from key ecological categories (i.e. trophic, reproduction, and tolerance) in order to generate IBIs that give an overall view of stream condition. Final Index scores in each region were evaluated with anthropogenic land-use and water quality parameters in which most indices were responsive.

The construction of fish based IBIs for West Virginia streams provides a starting point for evaluating the impacts of anthropogenic land-use patterns on stream fish communities. Further research can now be

conducted to compare the use of the benthic macroinvertebrate based index to a fish based index in the diverse geology of West Virginia. Finally, the hope is that the fish based index will be used in conjunction with a benthic macroinvertebrate based index to develop a holistic determination of aquatic condition for West Virginia streams and rivers for both preservation and remediation efforts.

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1.0 INTRODUCTION

Current water quality monitoring programs focus on specific thresholds and criteria to determine the health of an aquatic ecosystem. For example, Aquatic Life Criteria, developed under Section 304(a) of the Clean Water Act of 1972, are thresholds for water pollutants below which are thought to have no significant risk on the majority of species in that environment (US EPA 2002). These criteria are used by state and tribal agencies to set standards and control discharges and releases of certain pollutants into waterways. However, these state, or national, criteria normally cannot account for geographic variation or the synergistic effects of pollutants on groups of organisms. Since continuous water quality sampling is costly, most monitoring programs only get a snap-shot in time and may miss anthropogenic impairments such as channel/flow alteration and habitat degradation.

Bioassessment programs have been developed to enhance water quality monitoring. Bioassessment programs use the numbers, types, and conditions of organisms to provide precise information about the condition of an aquatic ecosystem (Barbour et al. 1999). Biological criteria thresholds are then used to describe the quality of organisms needed to support a healthy aquatic ecosystem. One major step in stream bioassessment is being able to compare elements of a stream (e.g. ecological, chemical, or physical) to unimpaired streams in the same region (Barbour et al. 1999, Hawkins et al. 2010). The reference condition approach is commonly used in biological assessment programs in which the effect of human activity on organisms at one site is compared to another site in the absence of human activity (Barbour et al. 1999, Stoddard et al. 2006).

1.1 REFERENCE CONDITION

There are four different types of reference condition used in bioassessment programs (Stoddard et al. 2006). The “historical condition” is the condition of a stream at some point in history, most often the point before any human disturbance (e.g. Pre-Columbian). The “minimally disturbed condition” is the condition of streams in the absence of significant human disturbance, which allows for minimal amounts

of disturbance. The “best attainable condition” is the predicted condition at a site if best management practices were used. Finally, the “least disturbed condition” is the best available condition given current landscape practices. Most bioassessment programs use minimally or least disturbed sites to compare biological conditions (Stoddard et al. 2006, Hawkins et al. 2010). However, the characteristics defining minimally or least disturbed sites can vary among regions due to human land use or natural variation (Stoddard et al. 2006). Also, ecological data within regions can vary among sites, so selecting reference sites regionally to help explain that variation can be important in setting baselines for bioassessment programs (Hawkins et al. 2010).

There are two different approaches for using the reference condition in bioassessment. First, the multimetric approach groups sites *a priori* based on geophysical attributes (Reynoldson et al. 1997). Reference sites are then chosen within those groups based on species composition (biotic) or physical attributes (abiotic) at those sites. The second approach uses multivariate analysis to cluster sites based on species composition and then uses probabilities to determine reference sites (Reynoldson et al. 1997). The multivariate approach differs from the multimetric approach because it does not make any *a priori* assumptions about the reference condition. Instead it uses the data collected to determine reference sites objectively. A traditional index of biotic integrity uses the multimetric approach to determine the reference condition (e.g. McCormick et al. 2001,). However, other bioassessment tools use the multivariate approach to generate expectations about a site and compare those with observed data (e.g Harget et al. 2007).

1.2 ORGANISMS USED FOR BIOASSESSMENT

There are many groups of organisms used in bioassessment programs. The most common types of organisms used are benthic macroinvertebrates, algae/diatoms, and fish, however other groups have been used (e.g., Mussels and Amphibians). Each group has many pros and cons which makes no one group the best in assessing aquatic ecosystem condition.

Diatoms are useful due to their position in the food chain and are one of the most specious organisms in rivers and streams, along with being the source of many nuisance algal problems and eutrophication (Kelley and Whitton, 1995; Vilmi et al. 2015). Diatoms are also found in every aquatic system so they can be sampled year around, even when streams are dry, and with minimal sampling equipment. Since diatoms are so diverse, numerous, and require microscopic identification, they take specialized taxonomic expertise, and time, to identify in the laboratory making the use of diatoms costly. Additionally, the life histories of diatoms are not well known, which is important for most bioassessment programs. Finally, using diatoms as indicators may be difficult to communicate to the general public.

Benthic macroinvertebrates are widely used in state biomonitoring programs. Benthic macroinvertebrates consist of several insect orders, gastropods, bivalves, oligochaetes, and crustaceans which covers many ecological functions due to the range in life histories. Due to their important roles in the food web, their high diversity, limited migration, and short life span, benthic macroinvertebrates serve as good indicators of localized stream conditions (Barbour et al. 1999). These characteristic along with simple sampling equipment and sample processing makes them highly used organisms. However, it does still require specialized taxonomic expertise, microscopy, and time, to identify them in the laboratory to family, genus, or species. Also, the life histories of all benthic macroinvertebrates are not completely well known, especially at the species level, which can be important for bioassessment programs. Like diatoms, using benthic macroinvertebrates may still be difficult to translate their meaning to the general public.

Lastly, many state bioassessment programs have incorporated fish community sampling to their protocols. Fish are being used as bioindicators because they represent a wide range of trophic levels and are commonly consumed by humans, making it necessary to assess contamination levels (Barbour et al. 1999). Also, many fish species are relatively long-lived and can be highly mobile making them somewhat better indicators of long-term and watershed wide effects (Barbour et al. 1999). If well trained professionals are collecting fishes, they can be sorted and mostly identified in the field, released live back

into the stream with some individuals retained for microscopy identification vouchers or reference specimens, which cannot be said for other target organisms. Also, the life histories of most fish species are well known and widely available (Barbour et al. 1999). Finally, fish, or fishery, condition can be readily communicated to the public. However, fish community sampling can be time consuming and costly due to the equipment used when compared to benthic macroinvertebrate and diatom sampling. Also, it takes highly trained and experienced professionals to identify all fish species in the field making laboratory voucher specimens necessary for most sampling events. In addition, fish are a generally less diverse group, with approximately 170 species found in West Virginia, making them easier to identify than both benthic macroinvertebrates and diatoms.

Even though there is no general consensus on which groups of organisms are to be used in bioassessment programs, biological criteria can link human disturbances to their impacts on water bodies (Karr and Yoder 2004). For example, total maximum daily load (TMDL) programs have been developed to restore impaired designated uses and tiered aquatic life uses established to protect waters that meet or exceed chemical, physical, and biological criteria. By adding biocriteria to these programs, managers can detect and quantify aquatic life impairments and identify stressors that may not be detected in chemical assessments (Karr and Yoder 2004).

1.3 INDEX OF BIOTIC INTEGRITY

In order to quantify ecological data in bioassessment programs, the concept of biological integrity was introduced (Angermeier and Karr 1986). Biological integrity is thought to be closely linked with overall environmental quality in which high integrity is demonstrated at a site that consists of native species interacting under natural ecosystem processes and functions. For example, streams in highly urbanized areas typically have low biological integrity due to channel and flow alterations, decreases in habitat quality, increases in water temperature, and increases in dissolved materials making it difficult for most

fish and benthic macroinvertebrates to inhabit these areas (Rogers et al. 2002). This “urban stream syndrome” is just one example in which biological integrity can be closely tied to overall stream health.

Karr (1981) enforced the idea that biological communities are better apt at reflecting watershed condition than traditional water quality monitoring. Since these communities are more sensitive to anthropogenic changes on the landscape and in stream, the index of biotic integrity was introduced as a relatively inexpensive bioassessment tool for evaluating stream health with fish assemblages (Karr et al. 1986). The general structure of an index of biotic integrity is a multimetric index in which metrics are numerical values summarizing different aspects of the fish community structure such as tolerance values, trophic status, species composition, diversity, and reproduction (Fausch et al. 1990). Since IBIs cover a broad range of ecological categories, it makes this approach firmly planted in ecological theory and provides a process for quantifying and assisting in decision making. IBIs were initially developed for warm water streams in Illinois since then IBIs for fishes have been developed and applied at regional and state levels worldwide (e.g. Lyons et al. 1995, Daniel et al. 2002, Bozzetti and Shulz 2004, Lyons 2012).

For example, the Mid-Atlantic Highlands region index of biotic integrity (MAH-IBI) was developed to assess the conditions of fish assemblages within the upland ecoregions of the Mid-Atlantic States, which includes West Virginia (McCormick et al. 2001). They evaluated 58 candidate fish community based metrics covering four general categories: taxonomic, trophic, reproductive, and tolerance. Out of those candidate metrics, only 9 made it through the metric screening process. These metrics are: number of native cyprinid species, number of native benthic species, proportion of individuals in family Cottidae, sensitive species richness, proportion of tolerant individuals, proportion of nonindigenous individuals, proportion of invertivore-piscivore individuals, proportion of macro-omnivores, and proportion of gravel spawning species. The resulting multimetric index was responsive to anthropogenic stressors (e.g., chlorides) but mean IBI scores did not significantly differ between the ecoregions (McCormick et al. 2001). However, another study found both ecoregional and basin differences in fish assemblages in the

Mid-Atlantic Highlands which was sufficient to develop separate IBI's for each region (Angermeier et al. 2000).

Even though the MAH-IBI is useful at the regional scale, once it was analyzed at the state scale, some additional factors were determined to be important for IBI development. Detenbeck and Cincotta (2008) reevaluated the MAH-IBI within West Virginia and found that by reducing the reference site variability and removing the biogeographically distinct Potomac River basin, they were able to detect ecoregional differences in fish IBI scores and metrics. Similarly, Hitt and Angermier (2011) added stream network position to the list of factors (i.e. ecoregion, basin, and stream size) that influence local fish community composition which in turn influence individual fish metrics in West Virginia streams. Hitt and Angermeier (2006, 2011) have shown that position within the stream network and size of adjacent streams has potential to influence bioassessment indices.

1.4 OBJECTIVES

Within West Virginia, regulatory agencies have been using benthic macroinvertebrates, paired with water quality, to enforce environmental laws and regulations. The addition of a fish based bioassessment tool could provide a more ecologically holistic measure of stream impairment while helping preserve the integrity of some of the larger scenic rivers where benthic macroinvertebrate data is either lacking or inappropriate due to sampling time frame. Currently, fisheries biologists within the state rely on the Mid-Atlantic Highlands IBI (McCormick et al. 2001), or its modification (Detenbeck and Cincotta 2008), to assess the condition of fish assemblages. However, the diverse geology and large scale anthropogenic land use changes across the state may require a finer scale index of biotic integrity to accurately quantify these impacts. Consequently, due to the lack of a cohesive IBI at the state level, the objectives of this project were to: 1) compile a comprehensive traits table for fish species in the state; 2) identify reference across a wide range of natural conditions; and 3) select metrics that are responsive to anthropogenic

stressors in order to construct and validate a fish based index of biotic integrity for wadeable West Virginia streams.

Based on previous IBI research conducted in this region, it was assumed fish metrics to be influenced by stream size, major basin, and ecoregion. By accounting for these larger regional processes, the response of fish metrics and IBI scores to local (segment level watershed) and watershed scale (HUC 8) landscape processes, stream position, and water quality was determined. It is expected for metrics and IBI scores to decrease (or increase depending on the type of metric) with anthropogenic landscape use and the level of cumulative upstream impacts. It is also expected for metrics and IBI scores to increase based on proximity to species pool (i.e., stream network position/swim distance) and to areas of low degradation.

2.0 METHODS

2.1 DEVELOPING THE DATABASE

2.1.1 Fish Sampling Locations

Statewide fish community data was combined from various sampling sources (Table 2.1-1). Sampling sites were selected for years 1997, 1998, and 2000 – 2013. Only electrofishing (backpack, parallel wires, and barge) sampling types were used (N=1089). Fish community data consisted of identification of each fish captured to species and their abundances. Hybrid species and individuals not identified to species were removed from the sample. If additional environmental (habitat and/or water quality) or benthic macroinvertebrates samples were taken at the time of sampling (paired samples) that data was also included in the dataset. Additional benthic macroinvertebrate, habitat, and water quality data were added to the dataset if they matched sampling locations and were sampled within two years of the fish collection. Benthic macroinvertebrate data was in the form of stream condition indices developed for West Virginia based on family (WVSCI; Gerritsen et al. 2000) or genus-level (GLIMPSS; Pond et al. 2012) identification. Habitat data consisted of a total habitat score from the EPA's Rapid Bioassessment Protocol Visual-Based habitat assessment (RBP-VBHA).

Sampling locations were then input to ArcGIS and joined with segment level watersheds (1:24,000). Locations of sampling points were evaluated against the National Hydrography Dataset (NHD-24 K) to ensure site locations were attributed to the correct segment-level watershed. In order to reduce pseudoreplication, sampling locations were further reduced by selecting the most recent sampling event within each segment level watersheds and by using only wadeable streams (>7 - 400 km²; 2.70 - 154.40 mi²; 1729.74 - 98842.2 acres). Each sampling location was assigned local and cumulative landscape attributes (see Section 2.1.2), major drainage basin (Monongahela, Ohio, Potomac, and Upper Kanawha), and Level 3 Ecoregion (Omerick 1987).

Table 2.1-1: A total of 1089 fish community samples were collected statewide from the sources listed.

Data Sources	Number of Samples
West Virginia University	128
West Virginia DEP	266
West Virginia DNR (Stream Classification Survey, REMAP)	525
Federal (MAHA, MAIA, NRSA, PEIS, EMAP)	38
Reports from Consulting Companies	135

2.1.2 Landscape Attributes

Landscape characteristics for all 1:24,000 segment-level watersheds (SLWs) within the state of West Virginia were quantified using spatial analysis functions in ArcGIS ArcMap 10.0 (Environmental Systems Research Institute, Redlands, California). In conjunction with flow tables, cumulative measures of several landscape attributes for each segment-level watershed (Strager et al. 2009) were quantified at the local (i.e., within individual SLWs) and cumulative (i.e., all SLWs upstream of a given sampling location) scale for each SLW.

Land cover classifications were derived from the 2009 and 2010 National Agriculture Imagery Program (NAIP) orthophotography with a 1 meter pixel resolution at a scale of 1:10,000. Land cover types included open water, forest, grass and agricultural lands, and barren development. The mining-permit boundaries layer developed by the Technical Applications in GIS (TAGIS) office within WVDEP enabled further differentiation into mining-related open water (i.e., slurry impoundments), barren (i.e., active mine lands) and grasslands (i.e., reclaimed mine lands) from non-mining land cover. All mining-related cover classes were summed into a measure of total surface mining. The density (#/km²) of surface mining, underground mining, sewage, and septic national pollution discharge elimination system (NPDES) permits were calculated from data obtained from WVDEP. The 2003 Statewide Addressing and Mapping Board structures layer (WV SAMB 2003) was used to calculate the density of residential and commercial structures (#/km²). Natural landscape variables for each SLW were summarized including basin area (km²), mean elevation (m), slope (%), Level III ecoregion (Omernik 1987), and swim distance (km). Swim distance was defined as the minimum downstream distance (km) to a SLW with a basin area ≥ 200 km² (Hitt and Angermeier 2011).

2.2 DEFINING BIOMONITORING REGIONS

West Virginia consists of 4 major drainage basins (Potomac, Ohio, Monongahela, and Upper Kanawha; Figure 2.2-1) and 3 Level III ecoregions (Ridge and Valley, Central Appalachians, and West Allegheny Plateau; Figure 2.2-2). Both of these spatial extents can greatly influence the distributions of fish species. Therefore, each sampling location was classified based on major basin and ecoregion. This classification resulted in 9 eco-basin groups: Ohio-Central Appalachians (OhioCA); Ohio-Western Allegheny Plateau (OhioWAP); Monongahela-Western Allegheny Plateau (MonWAP); Monongahela-Central Appalachians (MonCA); Monongahela-Ridge/Valley (MonRV); Potomac-Ridge/Valley (PotRV); Potomac-Central Appalachians (PotCA); Upper Kanawha-Ridge/Valley (UKRV); and Upper Kanawha-Central Appalachians (UKCA).

Since 9 different biomonitoring regions would be difficult to manage, a similarity analysis was conducted in order to determine which sections were more similar in terms of their fish assemblages and could be combined. Using the fish abundances at each site, the similarity of sites within eco-basin groups and between groups could be evaluated to determine which regions are most appropriate to combine for IBI development. Mean similarity analysis (MEANSIM) and analysis of similarity (ANOSIM) were used to make these comparisons. Each analysis was performed using the original set of reference sites defined by WV DEP (Table 2.2-1), commonly occurring fish species (>2.5% occurrence), log(x+1) transformed fish abundances, and Bray-Curtis distances. Significance for analysis was based on 1,000 permutations.

Table 2.2-1: Numbers of reference sites used in each ecoregion/basin combination for the similarity analysis.

Reference	Mon CA	Mon RV	Mon WAP	Ohio CA	Ohio WAP	UK RV	UK CA	Pot CA	Pot RV
N	4	17	3	29	8	17	8	11	2

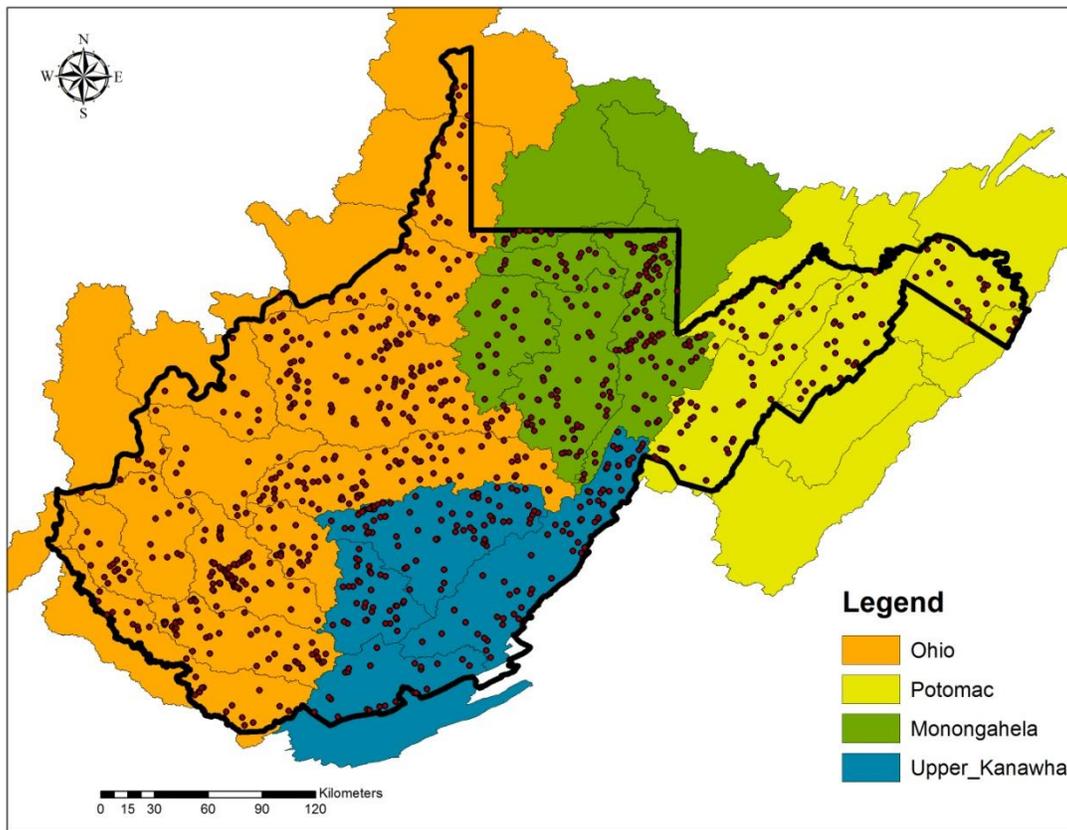


Figure 2.2-1: Grouping of HUC8 watersheds into major drainage basin categories.

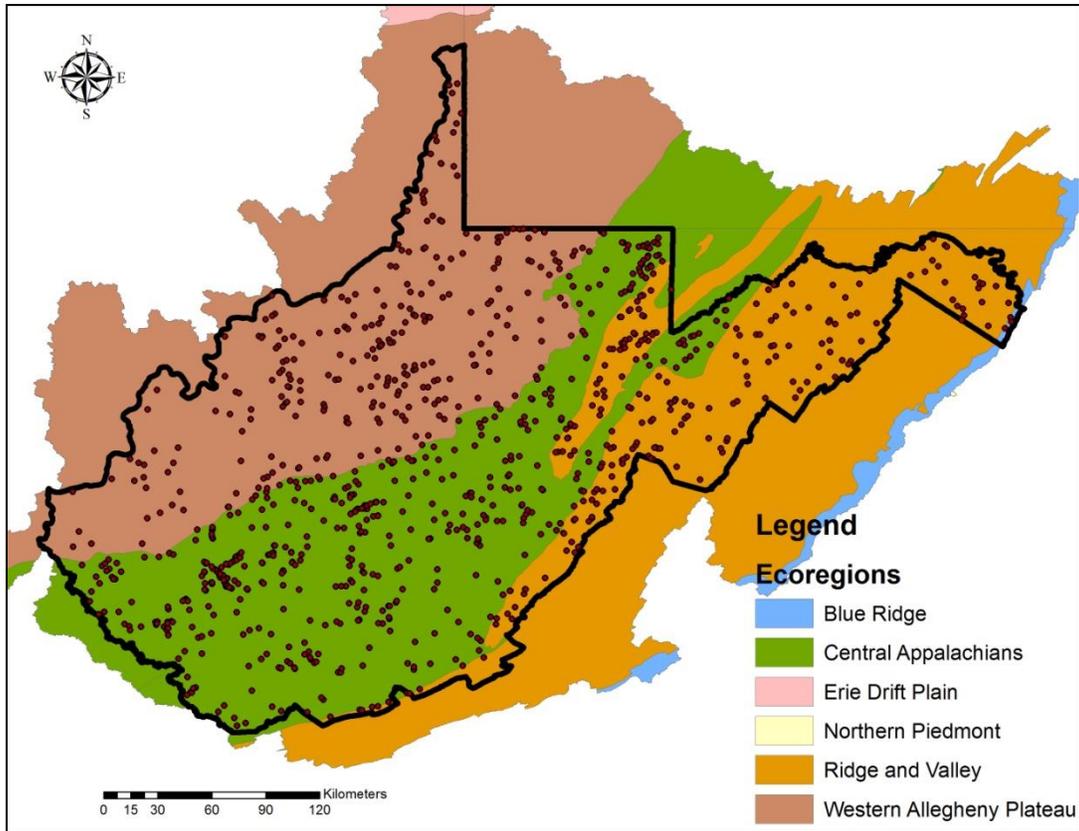


Figure 2.2-2: Spatial arrangement of sample sites by Level 3 ecoregions (Omernik 1987). The 3 main ecoregions used in IBI development were the Central Appalachians, Ridge and Valley, and the Western Allegheny Plateau.

2.3 CLASSIFICATION OF SITES

Stream ecological assessments rely on two major components: measurement of some ecological resource and a reference condition (Hawkins et al. 2010). A reference condition is considered a benchmark condition to which all other measurements are compared. Without a baseline condition, little can be inferred about the ecological condition due to natural variation among sites. In order to set reliable reference conditions, least disturbed reference sites were identified within each model region.

Identification of reference sites by the WVDEP were determined by a series of water quality and habitat characteristics along with identification of surrounding and upstream sources of pollution. However, the WVDEP identified reference sites did not encompass the range of drainage areas seen in the dataset (Figures 2.3-1 through 2.3-4), and so inferences outside of the range of reference sites would not have

been ecologically relevant. Supplemental reference sites were selected from the remaining pool of sites that were previously sampled using a human disturbance gradient and a series of water quality criteria.

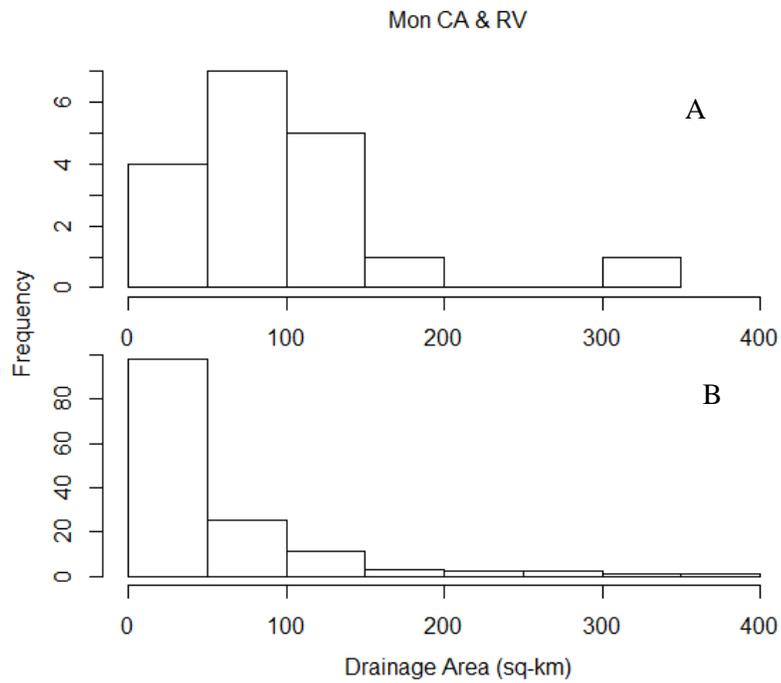


Figure 2.3-1: Frequency histogram of drainage areas within the original reference pool (A) and non-reference sites (B) within the Mon CA-RV region.

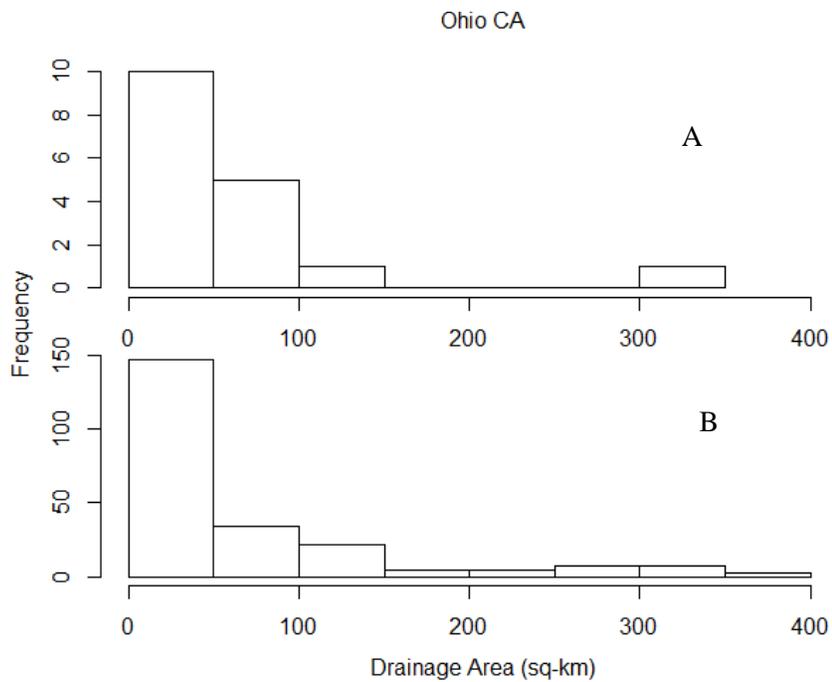


Figure 2.3-2: Frequency histogram of drainage areas within the original reference pool (A) and non-reference sites (B) within the Ohio CA region.

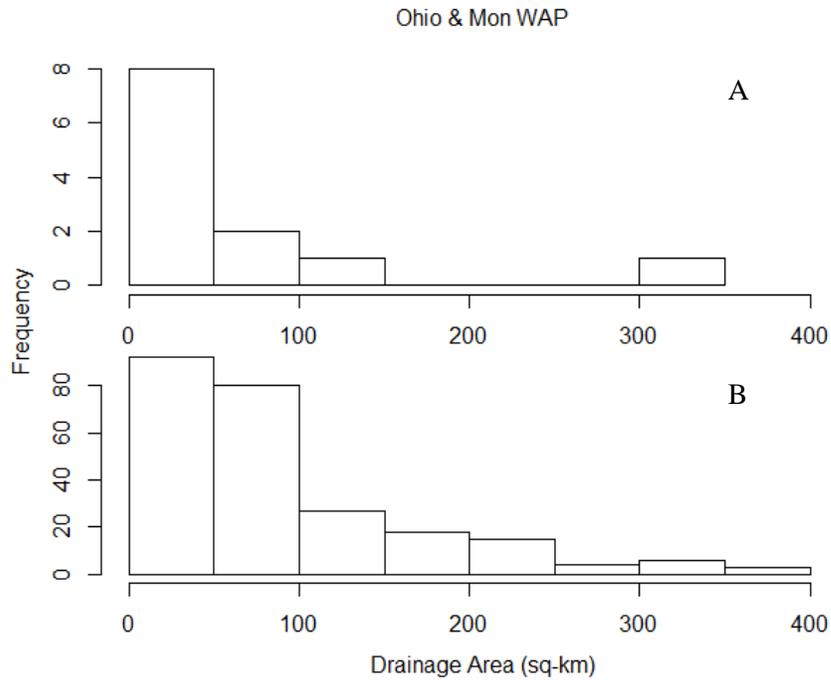


Figure 2.3-3: Frequency histogram of drainage areas within the original reference pool (A) and non-reference sites (B) within the Ohio & Mon WAP region.

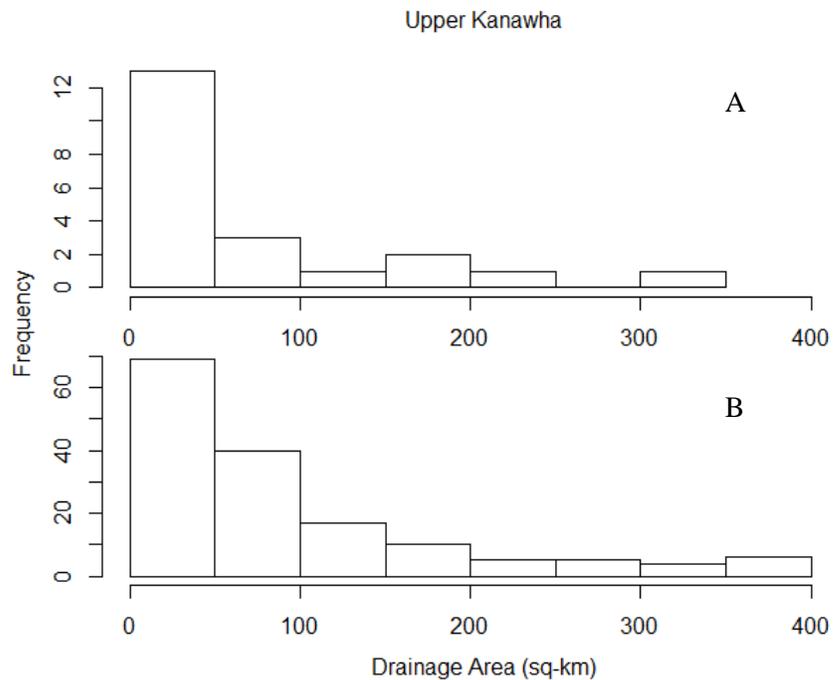


Figure 2.3-4: Frequency histogram of drainage areas within the original reference pool (A) and non-reference sites (B) within the Upper Kanawha region.

2.3.1 WVDEP Reference Site Classification

Reference conditions represent the characteristics of stream reaches that are least disturbed by human activities and are used to define attainable chemical, biological and habitat conditions for a region. The development of reference conditions is a key component of environmental impact evaluations. In most West Virginia streams, historic data were not collected prior to human disturbances and activities. Therefore, a logical method of determining the health of streams is to compare them to established reference conditions.

A considerable amount of time is invested each year in the process of selecting candidate reference sites, conducting field assessments on them, analyzing resultant data, and elevating them to full reference site status. This includes time spent to maintain the reference site database and improve methodologies used to identify them. The following outline provides the procedures used by WVDEP to establish reference sites.

Candidate reference sites are selected by examining past assessment data (if available), consulting with regional professionals of various agencies and entities that have knowledge of their local streams, and by examining landuse data from various map sources (primarily USGS 7.5-min. topographic maps) and GIS coverages. A customized GIS program called “WCMS” (Watershed Characterization Modeling System) was utilized to determine if a particular stream assessment site had the potential to be a reference site. This includes examining land use coverages for past and present disturbances and activities such as mining, urbanization, agriculture, NPDES permits, impoundments, proportion of forested land, etc. Digital Orthophoto Quadrangles (Color and Infrared aerial photo mosaics) are also examined as part of the initial step in the selection process. In general, if the drainage area above the candidate site has minimal disturbances and human activities the site may be considered a candidate. There are no stringent rules for percent forestland, agriculture, urban, or mining, land uses. However, preference is given to sites with minimal land cover disturbance, especially in the immediate stream corridor.

Because most reference sites currently in WVDEP's database are on first and second order streams, a concerted effort should be made to select some candidates on streams with larger watershed areas. It may be necessary to relax reference criteria to accommodate these larger streams since the potential for anthropogenic disturbance generally increases as stream size increases.

Establishing reference sites throughout all regions of West Virginia can be difficult. For example, few relatively undisturbed streams exist in the Western Allegheny Plateau section of the state. Conversely, the Ridge and Valley section has many relatively undisturbed streams located mostly in the mountains of the Monongahela National Forest. Therefore, the term "least disturbed" might describe more accurately the reference conditions in the Western Allegheny Plateau. Similar to selecting candidates on streams larger than first and second order, it may be necessary to relax reference criteria to accommodate least disturbed sites in regions where it is deemed necessary.

In order to address large streams and areas where reference sites are difficult to identify, WVDEP established a second level of reference condition (Level II). While Level I reference sites meet all reference site criteria described below, Level II reference sites fail to meet one or more of them by a narrow margin. For example, Level II reference sites may be deficient in one RBP habitat parameter. Level III reference site designations are generally reserved for rivers and large streams, primarily those with watershed areas exceeding 60 square miles. Level III reference sites generally meet RBP habitat and water quality criteria at the assessment site, but because of their size generally have point source discharges within their drainage or more land development and human disturbances than would be allowed for smaller streams designated as Level I or Level II. Level III reference sites are generally located in least disturbed segments of rivers and streams where local and upstream disturbances are minimized or distant to the site. It should be noted that best professional judgment by experienced personnel is an important part of the initial and final selection of Level I, Level II, and Level III reference sites.

Although selecting candidate reference sites a priori is the primary means of establishing reference conditions, a considerable number of sites meeting reference criteria are drawn from a pool of probabilistically selected and targeted sites that were not initially identified as potential reference sites. Both probabilistic sites and targeted sites must meet the criteria established for candidate sites.

2.3.2 WVDEP Reference Site Criteria

The reference site selection process begins in the field as WVDEP personnel will note based on site observations, if the sampled location could be a potential reference site. Following field assessments, all chemical, habitat, biological, and reconnaissance information for each site is entered into a relational database. Each site is then evaluated to see if it meets reference site criteria. If all of the criteria are met (see below), the site is given Level I reference site status. Full descriptions of criteria are in section 2.3.3.

1. No known significant point source discharges upstream of assessment site (i.e., NPDES)
2. Field evaluation of anthropogenic activities and disturbances at the assessment site by trained biologists and environmental resource specialists must be minimal
3. No obvious sources of non-point source pollution near assessment site
4. Primary WQ criteria:
 - a. D.O. \geq 5.0 mg/l
 - b. pH between 6.0 and 9.0 Std.Units
5. Secondary WQ criteria:
 - a. Conductivity $<$ 500 μ mhos/cm
 - b. Fecal coliform bacteria $<$ 800 colonies/100 ml
6. No known violations of state water quality criteria (e.g., metals)**
7. U.S. EPA-RBP VBHA metric scores:
 - a. 11 (lowest score possible for sub-optimal rating) for following:
 - i. epifaunal substrate
 - ii. channel alteration
 - iii. sediment deposition
 - b. 6 (lowest score possible for marginal rating) for following:
 - i. bank vegetative protection (right bank \geq 6 & left bank \geq 6)
 - ii. riparian vegetative zone width (right bank \geq 6 & left bank \geq 6)

- c. 130 (mid-suboptimal score) for following:
 - i. total RBP habitat score

2.3.3 Explanation of WVDEP Reference Site Criteria

1. Point source discharges - Because reference sites presumably represent least disturbed conditions, significant point source discharges (NPDES) located upstream of an assessment site generally disqualify it from becoming a reference site. WCMS and other GIS coverages provide easy access to the locations and chronology (e.g., was the permit active before, during, or after the sampling event) of many permitted point sources. However, extra reconnaissance effort is taken in the field to ensure that point sources do not exist above the site. Point source discharges may be acceptable for Level II reference site designations depending on the type, volume of discharge, and proximity to the assessment site. For example, a home aeration unit located in the headwaters of a stream may not exclude an assessment site near the mouth of the stream from becoming a Level II reference site.
2. Anthropogenic disturbances - The stream assessment area is evaluated visually for anthropogenic disturbances. Best professional judgement is employed to make reference site inclusions based on the number and type of disturbance(s). For example, a surface mine site would generally be considered a greater disturbance than an ATV trail and small road combined and could exclude the site from reference condition consideration. However, impacts from the ATV trail and/or road may be considered so minor that they do not exclude the site from reference consideration. This may be a case where best professional judgment dictates that the site be designated as a Level II reference site instead of a Level I site. The information gathered in the field on anthropogenic disturbance helps validate the GIS coverages used to select the candidate sites.
3. Non-Point Sources (NPS) - Obvious sources of NPS are documented within the assessment area. If sources of NPS are documented for areas above the assessment site, they are also considered. Livestock feedlots, parking lots, and road runoff are common sources of NPS. Best professional

judgment is employed to make reference site inclusions (Level I or Level II) based on the type location, and intensity of the NPS. For example, a livestock feedlot with direct drainage to the stream would likely exclude the site from reference consideration. In contrast, a small road drain may not be significant enough to exclude a site from consideration.

4. Primary WQ criteria:

- a. D.O. \geq 5.0 mg/l - The criterion for dissolved oxygen was taken from “WV Water Quality Standards” as developed by the State Water Resources Board (SWRB).
- b. pH between 6.0 and 9.0 Std.Units - The criterion for pH was taken from “WV Water Quality Standards” as developed by the State Water Resources Board (SWRB).

5. Secondary WQ criteria: (used as flag values)

- c. Conductivity $<$ 500 μ mhos/cm – Criterion for conductivity was established from analysis of WVDEP data and from best professional judgment of several experienced field employees. A value greater than 500 may indicate the presence of dissolved ions (such as sulfate, chlorides, and metals) exceeding the background levels for the area. It is important to note that a full water quality analysis that includes all possible chemical constituents is not within the resource pool of the program. Consequently, the conductivity reading of a site can be used as a means of flagging the site for further investigation before it can be considered a reference site. ** Region specific criteria for conductivity are currently being developed to address natural differences in ambient conductivity. This may result in having lower or higher conductivity thresholds based on ecoregion, watershed (8 digit HUC), etc. Currently, best professional judgment is used when conductivity is conspicuously higher than expected for the region.
- d. Fecal coliform bacteria $<$ 800 colonies/100 ml - The fecal coliform value of 800 colonies/100ml is double the maximum set by the WV Environmental Quality Board (WV EQB) which states that fecal coliform shall not exceed 400/100ml in more than 10

percent of all samples taken during the month. This value was raised to 800/100ml for reference criteria due to the lengthy holding times of fecal samples (24 hours in many cases). Additionally, experienced field personnel have encountered fecal coliform bacteria counts exceeding the standard in streams where no human impacts were known. Thus, a value of 800/100ml would decrease the possibility of excluding some undisturbed (anthropogenically) streams from reference consideration. Similar to the criterion for conductivity, fecal coliform bacteria can be used as a means of flagging the site for further investigation before it can be considered a reference site.

6. No known violations of state water quality standards – If there is a violation of a water quality criterion standard as established by the WV Environmental Quality Board (WV EQB), the site is eliminated from reference site consideration (with the exception of fecal coliform bacteria as described above). Because of their toxicity, metals are the primary consideration when evaluating data for violations.
7. RBP VBHA metric scores: The habitat criteria below are adapted from the US EPA-RBP VBHA procedures. These criteria were selected because they are considered most indicative of anthropogenic disturbance.

2.3.4 Warm/Cold water designation

Sites were identified as being either warm water or cold water by an evaluation of the fish community data. If enough coldwater species were collected, it was called cold. The threshold for the number of coldwater species present depended on overall richness. If species richness was less than or equal to five, then the presence of one cold indicator species resulted in the site being identified as a coldwater site; if richness was between 5 and 10 then the presence of two coldwater indicator species were required to be identified as coldwater; if richness was greater than ten, then 3 coldwater indicator species were required. Additionally, if sculpin species (*Cottus* spp.) were amongst the top three most numerous, the site was deemed to be coldwater. All other comparable samples were identified as warm water.

Coldwater indicator species used for this exercise were brook trout (*Salvelinus fontinalis*), mountain redbelly dace (*Chrosomus oreas*), longnose dace (*Rhinichthys cataractae*), and any sculpin spp. Final warm/cold water designation was determined by West Virginia Department of Environmental Protection.

2.3.5 Selection of Supplemental Reference Sites from Database

The removal of cold water sites from the total sample size in each biomonitoring region also reduced the number of reference sites available in the dataset. In order to offset the loss of reference sites, supplemental reference sites were chosen based on a human disturbance gradient. In order to identify stream segments that are least and most exposed to human activities, the variability of human activities within regions must be determined. Since field collections can be rather costly, remotely sensed human activity data (e.g., land use/land cover, permit locations, Table 2.3-1) were used as indicators of anthropogenic disturbance. Using Principal Component Analysis (PCA) a human activity gradient was established for each study region using anthropogenic based landscape variables only. PCA is a useful method for quantifying variation of human activity within each study region because it does not require *a priori* grouping of sites while also generating a continuous human activity score (PC scores) for each segment level watershed within the state (Yates and Bailey 2010). A human activity gradient, and water quality criteria, allowed the identification of least disturbed reference sites, and highly disturbed stressed sites, for specific drainage areas within each model region. The addition of larger (>100 km²) reference sites creates a benchmark that is more representative of natural environmental variation within each region.

The following steps were taken for the selection of additional reference sites:

1. If the site was originally listed as a reference site by the WVDEP, then it was kept as a reference site
2. Landscape characteristics summarized by Principle Components Analysis (PCA) of human activity gradients were used to identify candidate “least impacted” sample sites. The sites with

scores indicating the least amount of human activities were identified as candidates within each study region.

3. The list of candidate least impacted sites was then examined closely to determine if water quality, landuse information, and/or habitat quality data supported inclusion as a reference site. The process that DEP typically uses to evaluate sites as potential reference sites was followed where water quality and habitat data was available. Where these data were not available (not collected concurrent with the fish collection), data from other nearby sites on the same stream or within the drainage area were considered in addition to the various GIS data layers that are available statewide, including landuse, aerial photography, and NPDES outlets. The offsite data that was reviewed consisted of monitoring data (water quality, habitat, landuses, photographs, and potential watershed stressors) from targeted and probabilistic assessments and where available pre-TMDL development monitoring that included a 12 month data collection effort. If additional data supported inclusion, then the site was classified as a reference site.
4. The further use of PCA results was explored. Because the 2 primary PCA axes tend to measure distinct disturbance types (mining and human development) that were negatively correlated, the use of the sum of the 2 scores was abandoned. Consideration was given to development of independent criteria for each of the 2 axes to identify additional candidates for reference site evaluation. Doubts about the accuracy of the landuse information that was used influenced the decision to move to alternate methods of identifying candidate reference sites. Two additional processes were utilized to identify additional sites to further evaluate:
 - a. Proximity to DEP reference sites. A GIS exercise was undertaken to identify fish sites that were near (and on same stream) or in upstream drainage of previously identified DEP reference sites (considered for benthic macroinvertebrate IBI development). These candidates were then screened by process described above in step 3.
 - b. Sites within the Western Allegheny Plateau (ecoregion deemed most in need of additional reference sites) that had >92.5 % forest within their drainage area were identified as

candidates. These sites went through a preliminary screening and underwent further screening as described in step 3.

Table 2.3-1: Human activity groups, descriptors, and associated units used in principal component analysis to generate a human activity gradient. All variables are represented by local (segment level watershed) and cumulative (accumulation of upstream watershed) values.

Group	Descriptor	Units
Development	Development	% Area
	Grassland (non-agriculture)	% Area
	Structures	No./Area
	Septic and Sewage	No./Area
	NPDES Permits	No./Area
Mining	Surface Mining	% Area
	Underground Mine Permits	No./Area
	Surface Mine Permits	No./Area
Agriculture	Total Agriculture (pasture + crop)	% Area

2.3.6 Stressed Site Classification

Sites with pH < 5.0 were classified as stressed. Landscape characteristics (anthropogenic variables only) as summarized by PCA results were also used. Stressed sites were identified as sites with landscape characteristics that were highly divergent from reference sites. Additional habitat and water quality data were not used to classify stressed sites. However, only the most highly stressed sites were considered because there was no pressing need to maximize the stressed site sample size, as it was with reference sites. All candidate stressed sites were reviewed by DEP staff in order to add a quality control step and to gain confidence in the computer driven process. In addition, sites with no fish captured were not included as a stressed site in the dataset.

2.4 COMPILING CANDIDATE METRICS

Each fish species present in the total sampling database, which encompasses all stream sizes, were classified based on several natural history based traits. The traits included life history aspects such as: spawning, trophic guild, distribution, tolerance, and family classification. Traits for each individual species were collected from a variety of sources: Fish Traits database (Frimpong and Angermeier 2010), Freshwater Fishes of Virginia (Jenkins and Burkhead 1994), EPA's Rapid Bioassessment Protocols for Streams and Rivers (Barbour et al. 1999), with input from professionals from West Virginia Department of Environmental Protection, W.V. Division of Natural Resources, and U.S. Environmental Protection Agency (Region 3).

An extensive list of fish community and species based metrics were compiled from the Mid-Atlantic Highland IBI (McCormick et al. 2001) and its modification (Detenbeck and Cincotta 2008).

Modifications were made to several of the metrics to exclude tolerant or specific species. All metric calculations were conducted in program R (version 2.15.2 (R Development Team 2012) using matrix algebra and package vegan version 2.0-6 (Oksanen et al. 2013, used for richness calculations). Metrics for consideration in the IBI were then assigned an expected response to stressors: positive metrics decrease with increases in stressors, while negative metrics increase with increases in stressors.

2.5 SETTING BASELINE EXPECTATIONS

Fish community based metrics are commonly adjusted for watershed area during Index of Biotic Integrity construction (e.g. McCormick et al. 2001). Within West Virginia, other studies have demonstrated the importance of stream temperature, ecoregion, and distance to a source to fish community structure (Detenbeck and Cincotta 2008; Hitt and Angermeier 2011). However, little consideration has been given to adjusting metrics used in IBI development for these other natural environmental variables (i.e. elevation, distance to a source). Predictive models used in bioassessment programs allows for the comparison of observed fish community assemblages of a sampling location to what is expected in the

absence of human disturbance (Observed:Expected; Flotemersch et al. 2006). The expected assemblage is generated using linear models based on regionally specific reference sites. Specifically, fish community metrics can be predicted for all wadeable streams in West Virginia using reference site based models generated using surrounding landscape characteristics. This approach is based on the concept that any significant departure from the baseline reference condition (i.e. expected value under natural landscape conditions only) is indicative of a disturbed system.

For this IBI, only conditions of warm water streams on a regional basis was evaluated (i.e. biomonitoring regions; see Section 2.2). Each metric was evaluated within each biomonitoring region for its relationship with drainage area using linear models within the reference sites, in which drainage area was log₁₀ transformed. Some fish community metrics were also transformed (e.g. arc-sine or log₁₀(x+1)) depending on its check for normality with a Shapiro-Wilks test. Metrics with significant (p-value <0.05) relationships with drainage area were then predicted based on the linear model equation. Those metrics were then adjusted using the following formula:

$$\frac{(Observed\ Metric\ Value)}{(Predicted\ Metric\ Value)}$$

2.6 TESTING CANDIDATE METRICS

An initial set of 66 richness and 65 proportional potential fish community metrics, and one trophic diversity index (Shannon-Weaver Diversity index based on feeding guild), were analyzed for inclusion in regional IBI's following the guidelines set by Stoddard et al. (2008) with slight modifications. Selection of metrics was determined for each region by: 1) range, 2) discrimination between reference and stressed sites, 3) response to stressor gradient, and 4) redundancy (Stoddard et al. 2008, Pond et al. 2012). Again, range of each metric was evaluated prior to assessing their relationship with drainage area. The full description of each metric and their expected response to the stressor gradient can be seen in Table 2.6-1.

The range of each metric was evaluated based on the 25th percentile of the full distribution of the metric. If the 25th percentile was 0, then the metric was no longer considered for inclusion. If the metrics passed the range test, they were then evaluated for their relationship with drainage area using linear models (see Section 2.5). Metrics were adjusted for natural variables after the range test to allow rare metrics (i.e. metrics with too many zeros) within each region to be excluded and to ensure metrics have high enough variability to discriminate among sites in different conditions (Stoddard et al. 2008).

Raw (i.e. metrics not adjusted using linear models) and Adjusted (observed/expected) metrics were then evaluated for their discrimination efficiency (DE). For discrimination efficiency (i.e. responsiveness), the number of stressed sites that fell below the 25th percentile (for positive metrics) or fell above the 75th percentile were calculated (for negative metrics) of the reference distribution in each biomonitoring region (Blocksom and Johnson 2009). A metric had to exhibit a discrimination efficiency above 60% prior to further evaluation with anthropogenic stressors.

Each metric was then evaluated for their relationship with environmental stressors using Spearman's correlation. Metrics were correlated with % cumulative surface mining, structure density, total agriculture, development, and total forest along with pH, and specific conductance. Redundancy of metrics was evaluated with Spearman correlation. Any metric which was highly correlated ($>|0.90|$) with another metric was considered for removal from IBI development. This procedure produced a pool of potential metrics that are either correlated with human disturbance, highly discriminate, or both. From this pool, a selection of metrics, or all metrics, could be scored and combined to produce a final IBI.

Table 2.6-1: Description and expected response of each metric evaluated for IBI development. Each metric listed was summarized as a proportion (% individuals; P_) and as a richness (# of species; R_).

Metric	Expected Response	Description
Richness	+	Richness
Native	+	Native Status
Game	+	Classified Game fish from WV DNR
RGS	+	Rock and gravel spawning
GSS	+	Gravel and sand spawning
LSR	+	Lithophilic spawning
NGL	+	Non-guarding lithophilic spawning
MO	+	Macro-omnivore
IN	+	Invertivore
IP	+	Invertivore-Piscivore
ISEAT	+	Invertivore-Piscivore minus creek chub (SEAT)
Benthic	+	Benthic
Benthic_CACO	+	Benthic minus white sucker (CACO)
Cottid	+	Cottidae
Cyprinid	+	Cyprinidae
Cyprinid_BNDSEAT	+	Cyprinidae Family minus blacknose dace (RHOB & RHAT) and creek chub (SEAT)
Cyprinid_N	+	Native Cyprinidae
Cyprinid_NBNDSEAT	+	Native Cyprinidae Family minus blacknose dace (RHOB & RHAT) and creek chub (SEAT)
BND_CACO_SEAT	-	Blacknose Dace (RHOB & SEAT), white sucker (CACO), and creek chub (SEAT)
OH	-	Omnivore-Herbivore
OH_CAAN	-	Omnivore-Herbivore minus central stoneroller (CAAN)
OH_CAAN_CACO	-	Omnivore-Herbivore minus central stoneroller (CAAN) and white sucker (CACO)
Cold	+	Cold water specialists
Cold_SATR_ONMY	+	Cold water specialists minus brown (SATR) and rainbow (ONMY) trout
GameC	+	Reduced list of game fish
OH_NG	-	Non-game omnivore-herbivore
IBenthicNG	+	Benthic and non-game invertivore-piscivore
INonGameNB	+	Non-game and non-benthic invertivore-piscivore
DMS	+	Darter-madtom-sculplins
Percidae	+	Family Percidae
Sunfish	+	Sunfish (Family Centrarchidae)
Catfish	+	Family Ictaluridae
Catostomidae	+	Family Catostomidae
McC_CGS	+	Clean Gravel Spawning (Mc Cormick)
CGS_RGS	+	Clean Gravel & Rock-gravel Spawning
CavitySpawn	+	Cavity Spawning
Fish2.DEP	+	Fish minus tolerant
Native2.DEP	+	Native Status minus tolerant
RGS2.DEP	+	Rock and gravel spawning minus tolerant

Metric	Expected Response	Description
GSS2.DEP	+	Gravel and sand spawning minus tolerant
LSR2.DEP	+	Lithophilic spawning minus tolerant
NGL2.DEP	+	Non-guarding lithophilic spawning minus tolerant
IP2.DEP	+	Invertivore-Piscivore minus tolerant
Benthic2.DEP	+	Benthic minus tolerant
Cyprinid2.DEP	+	Family Cyprinidae minus tolerant
Cyprinid_N2.DEP	+	Native Family Cyprinidae minus tolerant
OH2.DEP	-	Omnivore-Herbivore minus tolerant
Cold2.DEP	+	Cold water specialist minus tolerant
Game2.DEP	+	Game fish minus tolerant
DMS2.DEP	+	Darter-madtom-sculpins minus tolerant
Tol.DEP	-	Tolerant
Mod.DEP	+	Moderate Tolerance
Int.DEP	+	Intolerant
Tol_Benthic.DEP	-	Tolerant Benthics
Int_Benthic.DEP	+	Intolerant Benthics
Tol_Cyprinid.DEP	-	Tolerant Family Cyprinidae
Int_Cyprinid.DEP	+	Intolerant Family Cyprinidae
Int_RGS.DEP	+	Intolerant Rock-gravel spawning
Int_GSS.DEP	+	Intolerant gravel-sand spawning
Int_LSR.DEP	+	Intolerant lithophilic spawning
Int_NGL.DEP	+	Intolerant non-guarding lithophilic spawning
McC_CGS2.DEP	+	Clean gravel spawning minus tolerant (Mc Cormick)
CGS_RGS2.DEP	+	Clean gravel and rock-gravel spawning minus tolerant
CavitySpawn2.DEP	+	Cavity Spawning minus tolerant
SW-Trophic	+	Shannon-Weaver Trophic Diversity Index

2.7 COMBINING METRICS INTO AN INDEX

The final pool of fish community based metrics represent a variety of ecological characteristics such as taxa richness, taxonomic composition, pollution tolerance, trophic groups, and spawning guilds. These metrics are in a variety of units such as percentages, richness, Shannon Diversity Trophic index, and ratios (adjusted metrics). In order to combine these metrics into a meaningful index, they must first be converted or standardized into unitless numbers or ratios. Different metric scoring methods could strengthen or weaken relationships with stressor gradients or influence the discrimination efficiency of

metrics (Blocksom 2003). The scoring method selected is based on an equation using the ceiling (95th percentile) and floor (5th percentile) of the entire distribution of sites for each metric (Blocksom 2003, Pond et al. 2012). The formula for standardization used depends on the expected direction of metric in response to stressor gradients.

For positive metrics:

$$\frac{\text{Observed value} - \text{lower threshold}}{\text{Upper} - \text{lower threshold}} \times 100$$

For negative metrics:

$$\frac{\text{Upper threshold} - \text{Observed value}}{\text{Upper} - \text{lower threshold}} \times 100$$

Scored metric values were averaged to get a final standardized index value ranging from 0 – 100.

2.8 IBI SCOPE OF IMPAIRMENT

The responsiveness of the IBI's in each biomonitoring region to stressors and landscape characteristics were evaluated by examining their relationships with water quality, cumulative landscape measures, and alternate stream condition measures (Table 2.8-1) using Pearson's Correlation. The ability of the IBI in each biomonitoring region to discriminate between site types (e.g. reference, other, and stress) was evaluated using Analysis of Variance (ANOVA) and Tukey's HSD post-hoc test to determine which groups were significantly different. In some regions, the temporal variance across years by comparing IBI scores from sites that were revisited in subsequent years was assessed. Finally, stream condition thresholds were generated using the distribution of IBI scores at reference sites (Table 2.8-2). Four condition classes (Excellent, Good, Degraded, Severely Degraded) were designated with either the 10th percentile (Mon CA & RV) or 25th percentile (Ohio CA, Ohio & Mon WAP, and UK) of the reference sites as the threshold between intact and impaired streams.

Table 2.8-1: Biotic, abiotic, and landscape measures that were evaluated for a relationship with final IBI scores in each biomonitoring region.

Biotic	Abiotic	Landscape
West Virginia Stream Condition Index (WVSCI)	Conductivity	Cumulative Forest (%)
Genus Level Index of the Most Probable Stream Status (GLIMPSS)	pH	Cumulative Development (%)
Total Fish Abundance	Drainage Area	Cumulative Surface Mining (%)
Mid-Atlantic Highlands IBI (MAH IBI)	Elevation	Cumulative Agriculture (%)
		Cumulative Structure Density (#/km ²)
		NPDES Permit Density (#/km ²)

Table 2.8-2: Stream condition classes based on the percentiles of the reference distribution in the Ohio CA, Ohio and Mon WAP, and Upper Kanawha biomonitoring region.

Condition	Percentile
Excellent	$\geq 75^{\text{th}}$
Good	$25^{\text{th}} \leq \text{IBI} < 75^{\text{th}}$
Degraded	$5^{\text{th}} \leq \text{IBI} < 25^{\text{th}}$
Severely Degraded	$< 5^{\text{th}}$

3.0 RESULTS

3.1 SELECTING BIOMONITORING REGIONS

Analysis of similarity (ANOSIM) indicated that classification using Major Basin, Ecoregion, and Ecoregion-Major Basin combinations all produced significant differences (p -value <0.05) between groups (Table 3.1-1). However, Ecoregion-Major Basin combinations produced the highest classification percentage (CS%), followed by Major Basin and then Ecoregion (13.5, 9.1, and 7.2%, respectively). This indicates that classification based solely on Basin or Ecoregion would be insufficient. Mean similarity analysis (MEANSIM) based on the Ecoregion-Major Basin classifications then indicated which groups were more similar based on their fish community structure (Table 3.1-1). Upon evaluation of the MEANSIM dendrogram (Figure 3.1-1), general regions were selected for Index of Biotic Integrity development and include: Upper Kanawha (UK); Potomac; Ohio and Monongahela-Western Allegheny Plateau (OhioMonWAP); Monongahela-Ridge/Valley and Central Appalachian (MonCARV); and Ohio-Central Appalachians (OhioCA). However, this initial classification resulted in regional boundaries that intersected watershed boundaries and were difficult to distinguish on the landscape. In order to make the regionalization more biologically relevant and amiable to interest groups that may utilize the index, either whole HUC8 watersheds were combined or distinct dividing lines were used, such as HUC12 outflows, based on which general region they intersected in order to form the biomonitoring regions (Table 3.1-2; Figure 3.1-3). The only HUC8 that was split based on a distinct dividing line was the Elk watershed. The dividing line for this watershed occurred at the outflow of the Suttonsville Dam, a HUC 12 outflow. Due to the low sample size ($N=105$) and uncertainty of reference condition, the Potomac biomonitoring region was removed from further analysis and IBI development.

Table 3.1-1: ANOSIM and MEANSIM results of the reference site analysis for region selection. The number of classification groups (n groups), within group similarity (W), and between group similarity (B) for each classification scenario. Classification strength (CS%) represents the difference of within group and between group similarities.

Classification	n groups	W	B	W-B (CS%)	p	R
Major Basin	4	0.35	0.26	9.10	0.001	0.26
Ecoregion	3	0.33	0.26	7.20	0.001	0.21
Ecoregion-Basin	9	0.40	0.27	13.50	0.001	0.38

Table 3.1-2: Final biomonitoring region classifications by HUC8 watershed name (HUC8 number) with total sample sizes (N=1089 statewide).

Mon CA & RV (N=253)	Ohio CA (N=254)	Ohio & Mon WAP (N=279)	UK (N=198)	Potomac (N=105)
Cheat (5020004)	Coal (5050009)	Big Sandy (5070204)	Gauley (5050005)	S. Branch Potomac (2070001)
Elk (5050007)	Elk (5050007)	Little Kanawha (5030203)	Greenbrier (5050003)	N. Branch Potomac (2070002)
Tygart Valley (5020001)	Tug (5070201)	Little Musringum- Middle Island (5030201)	Lower New (5050004)	Cacapon (2070003)
Youghiogheny (5020006)	Upper Guyandotte (5070101)	Lower Guyandotte (5070102)	Middle New (5050002)	Potomac (2070004)
	Upper Kanawha (5050006)	Lower Kanawha (5050008)		Shenandoah Hardy (2070006)
		Lower Monongahela (5020005)		Shenandoah Jefferson (2070007)
		Raccoon-Symmes (5090101)		
		Twelvepole (5090102)		
		Upper Monongahela (5020003)		
		Upper Ohio (5030101)		
		Upper Ohio-Shade (5030202)		
		Upper Ohio- Wheeling (5030106)		
		West Fork (5020002)		

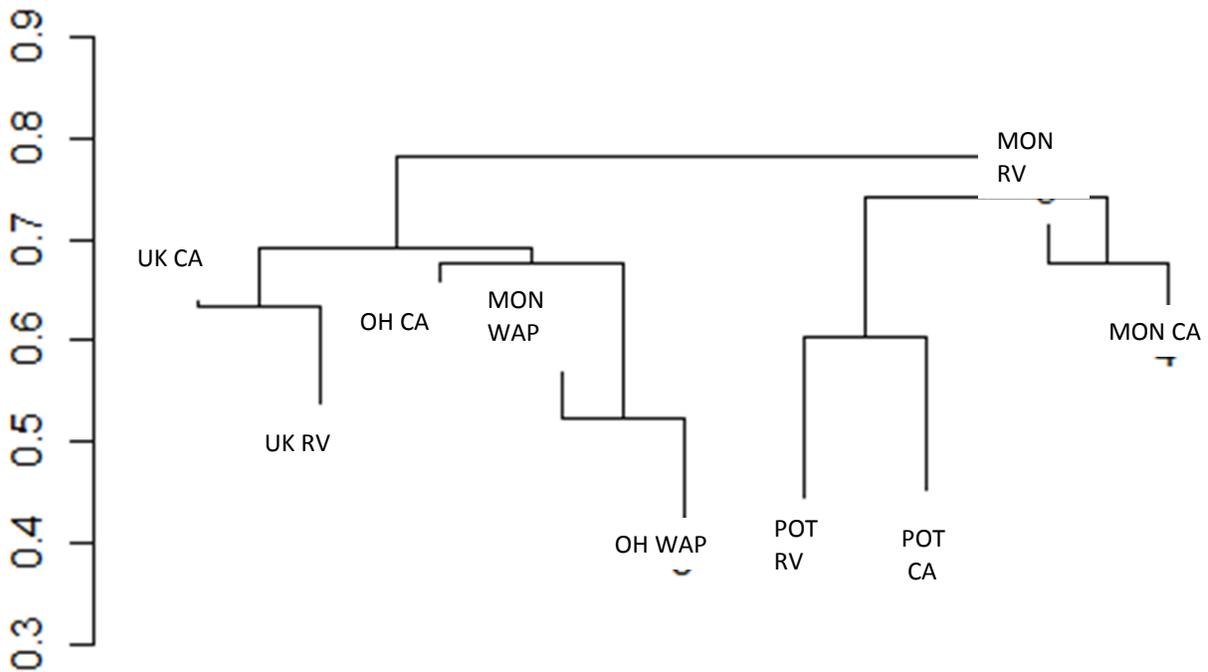


Figure 3.0.1-1: MEANSIM dendrogram groups based on fish species abundances at all reference sites. These results indicate that the Upper Kanawha Central Appalachians (UK CA) and Upper Kanawha Ridge/Valley (UK RV), Monongahela Ridge/Valley (MON RV) and Monongahela Central Appalachians (MON CA), Potomac Ridge/Valley (POT RV) and Potomac Central Appalachians (POT CA), Monongahela Western Allegheny Plateau (MON WAP) and Ohio Western Allegheny Plateau (OH WAP), and Ohio Central Appalachians (OH CA) should all be defined as separated study regions.

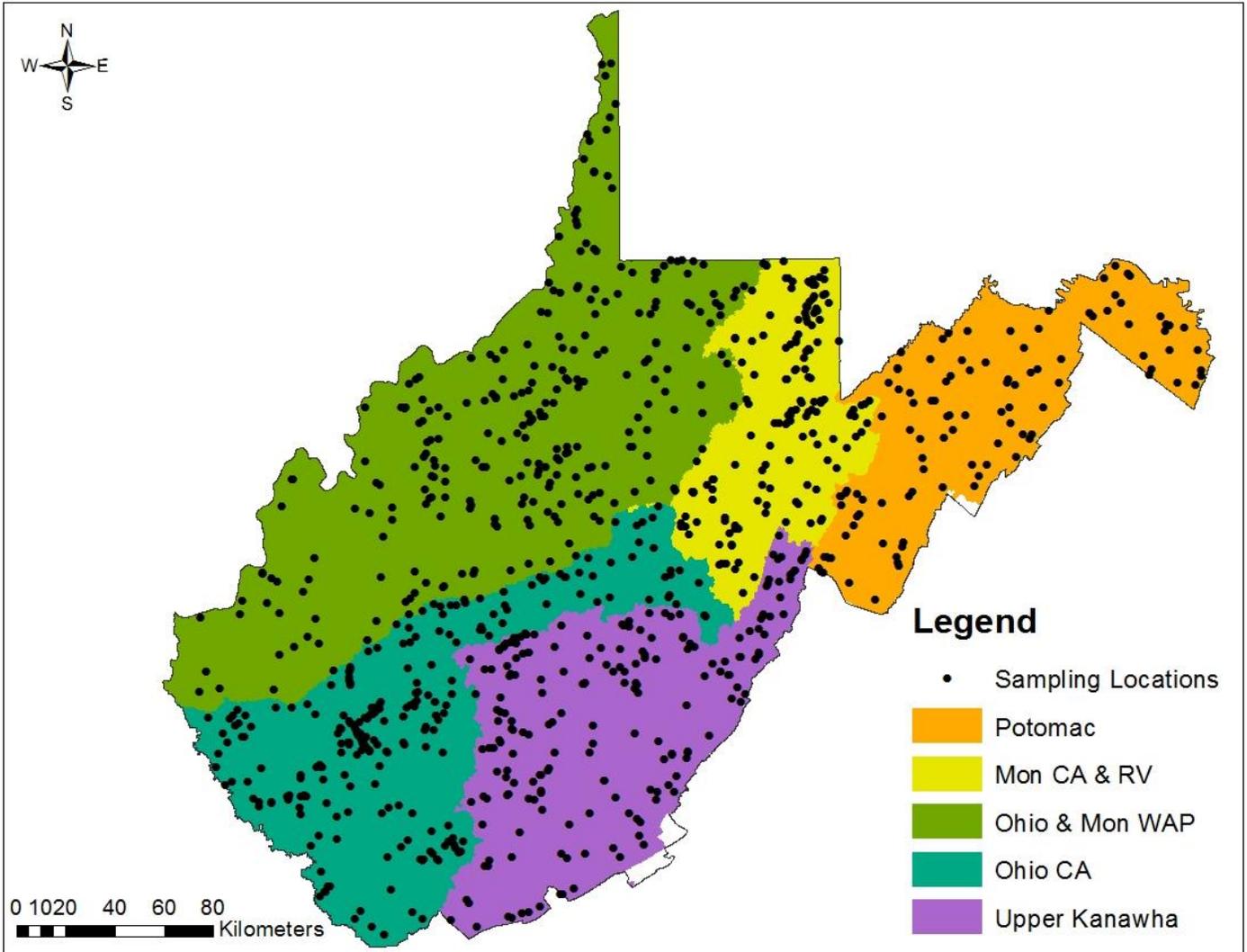


Figure 3.1-2: Selected model regions as a result of MEANSIM and ANOSIM analysis. Ohio CA region was selected as a separate region due to high sample size and professional opinion.

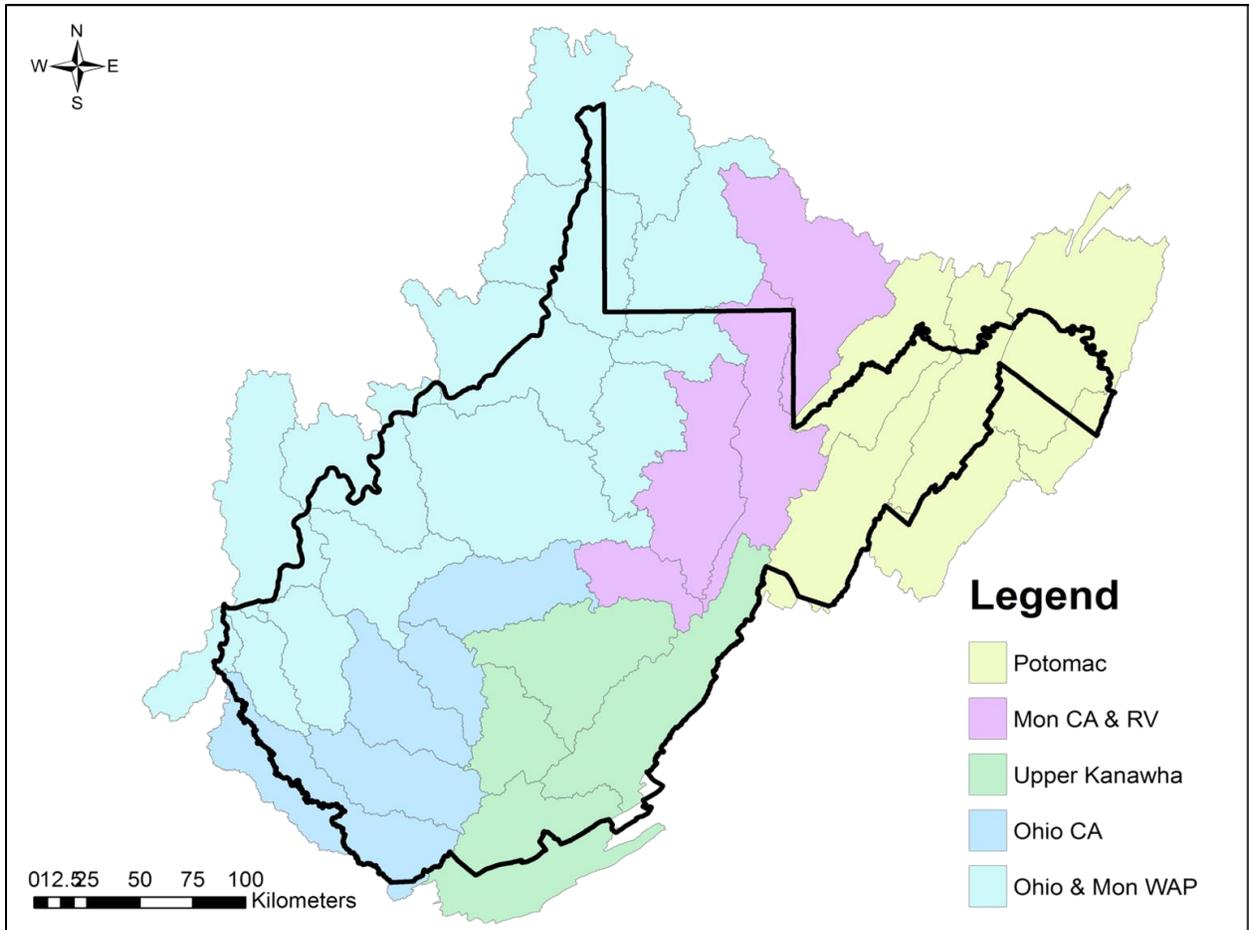


Figure 3.1-3: Final fish IBI biomonitoring regions based on similarity analysis and arrangement to include distinct watershed boundaries from whole HUC8 or HUC12 (Elk watershed) watersheds.

3.2 CLASSIFICATION OF SITE TYPES

Principal Component Analysis (PCA) was conducted on anthropogenic landscape variables within each model region serving as a human disturbance gradient. The human disturbance gradient in the Mon CA and R/V model region are represented by 3 main PC axes. In this region, each axis is represented as a development, development/mining, and mining only disturbance gradients, respectively (Table 3.2-1). The human disturbance gradient axes of the Ohio CA are represented by two developmental only axes and one development/mining axis (Table 3.2-2). The Ohio and Mon WAP region human disturbance gradients are represented by a development only axis, a mining only axis, an agriculture/development axis, and an agriculture/mining axis (Table 3.2-3). Similar to the other regions, the Upper Kanawha region human disturbance gradients are represented by a development only axis, a mining only axis, followed by an agriculture/development axis (Table 3.2-4).

Using the sum of PC scores of the first two Principal Component axes provided a quantitative way to select additional least disturbed reference sites from the database. Adding these additional sites into the reference pool broadened the reference conditions spanning from the smallest to the largest streams in the database which allowed us to make more appropriate inferences about the larger streams. Stressed sites were only classified using PCA and water quality criteria. The addition of these sites allow for the discrimination of IBI and metric values between reference and stressed sites to be determined.

Summary statistics for natural landscape variables for reference sites (Reference), stressed sites, and all other sites not classified as reference or stressed, as well as cold water streams (not used in IBI analysis) for the Mon CA and RV, Ohio CA, Ohio and Mon WAP, and Upper Kanawha are presented in Tables 3.2-4 to 3.2-7.

Table 3.2-1: PCA results for the development of a landscape based human stressor gradient in the Mon CARV region.

Mon CARV: Variables	PC 1	PC 2	PC 3
Cum. SD			
Cum. NPDES	-0.44		
Cum. Sur. Den.	-0.394	-0.387	
Cum. Und. Den.			
Cum. Sep & Sew. Den.	-0.394		
% Surface Mining			-0.612
% Grassland		0.388	
% Agriculture			
% Cum. Development		0.388	
% Cum. Grassland		0.447	
% Cum. Surface Mining			-0.599

Table 3.2-2: PCA results for the development of a landscape based human stressor gradient in the Ohio CA region.

Ohio CA: Variables	PC 1	PC 2	PC 3
Cum. SD	-0.41		
Cum. NPDES		-0.607	
Cum. Sur. Den.			
Cum. Und. Den.			
Cum. Sep & Sew. Den.	-0.372		-0.46
% Surface Mining			
% Grassland			0.382
% Agriculture			
% Cum. Development		-0.542	
% Cum. Grassland		-0.374	
% Cum. Surface Mining			0.37

Table 3.2-3: PCA results for the development of a landscape based human stressor gradient in the Ohio Mon WAP region.

Ohio Mon WAP:				
Variables	PC 1	PC 2	PC 3	PC4
Cum. SD	-0.421			
Cum. NPDES	-0.456			
Cum. Sur. Den.		-0.527		
Cum. Und. Den.		-0.522		
Cum. Sep & Sew. Den.	-0.386			
% Surface Mining				-0.557
% Grassland			-0.584	
% Agriculture			-0.604	0.406
% Cum. Development	-0.417			
% Cum. Grassland	-0.384			
% Cum. Surface Mining		-0.482		

Table 3.2-4: PCA results for the development of a landscape based human disturbance gradient in the Upper Kanawha region.

UK: Variables	PC 1	PC 2	PC 3
Cum. SD	-0.436		
Cum. NPDES	-0.44		
Cum. Sur. Den.		-0.43	
Cum. Und. Den.		-0.455	
Cum. Sep & Sew. Den.			-0.476
% Surface Mining		-0.395	
% Grassland			0.527
% Agriculture			0.47
% Cum. Development	-0.446		
% Cum. Grassland	-0.417		
% Cum. Surface Mining		-0.499	

Table 3.2-4: Natural landscape summary statistics and sample sizes for all site types in Mon CA and RV region. Values are presented as mean (minimum-maximum).

Site Type	N	Drainage Area (km ²)	Swim Distance (km)	Elevation (m)
Reference	23	82.76 (8.07 – 343.85)	51.91 (0 – 208.06)	628.58 (419 – 984)
Other	111	56.89 (7.95 – 357.60)	28.53 (0 – 120.08)	604.75 (320 – 1166)
Stressed	30	41.10 (7.47 – 234.63)	20.82 (0 – 81.11)	523.89 (354 – 1006)
Cold	77	29.31 (8.38 – 248.57)	41.37 (0 – 175.18)	725.37 (414 – 1232)

Table 3.2-5: Natural landscape summary statistics and sample sizes for all site types in Ohio CA region. Values are presented as mean (minimum-maximum).

Site Type	N	Drainage Area (km ²)	Swim Distance (km)	Elevation (m)
Reference	18	53.77 (7.34 – 307.98)	31.35 (0 – 66.98)	440.00 (251 – 703)
Other	202	73.48 (7.29 – 392.99)	27.85 (0 – 112.59)	364.72 (190 – 790)
Stressed	24	34.46 (7.34 – 154.18)	41.62 (1.7 – 109.5)	365.58 (249 – 575)
Cold	2	13.85 (13.45 – 14.25)	77.11 (59.37 – 94.84)	581.06

Table 3.2-6: Natural landscape summary statistics and sample sizes for all site types in Ohio and Mon WAP region. Values are presented as mean (minimum-maximum).

Site Type	N	Drainage Area (km ²)	Swim Distance (km)	Elevation (m)
Reference	21	79.20 (9.73 – 390.57)	32.10 (0 – 78.36)	310.97 (210 – 493)
Other	227	93.92 (7.38 – 384.85)	30.57 (0 – 137.51)	271.79 (175 – 506)
Stressed	27	98.68 (8.04 – 357.76)	24.89 (0 – 110.43)	302.56 (183 – 528)

Table 3.2-7: Natural landscape summary statistics and sample sizes for all site types in Upper Kanawha region. Values are presented as mean (minimum-maximum).

Site Type	N	Drainage Area (km ²)	Swim Distance (km)	Elevation (m)
Reference	21	74.02 (9.55 – 335.09)	37.39 (0 – 135.93)	745.38 (399 – 1035)
Other	147	96.81 (8.09 – 392.90)	35.95 (0 – 134.68)	645.95 (223 – 1074)
Stressed	11	65.74 (7.01 – 351.70)	21.21 (0 – 49.78)	632.98 (395 – 954)
Cold	19	42.75 (7.63 – 133.71)	66.22 (2.52 – 118.57)	931.20 (518 – 1137)

3.3 SETTING BASELINE EXPECTATIONS

A reduced set of metrics (i.e. passed range test) within each biomonitoring region were evaluated for their relationship with drainage area (log₁₀-transformed). Each metric, within each region, was modeled using linear models and drainage areas observed at the reference sites. Only significant models (p-value < 0.05) were used to predict metric values for all wadeable segment level watersheds in the Mon CA and RV (Table 3.3-1), Ohio CA (Table 3.3-2), Ohio and Mon WAP (Table 3.3-3), and Upper Kanawha (Table 3.3-4) in order to adjust raw metric values (Observed/Expected).

Table 3.3-1: All metrics that failed range test, adjusted using linear models, or were not adjusted (Raw).
Adjusted and Raw metrics were further considered in IBI development for the Mon CA and RV region.

Failed Range	Failed Range	Adjusted For DA	Adjusted For DA	Raw
P_Catfish	R_Cyprinid_BNDSEAT	R_Native	R_FISH	P_Native
P_Cold	R_Cyprinid_NBNDSEAT	R_CyprinidN	R_LSR	P_Game
P_Cold_SATR_ONMY	R_Cyprinid2.DEP	R_Native2.DEP	R_IN	P_Cyprinid
P_Cold2.DEP	R_CyprinidN2.DEP	P_CyprinidN	R_IP	P_GameC
P_Cottid	R_DMS	P_Cyprinid_NBNDSEAT	R_IP_SEAT	P_IP_BenthicNG
P_Cyprinid2.DEP	R_DMS2.DEP	P_Native2.DEP	R_Benthic	P_DMS
P_CyprinidN2.DEP	R_Game2.DEP	P_RGS	R_Benthic_CACO	P_Sunfish
P_GSS2.DEP	R_GameC	P_GSS	R_Cyprinid	P_Catostomidae
P_Int.DEP	R_GSS2.DEP	P_LSR	R_McC_CGS	P_McC_CGS
P_Int_Benthic.DEP	R_Int.DEP	P_NGL	R_CGS_RGS	P_Benthic2.DEP
P_Int_Cyprinid.DEP	R_Int_Benthic.DEP	P_MO	R_Fish2.DEP	P_Game2.DEP
P_Int_GSS.DEP	R_Int_Cyprinid.DEP	P_IN	R_IP2.DEP	P_DMS2.DEP
P_Int_LSR.DEP	R_Int_GSS.DEP	P_IP	R_Mod.DEP	P_Mod.DEP
P_Int_NGL.DEP	R_Int_LSR.DEP	P_IP_SEAT		R_Game
P_Int_RGS.DEP	R_Int_NGL.DEP	P_Benthic		R_GSS
P_LSR2.DEP	R_Int_RGS.DEP	P_Benthic_CACO		R_OH
P_NGL2.DEP	R_IP_BenthicNG	P_Cyprinid_BNDSEAT		R_OH_NG
P_OH2.DEP	R_LSR2.DEP	P_BND_CACO_SEAT		R_Tol.DEP
P_Percidae	R_McC_CGS2.DEP	P_OH		R_Tol_Cyprinid.DEP
R_Benthic2.DEP	R_MO	P_OH_CAAN		SW_TROPHIC
R_BND_CACO_SEAT	R_NGL	P_OH_CAAN_CACO		
R_Catfish	R_NGL2.DEP	P_OH_NG		
R_Catostomidae	R_OH_CAAN	P_IP_NonGameNB		
R_CavitySpawn	R_OH_CAAN_CACO	P_CGS_RGS		
R_CavitySpawn2.DEP	R_OH2.DEP	P_CavitySpawn		
R_CGS_RGS2.DEP	R_Percidae	P_Fish2.DEP		
R_Cold	R_RGS	P_RGS2.DEP		
R_Cold_SATR_ONMY	R_RGS2.DEP	P_IP2.DEP		
R_Cold2.DEP	R_Sunfish	P_Tol.DEP		
R_Cottid	R_Tol_Benthic.DEP	P_Tol_Benthic.DEP		
		P_Tol_Cyprinid.DEP		
		P_McC_CGS2.DEP		
		P_CGS_RGS2.DEP		
		P_CavitySpawn2.DEP		

Table 3.3-2: All metrics that failed range test, adjusted using linear models, or were not adjusted (Raw).
Adjusted and Raw metrics were further considered in IBI development for the Ohio CA region.

Failed Range	Failed Range	Adjusted For DA	Adjusted For DA	Raw
R_Game	P_Game	R_Benthic	P_Benthic	P_Benthic2.DEP
R_Cottid	P_Cottid	R_Benthic_CACO	P_Benthic_CACO	P_Catostomidae
R_Cold	P_Cold	R_Benthic2.DEP	P_BND_CACO_SEAT	P_Cyprinid
R_Cold_SATR_ONMY	P_Cold_SATR_ONMY	R_BND_CACO_SEAT	P_CavitySpawn	P_CyprinidN
R_GameC	P_GameC	R_CavitySpawn	P_CGS_RGS	P_DMS
R_Sunfish	P_Sunfish	R_Cyprinid	P_CGS_RGS2.DEP	P_DMS2.DEP
R_Catfish	P_Catfish	R_Cyprinid_BNDSEAT	P_Cyprinid_BNDSEAT	P_IP
R_GSS2.DEP	P_GSS2.DEP	R_CyprinidN	P_Cyprinid_NBNDSEAT	P_IP_BenthicNG
R_Cyprinid2.DEP	P_Cyprinid2.DEP	R_Cyprinid_NBNDSEAT	P_GSS	P_IP_NonGameNB
R_CyprinidN2.DEP	P_CyprinidN2.DEP	R_DMS	P_IN	P_LSR
R_OH2.DEP	P_OH2.DEP	R_DMS2.DEP	P_IP_SEAT	P_MO
R_Cold2.DEP	P_Cold2.DEP	R_GSS	P_IP2.DEP	P_Mod.DEP
R_Game2.DEP	P_Game2.DEP	R_IN	P_LSR2.DEP	P_Native
R_Int.DEP	P_Int.DEP	R_IP	P_McC_CGS	P_OH
R_Int_Benthic.DEP	P_Int_Benthic.DEP	R_IP_BenthicNG	P_McC_CGS2.DEP	P_OH_CAAN
R_Int_Cyprinid.DEP	P_Int_Cyprinid.DEP	R_IP_NonGameNB	P_Native2.DEP	P_OH_CAAN_CACO
R_Int_RGS.DEP	P_Int_RGS.DEP	R_IP_SEAT	P_NGL	P_OH_NG
R_Int_GSS.DEP	P_Int_GSS.DEP	R_IP2.DEP	P_NGL2.DEP	P_Percidae
R_Int_LSR.DEP	P_Int_LSR.DEP	R_LSR	P_RGS	P_Tol_Benthic.DEP
R_Int_NGL.DEP	P_Int_NGL.DEP	R_LSR2.DEP	P_RGS2.DEP	R_Catostomidae
R_CavitySpawn2.DEP	P_CavitySpawn2.DEP	R_MO	P_Tol.DEP	R_CGS_RGS
		R_Mod.DEP		R_CGS_RGS2.DEP
		R_Native		R_McC_CGS
		R_Native2.DEP		R_McC_CGS2.DEP
		R_NGL		R_RGS2.DEP
		R_NGL2.DEP		R_Tol_Benthic.DEP
		R_OH		SW_TROPHIC
		R_OH_CAAN		P_Benthic2.DEP
		R_OH_CAAN_CACO		P_Catostomidae
		R_OH_NG		
		R_Percidae		
		R_RGS		

Table 3.3-3: All metrics that failed range test, adjusted using linear models, or were not adjusted (Raw). Adjusted and Raw metrics were further considered in IBI development for the Ohio and Mon WAP region.

Failed Range	Adjusted for DA	Adjusted for DA	Adjusted for DA	Raw
P_Catfish	Richness	R_Mod.DEP	Proportion2.DEP	R_Game
P_Cold	R_Native	R_Int.DEP	P_Native2.DEP	R_OH
P_Cold_SATR_ONMY	R_RGS	R_Tol_Benthic.DEP	P_RGS2.DEP	R_OH_CAAN
P_Cold2.DEP	R_GSS	R_Int_Benthic.DEP	P_GSS2.DEP	R_OH_CAAN_CACO
P_Cottid	R_LSR	R_Int_RGS.DEP	P_LSR2.DEP	R_GameC
P_Int_Cyprinid.DEP	R_NGL	R_Int_LSR.DEP	P_NGL2.DEP	R_OH_NG
P_Int_GSS.DEP	R_MO	R_Int_NGL.DEP	P_IP2.DEP	R_Sunfish
P_OH2	R_IN	R_McC_CGS2.DEP	P_Cyprinid2.DEP	R_Catostomidae
R_Catfish	R_IP	R_CGS_RGS2.DEP	P_CyprinidN2.DEP	R_McC_CGS
R_Cold	R_IP_SEAT	R_CavitySpawn2.DEP	P_Tol.DEP	R_OH2.DEP
R_Cold_SATR_ONMY	R_Benthic	P_RGS	P_Int.DEP	R_Tol.DEP
R_Cold2.DEP	R_Benthic_CACO	P_GSS	P_Tol_Benthic.DEP	R_Tol_Cyprinid.DEP
R_Cottid	R_Cyprinid	P_NGL	P_Tol_Cyprinid.DEP	P_Native
R_Int_Cyprinid.DEP	R_Cyprinid_BNDSEAT	P_IN	P_Int_RGS.DEP	P_Game
	R_CyprinidN	P_IP_SEAT	P_Int_LSR.DEP	P_LSR
	R_CyprinidN_BNDSEAT	P_Benthic	P_Int_NGL.DEP	P_MO
	R_BND_CACO_SEAT	P_Cyprinid_BNDSEAT	P_McC_CGS2.DEP	P_IP
	R_IP_BenthicNG	P_CyprinidN_BNDSEAT	P_CGS_RGS2.DEP	P_Benthic_CACO
	R_IP_NonGameNB	P_BND_CACO_SEAT	P_CavitySpawn2.DEP	P_Cyprinid
	R_DMS	P_GameC		P_CyprinidN
	R_Percidae	P_Catostomidae		P_OH
	R_CGS_RGS			P_OH_CAAN
	R_CavitySpawn			P_OH_CAAN_CACO
	R_Richness2.DEP			P_OH_NG
	R_Native2.DEP			P_IP_BenthicNG
	R_RGS2.DEP			P_IP_NonGameNB
	R_GSS2.DEP			P_DMS
	R_LSR2.DEP			P_Percidae
	R_NGL2.DEP			P_Sunfish
	R_IP2.DEP			P_McC_CGS
	R_Benthic2.DEP			P_CGS_RGS
	R_Cyprinid2.DEP			P_CavitySpawn
	R_CyprinidN2.DEP			P_Benthic2.DEP
	R_Game2.DEP			P_OH2.DEP
	R_DMS2.DEP			P_Game2.DEP
				P_DMS2.DEP

Table 3.3-4: All metrics that failed range test, adjusted using linear models, or were not adjusted (Raw).
Adjusted and Raw metrics were further considered in IBI development for the Upper Kanawha region.

Failed Range	Adjusted for DA	Adjusted for DA	Raw	Raw
P_Catfish	Richness	P_Game	R_RGS	P_Native
P_Cold2.DEP	R_Native	P_NGL	R_GSS	P_RGS
P_Cottid	R_Game	P_MO	R_LSR	P_GSS
P_GSS2.DEP	R_NGL	P_IN	R_MO	P_LSR
P_Int_Benthic	R_IN	P_IP	R_CyprinidN	P_Cyprinid
P_Int_Cyprinid.DEP	R_IP	P_IP_SEAT	R_OH	P_CyprinidN
P_Int_GSS.DEP	R_IP_SEAT	P_Benthic	R_OH_CAAN	P_CyprinidN_BNDSEAT
P_Int_LSR.DEP	R_Benthic_CACO	P_Benthic_CACO	R_OH_CAAN_CACO	P_IP_BenthicNG
P_Int_NGL.DEP	R_Cyprinid	P_Cyprinid_BNDSEAT	R_Cold	P_DMS
P_NGL2.DEP	R_Cyprinid_BNDSEAT	P_BND_CACO_SEAT	R_Cold_SATR_ONMY	P_Percidae
P_OH2.DEP	R_CyprinidN_BNDSEAT	P_OH	R_OH_NG	P_McC_CGS
P_Sunfish	R_BND_CACO_SEAT	P_OH_CAAN	R_DMS	P_CGS_RGS
R_Catfish	R_GameC	P_OH_CAAN_CACO	R_Percidae	P_CavitySpawn
R_Cold2.DEP	R_IP_BenthicNG	P_Cold	R_Catostomidae	P_Native2.DEP
R_Cottid	R_IP_NonGameNB	P_Cold_SATR_ONMY	R_CavitySpawn	P_RGS2.DEP
R_GSS2.DEP	R_McC_CGS	P_GameC	R_RGS2.DEP	P_LSR2.DEP
R_Int_Cyprinid.DEP	R_CGS_RGS	P_OH_NG	R_LSR2.DEP	P_Benthic2.DEP
R_Int_GSS.DEP	R_Richness2.DEP	P_IP_NonGameNB	R_DMS2.DEP	P_CyprinidN2.DEP
R_Int_LSR.DEP	R_Native2.DEP	P_Catostomidae	R_Tol.DEP	P_DMS2.DEP
R_Int_NGL.DEP	R_IP2.DEP	Proportion2.DEP	R_Tol_Benthic.DEP	P_Int.DEP
R_NGL2.DEP	R_Benthic2.DEP	P_IP2.DEP	R_Tol_Cyprinid.DEP	P_Int_Benthic.DEP
R_OH2.DEP	R_Cyprinid2.DEP	P_Cyprinid2.DEP	R_Int_RGS.DEP	P_Int_RGS.DEP
	R_CyprinidN2.DEP	P_Game2.DEP	R_CavitySpawn2.DEP	P_McC_CGS2.DEP
	R_Game2.DEP	P_Tol.DEP		P_CGS_RGS2.DEP
	R_Mod.DEP	P_Mod.DEP		P_CavitySpawn2.DEP
	R_Int.DEP	P_Tol_Benthic.DEP		SW_TROPHIC
	R_Int_Benthic.DEP	P_Tol_Cyprinid.DEP		
	R_McC_CGS2.DEP			
	R_CGS_RGS2.DEP			

3.4 TESTING CANDIDATE METRICS

All candidate metrics (i.e. passed range test; see Section 3.3 for results) were further evaluated according to their discrimination and correlations with human disturbance. Smaller subsets of metrics were checked for redundancy within each model region.

The separation of metric values between reference and stressed sites (i.e. discrimination efficiency) was evaluated within each biomonitoring region. Within each of these regions, several metrics demonstrate high differences between reference and stressed sites (i.e. high discrimination) while some metrics showed little to no differences. A metric had to have a discriminate efficiency of $\geq 60\%$ before it could be further considered for inclusion in a final IBI.

Each metric was then evaluated for correlation with human disturbances and stressors. If a metric had a high correlation ($>|0.30|$) then it was flagged as a potential metric for inclusion in a final IBI. Each metric was correlated with: pH, specific conductance (SPC), cumulative structure density, and cumulative percentages of surface mining, agriculture, development, and forest. The results from the metric discrimination and correlation with human disturbance, as well as the 25th and 75th percentiles for the references distribution which was used in the calculation of DE, within the Mon CA and RV, Ohio CA, Ohio and Mon WAP, and Upper Kanawha are presented in Tables 3.4-1, 3.4-2, 3.4-3, 3.4-4, respectively.

A final list of potential metrics was generated if the metric had high to moderate discrimination (DE% > 60) and correlated with at least one human disturbance variable. Redundancy was then evaluated on shorter lists of metrics with Spearman correlations for all pair-wise comparisons of metrics. Only metrics that were highly correlated ($>|0.90|$) with at least one other metric was flagged. There was no strict criterion for excluding metrics based on redundancy, but correlations were considered during final metric selection.

Table 3.4-1: Spearman correlation coefficients for all metrics in the Mon CA-RV region with surface mining (%), development (%), grassland (%), agriculture (%), forest (%), structure density (#/km²; SD), specific conductance (SPC), and pH. Discrimination efficiency (%; DE) and 25th and 75th percentiles (reference distribution) were also calculated for each metric. Table is sorted by descending discrimination efficiency.

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
P_IP_BenthicNG	-0.352	-0.199	-0.158	-0.195	0.244	-0.011	-0.591	0.350	93.33	0.34	0.47
P_Benthic2.DEP*	-0.363	-0.208	-0.167	-0.202	0.253	-0.010	-0.575	0.314	93.33	0.35	0.47
Adj.P_Benthic	-0.324	-0.194	-0.106	-0.183	0.182	-0.142	-0.597	0.442	90.00	0.74	1.20
Adj.R_McC_CGS*	-0.304	-0.303	-0.198	-0.300	0.306	-0.150	-0.601	0.320	90.00	0.84	1.18
P_DMS	-0.337	-0.161	-0.151	-0.160	0.210	0.035	-0.544	0.381	90.00	0.21	0.38
P_DMS2.DEP	-0.337	-0.161	-0.151	-0.160	0.210	0.035	-0.544	0.381	90.00	0.21	0.38
Adj.P_Benthic_CACO	-0.351	-0.234	-0.139	-0.213	0.222	-0.149	-0.609	0.417	86.67	0.73	1.20
Adj.P_CavitySpawn2.DEP	-0.321	-0.181	-0.142	-0.134	0.199	-0.005	-0.564	0.359	86.67	0.62	1.38
Adj.R_Fish2	-0.310	-0.235	-0.201	-0.254	0.300	-0.015	-0.494	0.335	86.67	0.84	1.12
Adj.R_IP2	-0.312	-0.232	-0.199	-0.251	0.298	-0.014	-0.493	0.337	86.67	0.85	1.13
Adj.R_Benthic*	-0.323	-0.214	-0.106	-0.236	0.237	-0.001	-0.502	0.438	86.67	0.85	1.14
Adj.R_Fish2.DEP*	-0.302	-0.197	-0.118	-0.211	0.225	0.032	-0.543	0.354	86.67	0.89	1.11
P_McC_CGS	-0.223	-0.327	-0.189	-0.247	0.250	-0.176	-0.516	0.390	86.67	0.35	0.51
Adj.R_Native2	-0.277	-0.193	-0.169	-0.209	0.263	0.076	-0.512	0.338	83.33	0.74	1.21
Adj.P_McC_CGS2	-0.195	-0.256	-0.185	-0.204	0.252	-0.063	-0.460	0.224	83.33	0.61	1.36
Adj.R_IP_SEAT	-0.287	-0.122	-0.080	-0.146	0.162	0.106	-0.476	0.364	83.33	0.87	1.11
Adj.R_IP2.DEP	-0.307	-0.198	-0.123	-0.208	0.228	0.037	-0.548	0.354	83.33	0.89	1.13
P_Benthic2	-0.364	-0.220	-0.193	-0.200	0.265	-0.044	-0.578	0.312	83.33	0.23	0.46
Adj.R_CGS_RGS	-0.320	-0.250	-0.145	-0.238	0.260	0.006	-0.518	0.365	80.00	0.91	1.18
Adj.P_McC_CGS2.DEP	-0.184	-0.252	-0.185	-0.220	0.260	-0.050	-0.451	0.224	80.00	0.66	1.43
Adj.R_IP	-0.306	-0.156	-0.102	-0.182	0.193	0.064	-0.450	0.370	80.00	0.84	1.12
Adj.R_Benthic_CACO	-0.325	-0.170	-0.077	-0.209	0.207	0.070	-0.545	0.428	80.00	0.85	1.11
Adj.R_Mod	-0.291	-0.192	-0.153	-0.160	0.237	0.061	-0.424	0.322	80.00	0.78	1.20
Adj.R_Native2.DEP	-0.298	-0.186	-0.117	-0.197	0.222	0.090	-0.529	0.351	76.67	0.78	1.20
Adj.P_Native2	-0.201	-0.295	-0.352	-0.274	0.363	-0.028	-0.441	0.221	76.67	0.83	1.17
Adj.P_Native2.DEP	-0.202	-0.282	-0.321	-0.266	0.343	-0.002	-0.430	0.215	76.67	0.84	1.13
Adj.P_Fish2	-0.220	-0.295	-0.366	-0.271	0.368	-0.064	-0.418	0.207	76.67	0.84	1.17

Adj.P_IP2	-0.220	-0.295	-0.366	-0.271	0.368	-0.064	-0.418	0.207	76.67	0.84	1.17
Adj.P_Tol	0.074	0.071	0.145	0.125	-0.156	0.196	-0.047	0.386	76.67	0.65	1.31
Adj.P_CGS_RGS	-0.252	-0.352	-0.230	-0.284	0.306	-0.076	-0.448	0.471	76.67	0.85	1.12
Adj.P_CGS_RGS2	-0.199	-0.294	-0.227	-0.256	0.304	-0.035	-0.437	0.269	76.67	0.59	1.30
Adj.P_Fish2.DEP*	-0.218	-0.282	-0.337	-0.265	0.350	-0.034	-0.407	0.197	76.67	0.88	1.13
Adj.P_IP2.DEP	-0.219	-0.283	-0.338	-0.265	0.350	-0.037	-0.408	0.198	76.67	0.86	1.13
Adj.P_IN*	-0.225	-0.239	-0.282	-0.149	0.249	0.123	-0.361	0.321	73.33	0.86	1.16
Adj.P_Tol.DEP	0.055	0.053	0.102	0.110	-0.127	0.151	-0.066	0.404	73.33	0.70	1.26
Adj.R_IN	-0.298	-0.141	-0.110	-0.149	0.181	0.158	-0.440	0.404	73.33	0.75	1.14
Adj.R_Mod.DEP	-0.252	-0.176	-0.104	-0.158	0.206	0.180	-0.477	0.359	73.33	0.81	1.13
P_Mod.DEP	-0.165	-0.262	-0.288	-0.223	0.309	0.000	-0.464	0.180	73.33	0.39	0.67
Adj.P_CavitySpawn	-0.327	-0.118	-0.056	-0.019	0.101	0.125	-0.471	0.327	70.00	0.69	1.29
Adj.R_FISH	-0.318	-0.127	-0.076	-0.156	0.162	0.090	-0.438	0.388	70.00	0.82	1.16
Adj.R_LSR	-0.297	-0.157	-0.113	-0.189	0.203	0.053	-0.431	0.373	70.00	0.77	1.17
Adj.R_Cyprinid*	-0.324	-0.185	-0.098	-0.195	0.190	0.034	-0.437	0.408	70.00	0.68	1.13
P_Mod	-0.185	-0.230	-0.303	-0.185	0.291	-0.011	-0.408	0.192	70.00	0.32	0.54
Adj.P_CGS_RGS2.DEP	-0.213	-0.257	-0.151	-0.214	0.230	0.066	-0.403	0.324	66.67	0.58	1.28
Adj.R_CyprinidN	-0.330	-0.180	-0.089	-0.177	0.168	0.029	-0.427	0.384	63.33	0.68	1.20
Adj.P_RGS2.DEP	-0.114	-0.103	-0.198	-0.207	0.296	0.160	-0.351	0.180	63.33	0.40	1.19
Adj.R_Native	-0.313	-0.116	-0.061	-0.137	0.141	0.119	-0.419	0.379	60.00	0.78	1.18
R_IP_NonGameNB	-0.237	-0.153	-0.142	-0.187	0.225	0.059	-0.416	0.324	60.00	2.00	5.00
SW_TROPHIC	-0.281	-0.108	-0.018	-0.073	0.098	0.109	-0.443	0.432	60.00	2.76	3.24
Adj.P_MO	-0.232	-0.071	0.074	0.010	-0.018	0.013	-0.421	0.452	56.67	0.47	1.56
P_Catostomidae	-0.114	0.027	0.050	-0.001	0.039	0.110	-0.329	0.363	56.67	0.02	0.08
Adj.P_Cyprinid_NBNDSEAT	-0.192	-0.181	-0.143	-0.129	0.203	0.241	-0.315	0.360	53.33	0.74	1.26
Adj.P_RGS	-0.182	-0.198	-0.179	-0.180	0.256	0.265	-0.314	0.383	53.33	0.57	1.25
Adj.P_Cyprinid_BNDSEAT	-0.191	-0.179	-0.141	-0.128	0.202	0.243	-0.314	0.358	53.33	0.74	1.27
Adj.P_NGL	-0.217	0.021	0.084	-0.032	0.012	0.100	-0.276	0.437	50.00	0.57	1.35
P_Cyprinid	0.005	-0.200	-0.022	-0.081	0.048	-0.008	-0.215	0.446	50.00	0.47	0.70
Adj.P_IP_SEAT	-0.121	-0.215	-0.325	-0.192	0.279	0.068	-0.245	0.124	43.33	0.86	1.15
R_Game	-0.201	-0.023	-0.024	-0.046	0.057	0.095	-0.295	0.285	43.33	2.00	5.00

R_GSS	-0.176	-0.059	0.107	-0.024	-0.036	-0.008	-0.396	0.317	43.33	2.00	3.00
R_Game2	-0.217	-0.112	-0.085	-0.123	0.142	-0.013	-0.358	0.263	43.33	2.00	4.00
Adj.P_CyprinidN	-0.006	-0.181	0.016	-0.064	0.010	-0.011	-0.176	0.409	40.00	0.82	1.13
Adj.P_LSR	0.003	-0.144	-0.022	-0.088	0.029	0.006	-0.179	0.445	40.00	0.78	1.21
Adj.P_Tol_Cyprinid	-0.029	-0.033	0.075	0.023	-0.048	0.071	-0.226	0.499	40.00	0.60	1.43
Adj.P_Tol_Cyprinid.DEP	-0.050	-0.033	0.080	0.024	-0.050	0.073	-0.232	0.499	40.00	0.68	1.31
Adj.P_GSS	-0.021	-0.043	0.120	0.012	-0.095	-0.068	-0.265	0.317	40.00	0.58	1.32
P_GameC	-0.064	-0.110	-0.193	-0.043	0.141	0.026	-0.167	0.206	40.00	0.01	0.04
Adj.P_IP_NonGameNB	-0.004	-0.106	0.032	0.020	-0.043	0.112	-0.183	0.390	30.00	0.50	1.29
Adj.P_IP	0.024	-0.136	-0.217	-0.118	0.150	0.040	-0.138	0.086	30.00	0.90	1.12
P_Game2.DEP	-0.114	-0.049	-0.098	-0.053	0.112	0.116	-0.151	0.257	30.00	0.02	0.04
R_Tol.DEP	-0.241	0.044	0.066	0.024	-0.029	0.195	-0.293	0.380	30.00	3.50	6.00
Adj.P_BND_CACO_SEAT	-0.060	-0.127	0.061	-0.025	-0.018	-0.122	-0.330	0.308	26.67	0.31	1.39
P_Game2	-0.031	-0.008	-0.089	-0.078	0.108	0.106	-0.080	0.157	23.33	0.02	0.05
P_Sunfish	-0.024	0.037	0.002	0.031	-0.044	0.207	0.024	0.172	23.33	0.00	0.02
Adj.P_OH_CAAAN	-0.180	-0.129	0.021	0.039	0.001	0.072	-0.334	0.496	20.00	0.60	1.36
P_Game	0.011	-0.008	-0.109	-0.056	0.076	0.141	-0.030	0.144	20.00	0.02	0.05
R_Tol_Cyprinid.DEP	-0.309	-0.057	0.002	-0.052	0.058	0.159	-0.373	0.436	20.00	3.00	4.00
Adj.R_Tol_Benthic	-0.288	-0.118	0.017	-0.135	0.116	0.065	-0.460	0.380	16.67	0.86	1.18
R_Tol	-0.246	-0.010	0.032	-0.042	0.029	0.171	-0.352	0.412	16.67	5.00	8.00
R_Tol_Cyprinid	-0.282	-0.075	-0.017	-0.069	0.069	0.149	-0.379	0.463	16.67	3.00	4.00
Adj.P_OH	-0.206	-0.092	0.040	0.012	0.007	0.075	-0.371	0.495	13.33	0.55	1.47
Adj.P_OH_NG	-0.205	-0.099	0.036	0.008	0.012	0.070	-0.370	0.497	13.33	0.55	1.47
Adj.P_OH_CAAAN_CACO	-0.193	-0.174	0.003	0.011	0.035	0.066	-0.346	0.480	10.00	0.50	1.44
Adj.P_Tol_Benthic.DEP	-0.231	-0.099	-0.005	-0.077	0.058	-0.097	-0.465	0.468	10.00	0.45	1.72
P_Native	0.019	-0.097	-0.058	-0.024	-0.003	0.084	-0.177	0.227	10.00	0.94	1.00
R_OH	-0.272	-0.017	0.036	-0.029	0.020	0.145	-0.357	0.400	10.00	2.00	4.00
R_OH_NG	-0.270	-0.042	-0.042	-0.042	-0.042	-0.042	-0.042	-0.042	10.00	2.00	4.00
P_FISH	-0.121	-0.199	-0.174	-0.122	0.158	0.082	-0.409	0.534	0.00	1.00	1.00
P_Tol_Benthic	-0.252	-0.089	0.037	-0.084	0.045	-0.026	-0.458	0.505	0.00	0.16	0.34

Table 3.4-2: Spearman correlation coefficients for all metrics in the Ohio CA region with surface mining (%), development (%), grassland (%), agriculture (%), forest (%), structure density (#/km²; SD), specific conductance (SPC), and pH. Discrimination efficiency (%; DE) and 25th and 75th percentiles (reference distribution) were also calculated for each metric. Table is sorted by descending discrimination efficiency.

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
Adj.R_DMS*	-0.516	-0.084	0.331	0.264	0.311	0.430	-0.379	-0.074	95.83	0.61	1.30
Adj.R_Benthic2.DEP	-0.508	-0.055	0.315	0.242	0.300	0.462	-0.343	-0.052	95.83	0.66	1.32
Adj.R_DMS2.DEP	-0.516	-0.084	0.331	0.264	0.311	0.430	-0.379	-0.074	95.83	0.61	1.30
Adj.R_IP_BenthicNG	-0.496	-0.029	0.336	0.277	0.271	0.470	-0.334	-0.016	91.67	0.66	1.28
R_CavitySpawn	-0.528	0.315	0.315	0.315	0.315	0.315	0.315	0.315	91.67	2.00	5.00
Adj.P_CavitySpawn	-0.394	-0.079	0.301	0.296	0.215	0.325	-0.331	-0.096	87.50	0.76	1.18
Adj.R_GSS	-0.473	-0.133	0.342	0.163	0.263	0.291	-0.344	-0.166	83.33	0.62	1.27
Adj.R_Benthic_CACO	-0.482	-0.025	0.369	0.241	0.248	0.433	-0.348	-0.047	83.33	0.74	1.26
Adj.R_IP2.DEP	-0.474	-0.046	0.293	0.225	0.296	0.461	-0.316	-0.076	83.33	0.60	1.33
Adj.R_Mod.DEP	-0.478	-0.043	0.276	0.219	0.283	0.430	-0.306	-0.114	83.33	0.71	1.24
Adj.R_IN	-0.462	-0.054	0.323	0.226	0.259	0.450	-0.349	-0.120	79.17	0.62	1.39
Adj.R_IP	-0.471	-0.057	0.292	0.204	0.298	0.431	-0.296	-0.093	79.17	0.61	1.31
Adj.R_IP_SEAT	-0.473	-0.048	0.287	0.206	0.304	0.448	-0.290	-0.095	79.17	0.56	1.32
Adj.R_Percidae	-0.491	-0.062	0.340	0.242	0.293	0.438	-0.349	-0.087	79.17	0.53	1.36
Adj.R_Richness2.DEP*	-0.482	-0.040	0.316	0.229	0.291	0.474	-0.325	-0.102	79.17	0.58	1.30
Adj.R_Native2.DEP	-0.469	-0.042	0.312	0.210	0.277	0.453	-0.331	-0.114	79.17	0.60	1.33
Adj.R_RGS*	-0.446	-0.029	0.332	0.239	0.250	0.427	-0.352	-0.065	70.83	0.65	1.33
Adj.R_Benthic	-0.484	-0.010	0.406	0.268	0.230	0.461	-0.327	-0.020	70.83	0.68	1.24
R_Tol_Benthic.DEP	-0.233	0.123	0.413	0.152	0.009	0.345	-0.151	0.023	70.83	1.50	2.00
Adj.Richness	-0.466	-0.035	0.352	0.212	0.251	0.422	-0.307	-0.103	66.67	0.65	1.29
Adj.R_Native	-0.457	-0.042	0.348	0.196	0.245	0.402	-0.312	-0.110	66.67	0.66	1.29
R_CGS_RGS	-0.402	0.024	0.321	0.290	0.218	0.577	-0.223	0.023	66.67	4.00	11.00
P_Percidae	-0.157	-0.054	0.029	0.182	0.180	0.358	0.035	0.124	66.67	0.09	0.19
Adj.R_NGL	-0.397	0.031	0.363	0.215	0.189	0.459	-0.289	-0.035	62.50	0.58	1.32
P_DMS	-0.125	-0.099	-0.017	0.166	0.191	0.280	0.044	0.154	62.50	0.18	0.27
Adj.R_NGL2.DEP*	-0.349	0.060	0.306	0.223	0.171	0.409	-0.240	-0.002	58.33	0.65	1.70
Adj.P_IN*	-0.221	-0.235	0.001	0.128	0.276	0.234	-0.192	-0.114	58.33	0.81	1.29

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
Adj.P_IP_SEAT	-0.070	-0.185	-0.171	0.045	0.238	0.146	0.068	0.035	58.33	0.80	1.23
Adj.Proportion2.DEP	-0.074	-0.141	-0.099	0.119	0.207	0.241	0.006	0.055	58.33	0.80	1.21
Adj.P_Native2.DEP	-0.073	-0.139	-0.101	0.111	0.195	0.234	-0.039	0.040	58.33	0.82	1.22
P_IP	0.084	-0.151	-0.340	-0.096	0.198	-0.023	0.118	-0.026	58.33	0.58	0.80
P_IP2.DEP	-0.043	-0.068	-0.125	0.153	0.194	0.340	0.097	0.159	58.33	0.27	0.64
Adj.P_NGL	-0.257	-0.020	0.186	0.079	0.206	0.369	-0.092	0.020	54.17	0.58	1.43
Adj.P_RGS2.DEP	-0.136	-0.128	-0.007	0.095	0.187	0.212	-0.012	0.050	54.17	0.71	1.35
Adj.P_CGS_RGS2.DEP	-0.139	-0.148	-0.007	0.082	0.199	0.196	0.003	0.061	54.17	0.71	1.35
R_McC_CGS	-0.287	0.079	0.271	0.262	0.139	0.543	-0.064	0.135	54.17	2.50	5.00
R_RGS2.DEP	-0.425	-0.005	0.297	0.308	0.255	0.572	-0.256	-0.007	54.17	2.50	8.00
P_OH	-0.084	-0.084	-0.084	-0.084	-0.084	-0.084	-0.084	-0.084	54.17	0.20	0.42
P_OH_NG	-0.083	-0.083	-0.083	-0.083	-0.083	-0.083	-0.083	-0.083	54.17	0.20	0.42
P_IP_BenthicNG	-0.030	-0.040	-0.091	0.135	0.140	0.276	0.131	0.211	54.17	0.21	0.32
Adj.P_NGL2.DEP	-0.191	0.038	0.074	0.091	0.169	0.297	-0.065	0.018	50.00	0.32	2.24
Adj.P_Tol.DEP*	0.022	0.192	0.177	-0.007	-0.209	-0.056	0.016	-0.018	50.00	0.81	1.17
Adj.P_Tol_Cyprinid.DEP	0.014	0.177	0.187	0.010	-0.205	-0.054	-0.042	-0.037	50.00	0.84	1.18
P_Catostomidae	-0.088	0.075	0.009	0.093	0.130	0.386	0.162	0.194	50.00	0.02	0.06
Adj.R_MO	-0.340	0.068	0.446	0.169	0.071	0.294	-0.266	-0.077	45.83	0.85	1.35
P_IP_NonGameNB	-0.020	-0.154	-0.154	-0.154	-0.154	-0.154	-0.154	-0.154	45.83	0.26	0.50
Adj.R_LSR	-0.378	-0.036	0.335	0.194	0.181	0.344	-0.244	-0.085	41.67	0.60	1.33
Adj.R_LSR2.DEP	-0.341	-0.022	0.300	0.183	0.183	0.378	-0.182	-0.058	41.67	0.48	1.44
Adj.P_RGS	-0.113	0.023	0.072	0.021	0.083	0.100	-0.017	0.028	41.67	0.64	1.38
Adj.P_McC_CGS2.DEP	-0.081	-0.016	-0.017	0.060	0.144	0.221	0.265	0.220	41.67	0.31	1.74
Adj.P_CGS_RGS	0.069	-0.042	0.062	0.001	-0.127	-0.083	-0.013	0.091	37.50	0.85	1.13
Adj.P_LSR2.DEP	-0.100	-0.011	0.009	0.182	0.130	0.282	0.034	0.183	37.50	0.03	1.46
R_CGS_RGS2.DEP	-0.409	-0.003	0.285	0.298	0.242	0.577	-0.216	0.026	37.50	2.50	8.50
Adj.R_Cyprinid	-0.299	-0.032	0.330	0.083	0.116	0.191	-0.237	-0.134	33.33	0.72	1.37
Adj.R_Cyprinid_BNDSEAT	-0.250	0.032	0.261	0.130	0.095	0.222	-0.149	-0.047	33.33	0.28	1.93
Adj.R_CyprinidN	-0.301	-0.047	0.325	0.075	0.118	0.178	-0.257	-0.141	33.33	0.72	1.37

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
Adj.R_Cyprinid_NBNDSEAT	-0.253	0.129	0.129	0.129	0.129	0.129	0.129	0.129	33.33	0.28	1.93
Adj.R_IP_NonGameNB	-0.215	0.002	0.002	0.002	0.002	0.002	0.002	0.002	33.33	0.53	1.26
Adj.P_GSS	-0.113	-0.079	0.071	-0.090	0.084	-0.136	-0.073	-0.239	33.33	0.54	1.25
Adj.P_Benthic	0.153	0.158	-0.054	0.068	-0.113	0.186	0.254	0.301	33.33	0.79	1.12
Adj.P_Benthic_CACO	0.169	0.159	-0.089	0.051	-0.108	0.184	0.251	0.298	33.33	0.80	1.14
Adj.P_Cyprinid_BNDSEAT	-0.188	0.054	0.219	0.077	0.053	0.096	-0.136	0.006	33.33	0.31	1.43
Adj.P_Cyprinid_NBNDSEAT	-0.190	0.080	0.080	0.080	0.080	0.080	0.080	0.080	33.33	0.31	1.43
R_Catostomidae	-0.261	0.133	0.278	0.220	0.082	0.553	-0.087	0.127	33.33	1.00	2.00
P_Cyprinid	-0.004	0.016	0.153	-0.068	-0.138	-0.213	-0.211	-0.240	33.33	0.63	0.75
P_CyprinidN	-0.012	0.011	0.159	-0.057	-0.139	-0.223	-0.247	-0.219	33.33	0.63	0.75
Adj.P_BND_CACO_SEAT	0.075	0.052	0.133	-0.030	-0.187	-0.170	-0.040	-0.061	29.17	0.38	1.19
P_OH_CAAAN	-0.162	-0.162	-0.162	-0.162	-0.162	-0.162	-0.162	-0.162	29.17	0.15	0.40
P_OH_CAAAN_CACO	-0.154	-0.154	-0.154	-0.154	-0.154	-0.154	-0.154	-0.154	29.17	0.15	0.32
P_LSR	0.006	0.006	-0.104	0.006	0.065	0.074	0.128	-0.011	20.83	0.38	0.71
P_MO	0.017	0.131	0.247	0.008	-0.227	-0.104	0.018	0.105	20.83	0.09	0.39
Adj.R_OH	-0.344	0.050	0.431	0.174	0.076	0.311	-0.263	-0.095	16.67	0.71	1.28
Adj.P_McC_CGS	0.241	0.153	-0.061	-0.025	-0.214	0.002	0.327	0.290	16.67	0.64	1.37
Adj.R_OH_CAAAN	-0.376	-0.044	0.406	0.161	0.117	0.306	-0.294	-0.133	12.50	0.74	1.25
Adj.R_OH_CAAAN_CACO	-0.351	-0.053	0.358	0.124	0.112	0.220	-0.289	-0.148	12.50	0.65	1.30
R_OH_NG	-0.362	0.100	0.414	0.252	0.123	0.521	-0.179	-0.016	12.50	2.00	4.50
R_McC_CGS2.DEP	-0.281	0.038	0.178	0.258	0.176	0.541	-0.042	0.132	12.50	1.00	4.00
Adj.R_BND_CACO_SEAT	-0.196	0.089	0.349	0.175	0.009	0.336	-0.039	0.079	8.33	0.74	1.37
Adj.R_Tol.DEP	-0.330	0.033	0.375	0.117	0.112	0.283	-0.233	-0.079	8.33	0.79	1.35
Adj.R_Tol_Cyprinid.DEP	-0.289	0.010	0.360	0.074	0.081	0.176	-0.227	-0.095	8.33	0.71	1.38
P_Native	0.024	-0.041	0.041	-0.120	-0.063	-0.158	-0.111	-0.129	8.33	1.00	1.00

Table 3.4-3: Spearman correlation coefficients for metrics in the Ohio and Mon WAP region with surface mining (%), development (%), grassland (%), agriculture (%), forest (%), structure density (#/km²; SD), conductivity (SPC), and pH. Discrimination efficiency (%; DE) and 25th and 75th percentiles indicated.

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
Adj.R_CyprinidN	-0.196	-0.198	-0.111	-0.239	0.271	-0.118	-0.153	-0.090	92.59	0.930	1.169
Adj.R_CavitySpawn2.DEP	-0.201	-0.317	-0.259	-0.235	0.342	-0.218	-0.234	-0.146	88.89	0.906	1.183
Adj.R_CyprinidN2.DEP	-0.127	-0.223	-0.132	-0.263	0.267	-0.119	-0.129	-0.040	88.89	0.822	1.560
Adj.R_DMS*	-0.081	-0.388	-0.245	-0.193	0.297	-0.301	-0.176	-0.125	88.89	0.903	1.147
Adj.R_DMS2.DEP	-0.082	-0.390	-0.244	-0.194	0.297	-0.302	-0.176	-0.125	88.89	0.903	1.147
Adj.R_IP_BenthicNG	-0.195	-0.380	-0.215	-0.145	0.280	-0.317	-0.198	-0.141	88.89	0.928	1.142
Adj.R_NGL2.DEP	-0.140	-0.301	-0.152	-0.098	0.227	-0.232	-0.158	-0.150	88.89	0.758	1.305
Adj.P_CyprinidN2.DEP	-0.166	-0.258	-0.188	-0.204	0.265	-0.105	-0.191	0.087	85.19	0.343	1.789
Adj.P_Int_LSR.DEP	-0.152	-0.290	-0.250	-0.250	0.291	-0.214	-0.217	-0.137	85.19	0.405	1.805
Adj.R_CavitySpawn	-0.241	-0.250	-0.208	-0.197	0.295	-0.157	-0.235	-0.091	85.19	0.928	1.071
Adj.R_Int_Benthic.DEP	-0.184	-0.332	-0.223	-0.129	0.317	-0.254	-0.201	-0.195	85.19	0.812	1.162
Adj.R_Int_RGS.DEP	-0.064	-0.360	-0.220	-0.216	0.317	-0.263	-0.196	-0.058	85.19	0.791	1.159
Adj.R_RGS2.DEP	0.019	-0.292	-0.176	-0.221	0.248	-0.179	-0.157	-0.022	85.19	0.786	1.192
Adj.R_Cyprinid_BNDSEAT*	-0.122	-0.198	-0.072	-0.199	0.237	-0.104	-0.122	-0.120	81.48	0.918	1.144
Adj.R_Cyprinid2.DEP	-0.018	-0.196	-0.072	-0.234	0.219	-0.083	-0.098	0.009	81.48	0.859	1.145
Adj.R_Int_LSR.DEP	-0.181	-0.331	-0.267	-0.254	0.370	-0.274	-0.194	-0.134	81.48	0.728	1.141
Adj.R_Benthic	-0.169	-0.307	-0.196	-0.158	0.275	-0.254	-0.209	-0.133	77.78	0.905	1.155
Adj.R_Benthic2.DEP	-0.202	-0.359	-0.222	-0.147	0.307	-0.299	-0.192	-0.223	77.78	0.812	1.188
Adj.R_RGS*	0.021	-0.279	-0.171	-0.239	0.249	-0.174	-0.139	-0.048	77.78	0.815	1.181
Adj.P_Int_RGS.DEP	-0.014	-0.235	-0.163	-0.081	0.180	-0.097	-0.120	-0.178	74.07	0.626	1.432
Adj.R_Benthic_CACO	-0.190	-0.287	-0.160	-0.101	0.242	-0.225	-0.178	-0.176	74.07	0.874	1.188
Adj.R_CGS_RGS2.DEP	-0.117	-0.350	-0.215	-0.176	0.288	-0.281	-0.209	-0.112	74.07	0.840	1.150
Adj.R_Cyprinid	-0.105	-0.144	-0.036	-0.184	0.197	-0.062	-0.146	-0.064	74.07	0.895	1.115
Adj.R_IP_NonGameNB	-0.137	-0.216	-0.085	-0.220	0.257	-0.113	-0.171	-0.082	74.07	0.824	1.252
Adj.R_IP2.DEP	-0.213	-0.284	-0.146	-0.174	0.284	-0.209	-0.154	-0.150	74.07	0.814	1.188
Adj.R_LSR2.DEP	-0.103	-0.370	-0.247	-0.253	0.352	-0.293	-0.170	-0.156	74.07	0.691	1.147
Adj.R_NGL	-0.152	-0.274	-0.125	-0.124	0.216	-0.214	-0.153	-0.132	74.07	0.771	1.299

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
Adj.P_CavitySpawn2.DEP	-0.145	-0.109	-0.120	0.014	0.104	-0.122	-0.111	-0.178	70.37	0.570	1.105
Adj.R_CGS_RGS	-0.100	-0.295	-0.196	-0.195	0.280	-0.232	-0.225	-0.112	70.37	0.877	1.181
Adj.R_Int.DEP*	-0.136	-0.293	-0.161	-0.161	0.290	-0.191	-0.209	-0.107	70.37	0.626	1.270
Adj.R_Mod.DEP	-0.208	-0.181	-0.059	-0.109	0.201	-0.155	-0.113	-0.147	70.37	0.827	1.275
Adj.R_Native2.DEP	-0.251	-0.294	-0.171	-0.170	0.309	-0.236	-0.171	-0.176	70.37	0.769	1.306
Adj.R_Percidae	-0.067	-0.335	-0.214	-0.129	0.248	-0.303	-0.150	-0.110	70.37	0.811	1.119
Adj.R_Richness2.DEP*	-0.212	-0.265	-0.137	-0.159	0.284	-0.195	-0.162	-0.167	70.37	0.768	1.291
Adj.P_Cyprinid_NBNDSEAT	-0.140	-0.121	-0.049	-0.198	0.212	-0.050	-0.128	-0.013	66.67	0.927	1.161
Adj.P_Cyprinid2.DEP	-0.008	-0.184	-0.111	-0.096	0.157	-0.023	-0.169	0.166	66.67	0.301	1.747
Adj.P_Native2.DEP	-0.053	-0.152	-0.131	-0.091	0.108	-0.069	-0.177	-0.197	66.67	0.747	1.171
Adj.R_Cyprinid_NBNDSEAT	-0.189	-0.240	-0.133	-0.243	0.299	-0.148	-0.148	-0.148	66.67	0.641	1.225
Adj.R_GSS	-0.230	-0.242	-0.199	-0.176	0.295	-0.257	-0.163	-0.202	66.67	0.780	1.129
Adj.R_GSS2.DEP	-0.275	-0.325	-0.310	-0.179	0.377	-0.327	-0.223	-0.276	66.67	0.660	1.305
Adj.R_IN	-0.207	-0.304	-0.122	-0.144	0.266	-0.223	-0.176	-0.167	66.67	0.795	1.152
Adj.R_LSR	-0.083	-0.304	-0.181	-0.267	0.301	-0.242	-0.117	-0.121	66.67	0.802	1.174
P_Native	-0.358	-0.236	-0.245	-0.273	0.319	-0.276	-0.129	-0.157	66.67	1.000	1.000
Adj.P_CGS_RGS2.DEP	0.104	-0.179	-0.144	-0.143	0.139	-0.116	-0.140	-0.220	62.96	0.765	1.291
Adj.P_Int.DEP	0.086	-0.245	-0.214	-0.207	0.210	-0.209	-0.134	-0.074	62.96	0.549	1.738
Adj.P_IP2	-0.184	-0.213	-0.130	-0.190	0.160	-0.118	-0.099	-0.091	62.96	0.468	1.434
Adj.R_Int_NGL.DEP	-0.157	-0.268	-0.098	-0.039	0.196	-0.211	-0.165	-0.117	62.96	0.708	1.662
Adj.R_IP	-0.255	-0.247	-0.114	-0.108	0.245	-0.199	-0.156	-0.173	62.96	0.780	1.181
Adj.R_IP_SEAT	-0.267	-0.252	-0.117	-0.106	0.249	-0.199	-0.158	-0.176	62.96	0.758	1.199
P_CyprinidN	-0.146	-0.131	-0.110	-0.217	0.234	-0.070	-0.070	0.162	62.96	0.674	0.762
P_OH_CAAN	-0.035	-0.035	-0.035	-0.035	-0.035	-0.035	-0.035	-0.035	62.96	0.142	0.301
Adj.P_Cyprinid_BNDSEAT	-0.058	-0.078	0.005	-0.113	0.139	0.000	-0.107	0.048	59.26	0.928	1.128
Adj.P_GSS2.DEP	-0.130	-0.220	-0.254	-0.145	0.247	-0.321	-0.115	-0.255	59.26	0.627	1.348
Adj.P_RGS	0.042	-0.146	-0.045	-0.166	0.149	-0.039	0.016	-0.022	59.26	0.731	1.220
Adj.R_Native	-0.276	-0.203	-0.094	-0.099	0.220	-0.175	-0.134	-0.168	59.26	0.839	1.163
Adj.R_Tol_Benthic.DEP	0.080	0.183	0.163	0.157	-0.201	0.177	0.019	0.188	59.26	0.830	1.298

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
P_Int_Benthic.DEP	0.019	-0.111	-0.140	0.017	0.066	-0.154	-0.074	-0.198	59.26	0.083	0.162
P_LSR	0.037	-0.141	-0.125	-0.194	0.191	-0.014	-0.030	0.099	59.26	0.623	0.708
Adj.P_Int_NGL.DEP	0.095	-0.153	-0.055	0.008	0.022	-0.193	-0.125	-0.051	55.56	0.553	1.338
Adj.P_IP_SEAT	-0.020	-0.117	-0.069	-0.016	0.041	-0.041	-0.076	-0.235	55.56	0.718	1.132
Adj.P_LSR2.DEP	0.146	-0.239	-0.208	-0.231	0.231	-0.102	-0.162	-0.018	55.56	0.654	1.535
Adj.P_RGS2.DEP	0.109	-0.143	-0.071	-0.106	0.103	-0.035	-0.100	-0.161	55.56	0.645	1.332
Adj.R_McC_CGS2.DEP	-0.053	-0.203	-0.182	-0.136	0.195	-0.213	-0.165	-0.137	55.56	0.752	1.188
Adj.Richness	-0.234	-0.166	-0.045	-0.068	0.173	-0.129	-0.124	-0.151	55.56	0.823	1.199
P_IP	0.025	-0.054	-0.088	-0.005	-0.003	-0.002	-0.030	-0.200	55.56	0.498	0.692
P_IP_NonGameNB	-0.024	-0.026	-0.026	-0.026	-0.026	-0.026	-0.026	-0.026	55.56	0.247	0.471
P_OH	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	55.56	0.308	0.502
P_OH_CAAN_CACO	-0.062	-0.062	-0.062	-0.062	-0.062	-0.062	-0.062	-0.062	55.56	0.103	0.301
P_OH_NG	-0.024	0.018	0.018	0.018	0.018	0.018	0.018	0.018	55.56	0.308	0.502
Adj.P_BND_CACO_SEAT	0.034	0.084	0.023	0.052	-0.122	0.012	0.107	0.056	51.85	0.561	1.286
Adj.P_NGL2.DEP*	0.120	-0.162	-0.115	-0.042	0.054	-0.196	-0.074	-0.109	51.85	0.665	1.306
Adj.P_Tol.DEP*	-0.014	0.089	0.041	0.016	-0.040	-0.044	0.177	0.173	51.85	0.788	1.135
Adj.P_Tol_Cyprinid.DEP	0.021	0.009	-0.028	-0.074	0.043	-0.085	0.093	0.192	51.85	0.765	1.162
Adj.Proportion2.DEP	0.070	-0.085	-0.058	0.009	0.006	0.010	-0.166	-0.125	51.85	0.746	1.161
Adj.R_BND_CACO_SEAT	0.132	0.172	0.088	0.055	-0.145	0.095	-0.065	0.173	48.15	0.811	1.386
P_CGS_RGS	0.096	-0.143	-0.085	-0.149	0.107	-0.057	0.106	0.070	48.15	0.415	0.671
P_Cyprinid	-0.053	-0.075	-0.057	-0.126	0.157	-0.007	-0.059	0.190	48.15	0.674	0.787
Adj.P_GSS	-0.013	-0.032	-0.146	-0.049	0.050	-0.121	0.022	-0.026	44.44	0.732	1.076
Adj.P_IP2.DEP	0.043	-0.131	-0.077	-0.080	0.049	-0.047	-0.082	-0.196	44.44	0.648	1.192
P_DMS	0.171	-0.017	-0.048	-0.012	-0.034	-0.044	-0.063	-0.166	44.44	0.120	0.244
P_DMS2.DEP	0.171	-0.017	-0.048	-0.012	-0.034	-0.044	-0.063	-0.166	44.44	0.120	0.244
P_IP_BenthicNG	0.153	-0.036	-0.050	-0.002	-0.060	-0.071	-0.066	-0.132	44.44	0.173	0.287
SW_TROPHIC.cor	-0.097	-0.072	-0.025	0.041	0.009	-0.037	0.004	-0.225	44.44	2.720	2.982
Adj.P_IN	-0.014	-0.174	-0.049	-0.019	0.103	-0.049	-0.160	-0.141	40.74	0.810	1.137
Adj.P_McC_CGS2.DEP	0.196	-0.069	-0.096	-0.154	0.049	-0.070	-0.055	-0.053	40.74	0.538	1.336

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
P_Benthic2.DEP	0.158	-0.048	-0.068	-0.018	-0.043	-0.098	-0.063	-0.179	40.74	0.180	0.293
P_McC_CGS	0.116	-0.063	-0.033	-0.131	0.017	-0.025	0.125	0.092	40.74	0.204	0.334
P_Percidae	0.201	-0.014	-0.038	0.038	-0.012	-0.057	-0.057	-0.166	40.74	0.100	0.216
R_McC_CGS	0.007	-0.097	-0.125	-0.082	0.115	-0.112	-0.157	0.009	40.74	4.000	6.000
Adj.P_Benthic	0.115	-0.048	-0.041	-0.051	-0.044	-0.041	0.125	0.024	37.04	0.715	1.182
Adj.P_NGL	0.026	-0.028	0.041	-0.025	-0.056	-0.023	0.073	-0.030	37.04	0.704	1.120
Adj.P_Tol_Benthic.DEP	-0.014	-0.020	0.018	-0.012	-0.024	0.015	0.177	0.161	37.04	0.687	1.206
P_Benthic_CACO	0.074	-0.105	-0.063	-0.101	0.030	-0.068	0.097	-0.041	37.04	0.301	0.542
P_CavitySpawn	-0.074	0.058	0.054	-0.003	-0.049	-0.050	-0.127	-0.090	37.04	0.124	0.233
R_Catostomidae	-0.205	-0.160	-0.051	0.002	0.096	-0.160	-0.144	0.016	37.04	2.000	3.000
Adj.P_Catostomidae	-0.136	-0.039	0.022	-0.013	-0.040	-0.026	-0.014	-0.143	33.33	0.657	1.429
Adj.R_MO	-0.016	0.148	0.100	0.065	-0.098	0.092	0.008	0.098	33.33	0.822	1.150
P_Mod.DEP	0.055	0.059	0.029	0.155	-0.129	0.087	-0.136	-0.072	33.33	0.199	0.342
P_OH2.DEP	-0.005	-0.098	-0.063	0.049	0.089	-0.094	-0.124	0.009	25.93	0.000	0.089
R_Tol.DEP	-0.162	0.098	0.117	0.148	-0.109	0.032	-0.015	0.021	25.93	6.000	9.000
P_MO	-0.044	0.107	0.130	-0.030	-0.064	0.048	0.131	0.154	22.22	0.177	0.411
R_Tol_Cyprinid.DEP	-0.134	-0.014	0.026	0.032	0.024	-0.022	-0.166	0.038	22.22	5.000	6.000
P_Game	-0.141	0.075	0.072	0.194	-0.079	0.020	0.124	-0.148	11.11	0.001	0.030
R_OH	-0.074	0.066	0.088	0.009	-0.003	0.050	-0.050	-0.001	11.11	4.000	7.000
R_OH_CAAN	-0.087	0.066	0.093	0.021	-0.006	0.050	-0.061	-0.007	11.11	3.000	6.000
R_OH_CAAN_CACO	-0.076	0.056	0.064	0.031	-0.002	0.040	-0.044	-0.011	11.11	2.000	5.000
R_OH_NG	-0.080	-0.003	-0.003	-0.108	0.122	-0.020	-0.134	-0.040	11.11	4.000	6.000
R_Game	-0.171	0.090	0.081	0.198	-0.094	0.010	0.048	-0.046	3.70	1.000	4.000
Adj.P_GameC	-0.230	0.074	0.116	0.137	-0.036	0.067	0.129	-0.164	0.00	0.000	2.232
Adj.R_Game2.DEP	-0.284	0.002	0.049	0.004	0.095	0.015	-0.058	-0.194	0.00	0.000	1.658
P_Game2.DEP	-0.198	0.035	0.050	0.106	-0.009	0.020	0.071	-0.163	0.00	0.000	0.020
P_Sunfish	-0.195	-0.027	-0.021	0.121	0.044	-0.111	0.035	-0.188	0.00	0.000	0.010
R_GameC	-0.123	0.148	0.084	0.136	-0.104	0.035	0.047	0.021	0.00	0.000	2.000
R_OH2.DEP	-0.058	-0.100	-0.054	-0.037	0.138	-0.082	-0.085	-0.030	0.00	0.000	2.000

Table 3.4-4: Spearman correlation coefficients for metrics in the Upper Kanawha region with surface mining (%), development (%), grassland (%), agriculture (%), forest (%), structure density (#/km²; SD), conductivity (SPC), and pH. Discrimination efficiency (%; DE) and 25th and 75th percentiles indicated. Table is sorted by descending discrimination efficiency.

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
Adj.P_Cold	-0.010	-0.082	-0.021	-0.022	0.137	0.034	-0.123	-0.165	81.82	0.638	1.404
P_Int_RGS.DEP	-0.188	-0.359	-0.182	-0.147	0.351	-0.363	-0.255	-0.173	81.82	0.128	0.324
Adj.P_Cold_SATR_ONMY	-0.022	-0.107	-0.043	-0.030	0.128	0.000	-0.139	-0.193	72.73	0.370	1.494
P_Native2	-0.176	-0.258	-0.067	-0.041	0.274	-0.146	-0.102	0.192	72.73	0.216	0.368
P_IP_BenthicNG	-0.048	-0.391	-0.316	-0.168	0.189	-0.390	-0.100	0.049	72.73	0.192	0.396
Adj.R_IP_BenthicNG	-0.249	-0.404	-0.376	0.000	0.167	-0.294	-0.218	0.083	63.64	0.594	1.357
Adj.R_Int	-0.340	-0.370	-0.185	0.058	0.251	-0.123	-0.336	0.006	63.64	0.441	1.645
Adj.R_Int.DEP*	-0.354	-0.307	-0.082	-0.007	0.287	-0.133	-0.399	0.056	63.64	0.555	1.464
Adj.R_Int_Benthic.DEP	-0.319	-0.490	-0.333	0.006	0.223	-0.396	-0.326	-0.058	63.64	0.708	1.210
Adj.P_Tol.DEP*	0.155	0.425	0.271	0.323	-0.418	0.400	0.318	0.091	63.64	0.577	1.166
P_Cyprinid2	-0.127	-0.164	-0.026	-0.065	0.260	-0.121	-0.142	0.138	63.64	0.124	0.317
P_CyprinidN2	-0.119	-0.154	-0.014	-0.059	0.246	-0.113	-0.137	0.137	63.64	0.124	0.317
P_DMS	-0.128	-0.439	-0.377	-0.146	0.193	-0.421	-0.165	-0.006	63.64	0.108	0.370
P_RGS2.DEP	-0.043	-0.340	-0.279	-0.314	0.295	-0.467	-0.283	-0.028	63.64	0.233	0.484
P_Benthic2.DEP	-0.026	-0.354	-0.284	-0.167	0.159	-0.378	-0.084	0.061	63.64	0.192	0.396
P_CyprinidN2.DEP	-0.144	-0.167	-0.005	-0.045	0.249	-0.104	-0.142	0.159	63.64	0.124	0.317
P_DMS2.DEP	-0.128	-0.439	-0.377	-0.146	0.193	-0.421	-0.165	-0.006	63.64	0.108	0.370
P_Int_Benthic.DEP	-0.246	-0.449	-0.297	-0.118	0.292	-0.491	-0.309	-0.176	63.64	0.066	0.179
Adj.R_Native	-0.262	-0.332	-0.323	0.066	0.117	-0.236	-0.267	0.101	54.55	0.755	1.154
Adj.R_IN	-0.245	-0.308	-0.300	0.019	0.111	-0.234	-0.218	0.079	54.55	0.668	1.298
R_Benthic*	-0.220	-0.220	-0.220	-0.220	-0.220	-0.220	-0.220	-0.220	54.55	4.000	6.000
Adj.R_Benthic_CACO	-0.236	-0.340	-0.343	0.026	0.118	-0.254	-0.226	0.134	54.55	0.626	1.278
Adj.R_CGS_RGS*	-0.214	-0.316	-0.328	-0.035	0.120	-0.288	-0.340	0.068	54.55	0.703	1.214
Adj.R_CGS_RGS2.DEP	-0.248	-0.354	-0.302	-0.060	0.170	-0.303	-0.368	0.002	54.55	0.732	1.224
Adj.P_Cyprinid2.DEP	-0.252	-0.204	-0.055	-0.063	0.307	-0.086	-0.163	0.052	54.55	0.744	1.536
R_CyprinidN	-0.221	-0.132	-0.124	0.156	0.043	-0.038	-0.205	0.226	54.55	3.000	5.000
P_Fish2	-0.165	-0.191	-0.014	-0.049	0.254	-0.081	-0.072	0.197	54.55	0.217	0.444

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
P_Tol	0.132	0.185	0.029	0.074	-0.232	0.101	0.072	-0.197	54.55	0.556	0.783
P_Percidae	-0.086	-0.397	-0.388	-0.293	0.205	-0.539	-0.281	-0.144	54.55	0.078	0.233
P_CGS_RGS2	-0.098	-0.119	0.017	-0.115	0.277	-0.099	-0.201	0.116	54.55	0.131	0.289
P_Native2.DEP	-0.114	-0.396	-0.230	-0.206	0.307	-0.389	-0.223	0.059	54.55	0.321	0.589
P_Int.DEP	-0.196	-0.196	0.024	-0.083	0.319	-0.081	-0.292	0.068	54.55	0.015	0.270
P_CGS_RGS2.DEP	-0.013	-0.313	-0.219	-0.298	0.307	-0.411	-0.265	-0.040	54.55	0.269	0.545
P_CavitySpawn2.DEP	-0.224	-0.443	-0.266	0.017	0.264	-0.330	-0.149	-0.039	54.55	0.072	0.243
Adj.R_FISH*	-0.225	-0.217	-0.219	0.094	0.032	-0.140	-0.199	0.172	45.45	0.764	1.212
Adj.R_Benthic2	-0.211	-0.253	-0.206	0.098	0.071	-0.088	-0.043	0.204	45.45	0.645	1.619
Adj.R_Cyprinid*	-0.241	-0.164	-0.173	0.149	0.025	-0.065	-0.214	0.158	45.45	0.791	1.136
Adj.R_Cyprinid_BNDSEAT	-0.240	-0.173	-0.152	0.151	-0.003	-0.052	-0.213	0.126	45.45	0.747	1.496
Adj.R_McC_CGS	-0.131	-0.248	-0.306	-0.032	0.079	-0.224	-0.283	0.067	45.45	0.646	1.455
Adj.R_Native2.DEP	-0.347	-0.398	-0.320	0.055	0.189	-0.270	-0.311	0.067	45.45	0.580	1.255
Adj.R_IP2.DEP	-0.276	-0.286	-0.233	0.015	0.119	-0.189	-0.257	0.059	45.45	0.721	1.294
Adj.R_Benthic2.DEP	-0.228	-0.356	-0.349	-0.008	0.122	-0.272	-0.205	0.088	45.45	0.541	1.281
Adj.R_McC_CGS2.DEP	-0.086	-0.263	-0.295	-0.111	0.077	-0.274	-0.300	-0.064	45.45	0.649	2.176
Adj.P_IN*	-0.059	-0.394	-0.280	-0.293	0.327	-0.406	-0.228	0.010	45.45	0.671	1.372
Adj.P_Benthic_CACO	-0.016	0.079	0.019	0.142	-0.142	0.126	0.126	0.195	45.45	0.799	1.186
Adj.P_Catostomidae	0.324	-0.006	-0.149	-0.179	-0.022	-0.122	0.121	0.232	45.45	0.148	1.925
Adj.P_Fish2.DEP	-0.201	-0.397	-0.225	-0.229	0.382	-0.317	-0.270	-0.028	45.45	0.771	1.309
Adj.P_Mod.DEP	-0.114	-0.348	-0.257	-0.200	0.231	-0.300	-0.164	0.032	45.45	0.587	1.482
Adj.P_Tol_Cyprinid.DEP	0.135	0.391	0.243	0.330	-0.408	0.369	0.284	0.125	45.45	0.618	1.199
R_RGS	-0.237	-0.229	-0.274	0.065	0.072	-0.192	-0.235	0.203	45.45	3.000	6.000
R_RGS2.DEP	-0.271	-0.273	-0.273	0.053	0.117	-0.217	-0.258	0.169	45.45	2.000	5.000
P_Benthic2	-0.053	-0.157	-0.064	0.004	0.096	-0.079	0.083	0.290	45.45	0.042	0.177
P_Cyprinid_NBNDSEAT	-0.062	0.140	0.166	0.210	-0.173	0.198	0.132	0.397	45.45	0.129	0.362
P_Tol_Cyprinid	0.062	0.297	0.174	0.247	-0.262	0.328	0.150	-0.029	45.45	0.385	0.613
P_CGS_RGS	0.018	0.018	-0.031	-0.103	0.046	-0.040	-0.119	-0.019	45.45	0.598	0.780
P_CavitySpawn	-0.209	-0.330	-0.152	0.155	0.088	-0.222	-0.071	0.048	45.45	0.072	0.243

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
Adj.R_Native2	-0.328	-0.363	-0.267	0.070	0.194	-0.186	-0.262	0.087	36.36	0.533	1.223
Adj.R_Game	-0.018	0.126	0.161	0.187	-0.177	0.094	0.000	0.259	36.36	0.000	1.445
Adj.R_NGL	-0.028	-0.183	-0.280	-0.007	0.028	-0.121	-0.115	0.101	36.36	0.616	1.393
Adj.R_IP	-0.224	-0.243	-0.201	0.011	0.084	-0.184	-0.215	0.092	36.36	0.673	1.263
Adj.R_Cyprinid2	-0.308	-0.223	-0.146	0.107	0.138	-0.088	-0.233	0.074	36.36	0.509	1.210
Adj.R_CyprinidN2	-0.297	-0.217	-0.141	0.106	0.136	-0.086	-0.231	0.073	36.36	0.468	1.233
Adj.R_Mod	-0.097	-0.132	-0.171	0.052	-0.018	-0.057	-0.021	0.198	36.36	0.614	1.702
Adj.R_Fish2.DEP	-0.336	-0.304	-0.237	0.070	0.121	-0.173	-0.265	0.094	36.36	0.590	1.248
Adj.R_Cyprinid2.DEP	-0.361	-0.212	-0.106	0.159	0.142	-0.036	-0.241	0.091	36.36	0.626	1.513
Adj.R_CyprinidN2.DEP	-0.326	-0.196	-0.083	0.159	0.120	-0.033	-0.218	0.094	36.36	0.615	1.614
Adj.P_NGL	0.000	-0.225	-0.288	-0.139	0.135	-0.166	-0.040	0.074	36.36	0.141	1.838
Adj.P_Cyprinid_BNDSEAT	-0.106	0.017	0.040	0.148	-0.067	0.133	0.054	0.248	36.36	0.716	1.365
Adj.P_OH	-0.083	0.269	0.162	0.415	-0.301	0.385	0.162	0.207	36.36	0.611	1.318
Adj.P_OH_NG	-0.084	0.268	0.161	0.415	-0.300	0.385	0.160	0.208	36.36	0.611	1.318
Adj.P_IP2.DEP	-0.084	-0.345	-0.194	-0.297	0.338	-0.329	-0.207	-0.030	36.36	0.686	1.422
Adj.P_Tol_Benthic.DEP	-0.005	0.245	0.120	0.239	-0.208	0.320	0.156	0.126	36.36	0.685	1.473
R_LSR	-0.180	-0.168	-0.253	0.049	0.067	-0.129	-0.195	0.250	36.36	3.000	6.000
R_MO	-0.192	-0.081	-0.140	0.252	-0.118	0.033	-0.069	0.186	36.36	2.000	4.000
R_Percidae	-0.237	-0.330	-0.305	-0.033	0.150	-0.300	-0.275	0.046	36.36	1.000	2.000
R_Catostomidae	0.134	-0.024	-0.197	0.003	-0.092	-0.067	0.000	0.302	36.36	1.000	1.000
R_CavitySpawn2.DEP	-0.358	-0.464	-0.348	0.096	0.213	-0.319	-0.241	0.024	36.36	1.000	2.000
P_Native	0.079	0.051	0.062	-0.024	-0.136	-0.079	0.190	0.166	36.36	0.586	0.795
P_RGS	-0.028	-0.035	-0.093	-0.054	-0.041	-0.101	-0.057	0.230	36.36	0.233	0.536
P_IP2	-0.028	-0.107	0.026	-0.116	0.205	-0.080	-0.012	0.240	36.36	0.131	0.343
P_Cyprinid	0.021	0.252	0.233	0.182	-0.097	0.319	0.074	-0.013	36.36	0.573	0.796
P_Mod	-0.080	-0.079	-0.048	0.048	0.052	0.011	0.066	0.278	36.36	0.007	0.154
P_McC_CGS2.DEP	0.099	-0.019	-0.110	-0.133	-0.017	-0.136	-0.065	0.164	36.36	0.007	0.199
SW_TROPHIC	0.086	-0.093	-0.097	-0.050	0.022	-0.176	-0.101	0.072	36.36	2.360	3.068
Adj.R_Fish2	-0.310	-0.263	-0.180	0.064	0.146	-0.090	-0.211	0.117	27.27	0.514	1.331

Metric	Surface Mining	Development	Grassland	Agriculture	Forest	SD	SPC	pH	DE	25th	75th
Adj.R_IP2	-0.162	-0.224	-0.173	-0.029	0.118	-0.160	-0.211	0.103	27.27	0.788	1.500
Adj.R_IP_SEAT	-0.237	-0.242	-0.197	0.011	0.092	-0.167	-0.215	0.041	27.27	0.708	1.289
Adj.R_GameC	-0.036	0.100	0.133	0.171	-0.112	0.074	-0.032	0.269	27.27	0.000	1.632
Adj.R_Game2	0.022	0.058	0.077	0.101	-0.104	0.028	-0.060	0.261	27.27	0.000	1.501
Adj.R_IP_NonGameNB	-0.161	-0.165	-0.145	-0.036	0.111	-0.157	-0.266	0.008	27.27	0.644	1.423
Adj.R_CGS_RGS2	-0.228	-0.232	-0.118	0.025	0.131	-0.114	-0.290	0.068	27.27	0.691	1.549
Adj.R_Game2.DEP	0.009	0.036	0.055	0.077	-0.086	-0.008	-0.099	0.277	27.27	0.000	1.545
Adj.R_Mod.DEP	-0.240	-0.178	-0.196	0.114	-0.017	-0.104	-0.131	0.104	27.27	0.501	1.318
Adj.P_MO	-0.096	0.258	0.174	0.407	-0.283	0.390	0.166	0.191	27.27	0.616	1.321
Adj.P_IP_SEAT	-0.075	-0.326	-0.191	-0.298	0.337	-0.322	-0.251	-0.086	27.27	0.682	1.412
Adj.P_Benthic	-0.014	0.082	0.022	0.133	-0.134	0.130	0.161	0.183	27.27	0.752	1.253
Adj.P_BND_CACO_SEAT	0.166	0.035	0.019	-0.119	0.023	-0.046	0.046	-0.294	27.27	0.436	1.262
R_RGS2	-0.264	-0.161	-0.145	0.129	0.078	-0.052	-0.187	0.226	27.27	1.000	3.000
R_LSR2	-0.245	-0.213	-0.185	0.079	0.122	-0.103	-0.213	0.250	27.27	1.000	3.000
R_Cold_SATR_ONMY	-0.155	-0.199	-0.113	0.020	0.161	-0.072	-0.237	0.014	27.27	1.000	2.000
R_DMS	-0.332	-0.370	-0.326	0.066	0.163	-0.265	-0.214	0.132	27.27	1.000	3.000
R_Tol_Cyprinid	-0.094	-0.021	-0.126	0.145	-0.099	-0.002	-0.075	0.311	27.27	2.000	3.000
R_CavitySpawn	-0.338	-0.365	-0.261	0.176	0.105	-0.193	-0.163	0.134	27.27	1.000	2.000
R_LSR2.DEP	-0.260	-0.211	-0.225	0.068	0.121	-0.113	-0.199	0.223	27.27	1.000	4.000
R_DMS2.DEP	-0.332	-0.370	-0.326	0.066	0.163	-0.265	-0.214	0.132	27.27	1.000	3.000
P_GSS	0.041	-0.081	-0.096	-0.093	0.137	-0.121	0.032	-0.110	27.27	0.073	0.312
P_RGS2	-0.163	-0.163	-0.085	-0.120	0.244	-0.158	-0.195	0.177	27.27	0.022	0.270

3.5 COMBINING METRICS INTO AN INDEX

Final metric selections for each biomonitoring region were determined based on their correlation with landscape and water quality variables as well as their discrimination efficiency between reference and stressed sites. Metrics were hand selected to represent varying trophic, reproductive, and diversity characteristics of the fish communities. A total of 7 metrics were selected for the Mon CA-RV (Table 3.5-1), Ohio CA (Table 3.5-2), and Upper Kanawha (Table 3.5-4) biomonitoring regions. The Ohio and Mon WAP had a total of 8 metrics selected (Table 3.5-3).

Thresholds for each scoring method for final metrics within the Mon CA and RV, Ohio CA, Ohio and Mon WAP, and Upper Kanawha are presented in Tables 3.5-1, 3.5-2, 3.5-3, and 3.5-4, respectively. For each region, final IBI scores were calculated based on the 5th (floor) and 95th (ceiling) of the full distribution of sites, minus sites with zero individuals.

Table 3.5-1: Final metrics selected for the Mon CA-RV region with metric description and direction. Metrics direction is either positive (decreases with increases in stress) or negative (increases with increases in stress). The Ceiling (95th percentile) and Floor (5th percentile) were used for scoring criteria.

Metric	Description	Direction	Ceiling	Floor
P_Benthic2.DEP	Proportion of benthic individuals minus tolerant	Positive	0.683	0
Adj.R_Fish2.DEP	Adjusted species richness minus tolerant	Positive	1.215	0
Adj.R_McC_CGS	Adjusted clean gravel spawner richness	Positive	1.326	0
Adj.P_Fish2.DEP	Adjusted proportion of non-tolerant individuals	Positive	1.537	0
Adj.P_IN	Adjusted proportion of invertivore individuals	Positive	1.506	0
Adj.R_Benthic	Adjusted benthic species richness	Positive	1.370	0
Adj.R_Cyprinid	Adjusted Cyprinidae richness	Positive	1.326	0

Table 3.5-2: Final metrics selected for the Ohio CA region with metric description and direction. Metrics direction is either positive (decreases with increases in stress) or negative (increases with increases in stress). The Ceiling (95th percentile) and Floor (5th percentile) were used for scoring criteria.

Metric	Description	Direction	Ceiling	Floor
Adj.R_Fish2.DEP	Adjusted non-tolerant species richness	Positive	1.766	0
Adj.P_IN	Adjusted proportion invertivore individuals	Positive	1.751	0

Adj.R_DMS	Adjusted darter-madtom-sculpin richness	Positive	1.325	0
Adj.P_Tol	Adjusted proportion of tolerant individuals	Negative	1.702	0.413
Adj.R_RGS	Adjusted rock-gravel spawner richness	Positive	1.447	0
Adj.R_NGL2	Adjusted non-guarding lithophils minus tolerant richness	Positive	1.950	0
SW_TROPHIC	Shannon-Weaver Trophic diversity index	Positive	3.455	1.689

Table 3.5-3: Final metrics selected for the Ohio-Mon WAP region with metric description and direction. Metrics direction is either positive (decreases with increases in stress) or negative (increases with increases in stress). The Ceiling (95th percentile) and Floor (5th percentile) were used for scoring criteria.

Metric	Description	Direction	Ceiling	Floor
Adj.R_Fish2.DEF	Adjusted non-tolerant species richness	Positive	1.339	0.371
Adj.R_DMS	Adjusted darter-madtom-sculpin richness	Positive	1.158	0.265
Adj.R_Int	Adjusted intolerant species richness	Positive	1.640	0
Adj.P_Tol	Adjusted proportion tolerant individuals	Negative	1.675	0.726
Adj.R_RGS	Adjusted rock-gravel spawner richness	Positive	1.191	0.358
Adj.R_NGL2	Adjusted non-guarding lithophil richness minus tolerant species	Positive	1.454	0
P_OH_CAAN	Aroportion of omnivore-herbivore minus Central Stoneroller	Negative	0.655	0.119
Adj.R_Cyprinid_BNDSEAT	Adjusted Cyprinidae richness minus blacknose dace and Creek Chub	Positive	1.412	0.276

Table 3.5-4: Final metrics selected for the Upper Kanawha region with metric description and direction. Metrics direction is either positive (decreases with increases in stress) or negative (increases with increases in stress). The Ceiling (95th percentile) and Floor (5th percentile) were used for scoring criteria.

Metric	Description	Direction	Ceiling	Floor
Adj.P_IN	Adjusted proportion of invertivore individuals	Positive	1.808	0.011
Adj.R_Int	Adjusted intolerant species richness	Positive	2.952	0
Adj.P_Tol	Adjust proportion of tolerant individuals	Negative	2.412	0.269
R_Benthic	Benthic species richness	Positive	1.850	10
Adj.Richness	Adjusted total species richness	Positive	2.001	0.405
Adj.R_CGS_RGS	Adjusted clean and rock-gravel spawning species richness	Positive	1.735	0.354
Adj.R_Cyprinid	Adjusted Cyprinidae species richness	Positive	2.067	0.426

The final IBI scores within each region were compared between known reference, stressed, and other sites to determine the ability of the IBI to discriminate between stressed and not stressed sites. Figures 3.5-1, 3.5-2, 3.5-3, and 3.5-4 show the ranges of final IBI scores for each site type within the Mon CA-RV, Ohio CA, Ohio and Mon WAP, and Upper Kanawha, respectively. Based on ANOVA results and a post-hoc test (TukeyHSD) showed that the Mon CA-RV region has distinct separation in mean IBI scores between reference-stressed and reference-other sites, with no statistical distinction between stressed and other sites (Figure 3.5-1). The Ohio CA and Ohio and Mon WAP biomonitoring regions found all three groups to be statistically difference from one another (Figures 3.5-2 and 3.5-3). However, the Upper Kanawha biomonitoring region showed no significant difference between any of the site types (Figure 3.5-4).

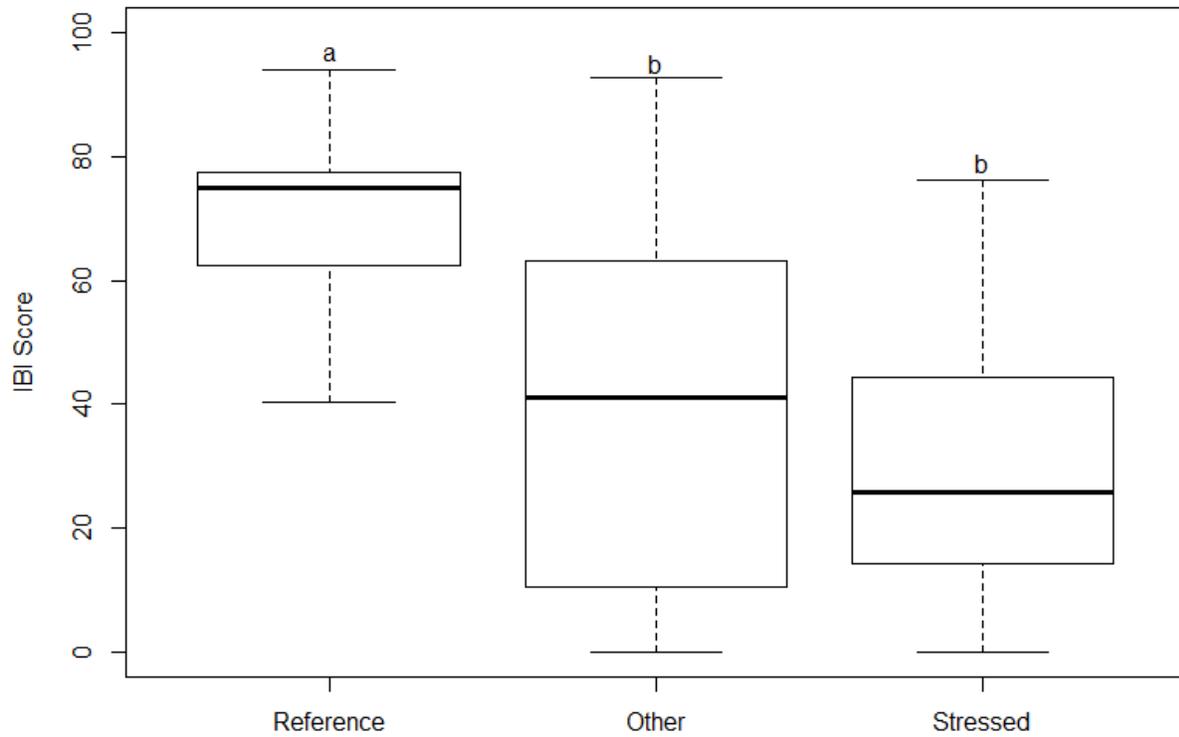


Figure 3.5-1: Final IBI scores for the Mon CA-RV biomonitoring region. Different letters indicate a significant difference between groups (Reference, Stressed, or Other) based on ANOVA results.

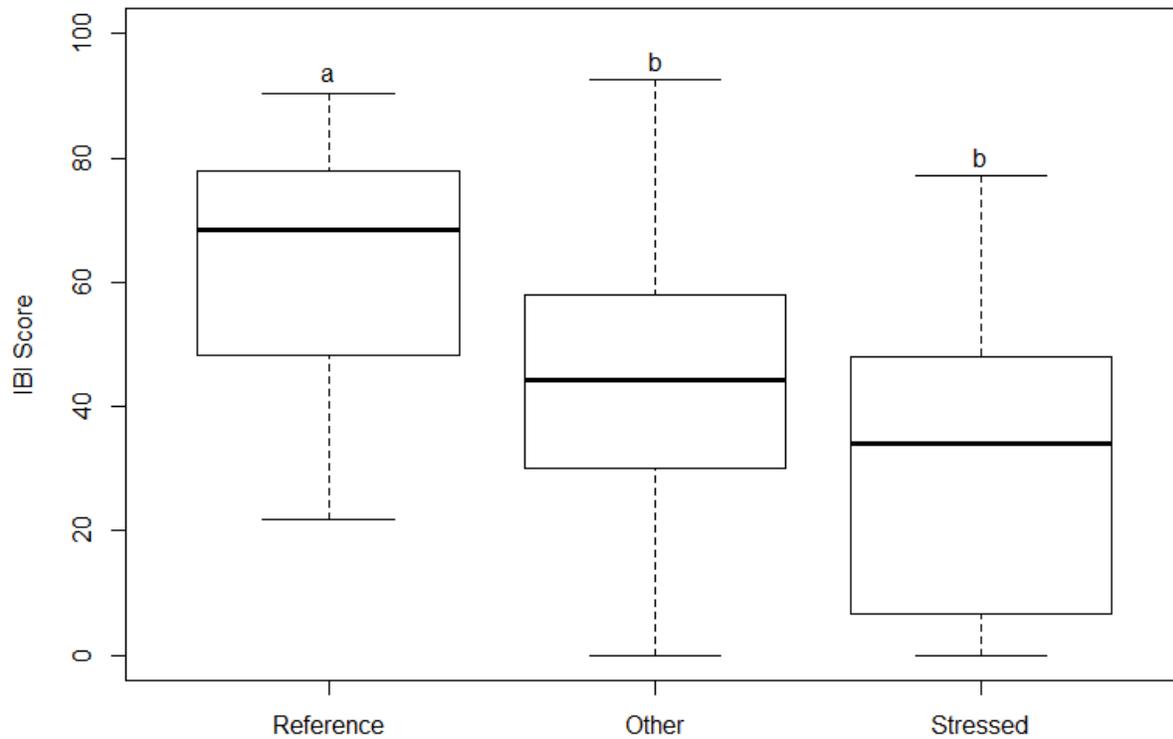


Figure 3.5-2: Final IBI scores for the Ohio CA biomonitoring region. Different letters indicate a significant difference between groups (Reference, Stress, or Other) based on ANOVA results.

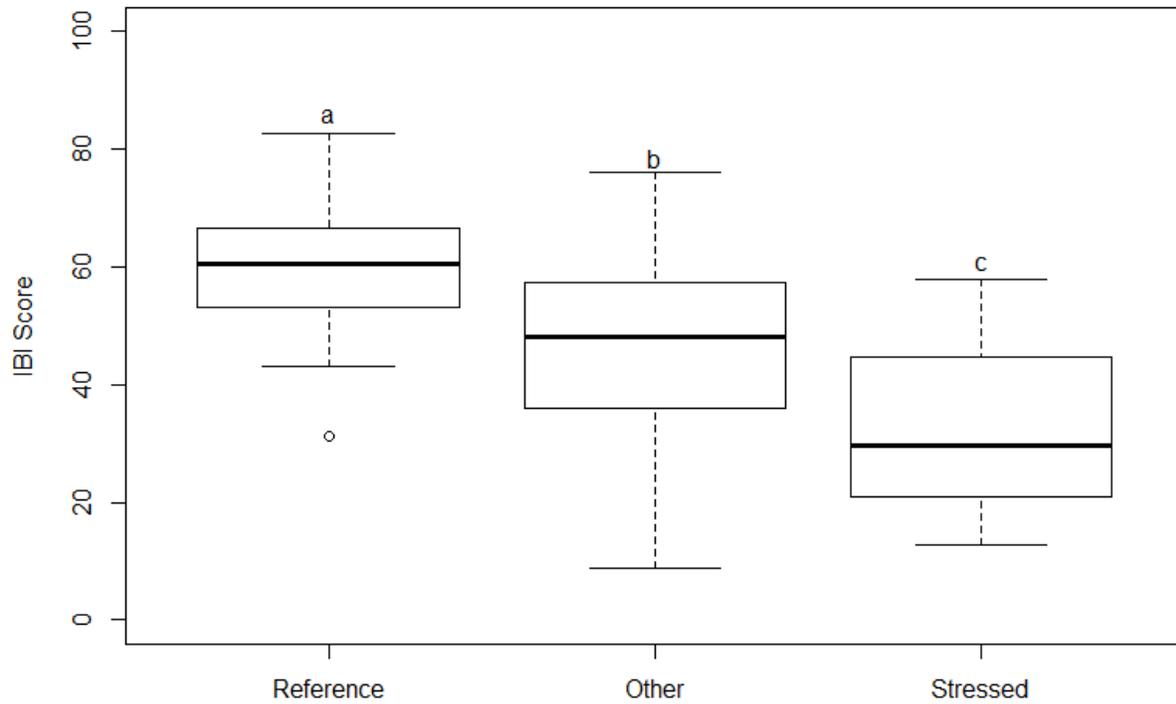


Figure 3.5-3: Final IBI scores for the Ohio and Mon WAP biomonitoring region. Different letters indicate a significant difference between groups (Reference, Stress, or Other) based on ANOVA results.

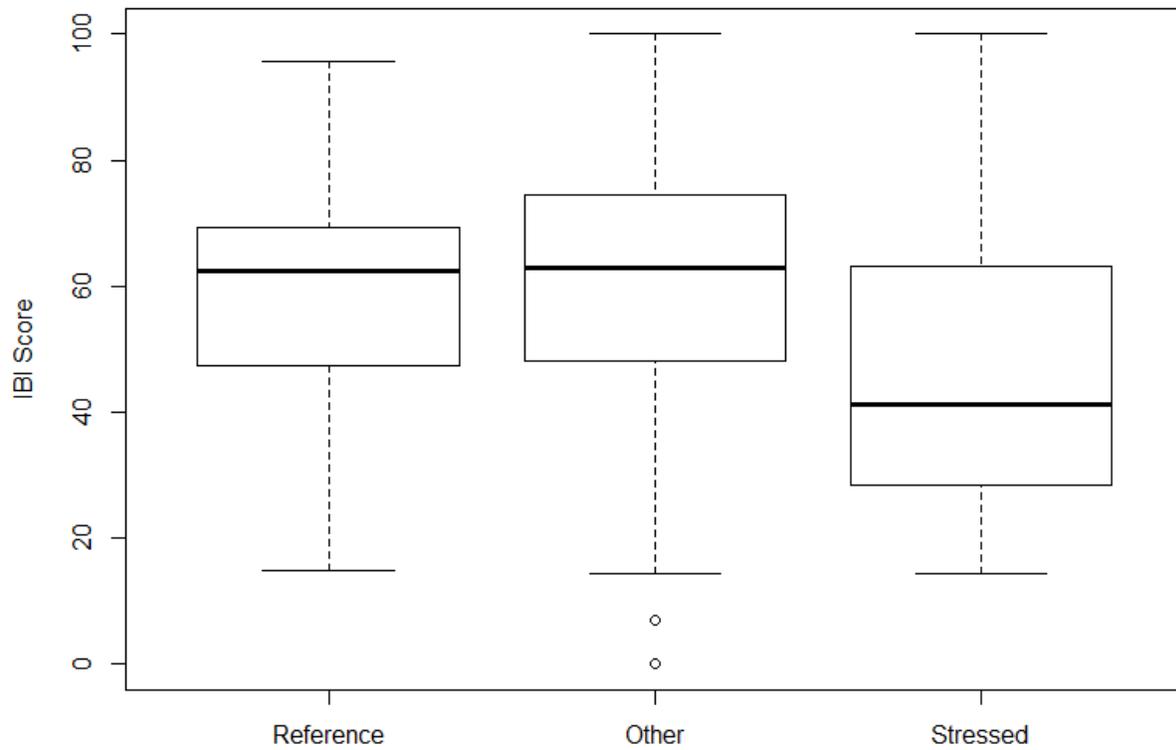


Figure 3.5-4: Final IBI scores for the Upper Kanawha biomonitoring region. There is no significant difference between groups based on the ANOVA results.

3.7 SCOPE OF IMPAIRMENT

3.7.1 Relationship of IBI to Abiotic and Biotic Stream Characteristics

Spearman correlations for final IBI scores within biomonitoring region against stream characteristics indicate that in some regions the IBI is responsive to anthropogenic land use patterns as well as to other measures of biotic conditions (Table 3.7-1). The Mon CA-RV region showed the strongest positive correlation, among all the regions, with biotic measures of stream conditions (WVSCI, GLIMPSS, Fish Abundance, and MAH IBI). All regions exhibited negative relationships, of varying strength, with specific conductance (SPC), as well as cumulative percentages of surface mining (C. Surface Mining) and development (C. Development). Strong relationships with drainage area and elevation were not detected in any of the regions, indicating that IBI scores are not biased towards large or low elevation streams. All regions exhibited positive relationships, of varying strength, with cumulative percent forest (C. Forest).

3.7.2 Temporal Variability

Duplicate samples (i.e. samples within the same segment level watershed) were retained within the Mon CA-RV (N=12), Ohio CA (N=4), and Ohio and Mon WAP (N=4) biomonitoring regions to evaluate the temporal variation in IBI scores between years. These duplicate samples were taken in different years and were not used to construct the final IBIs. The Mon CA-RV duplicate samples deviate strongly from the 1-to-1 relationship that was expected (Figure 3.7-1). Three of the 12 samples in the Mon CA-RV had initial IBI scores of 0 due to no fish being captured during the original sampling. Duplicate samples were taken in 2013 following chemical stream restoration of acid mine drainage (AMD) in the Three Forks watershed. The duplicate samples in the Ohio CA (Figure 3.7-2) and Ohio and Mon WAP (Figure 3.7-3) show little deviation from the 1-to-1 relationship that was expected. No duplicate samples were located in the Upper Kanawha biomonitoring region.

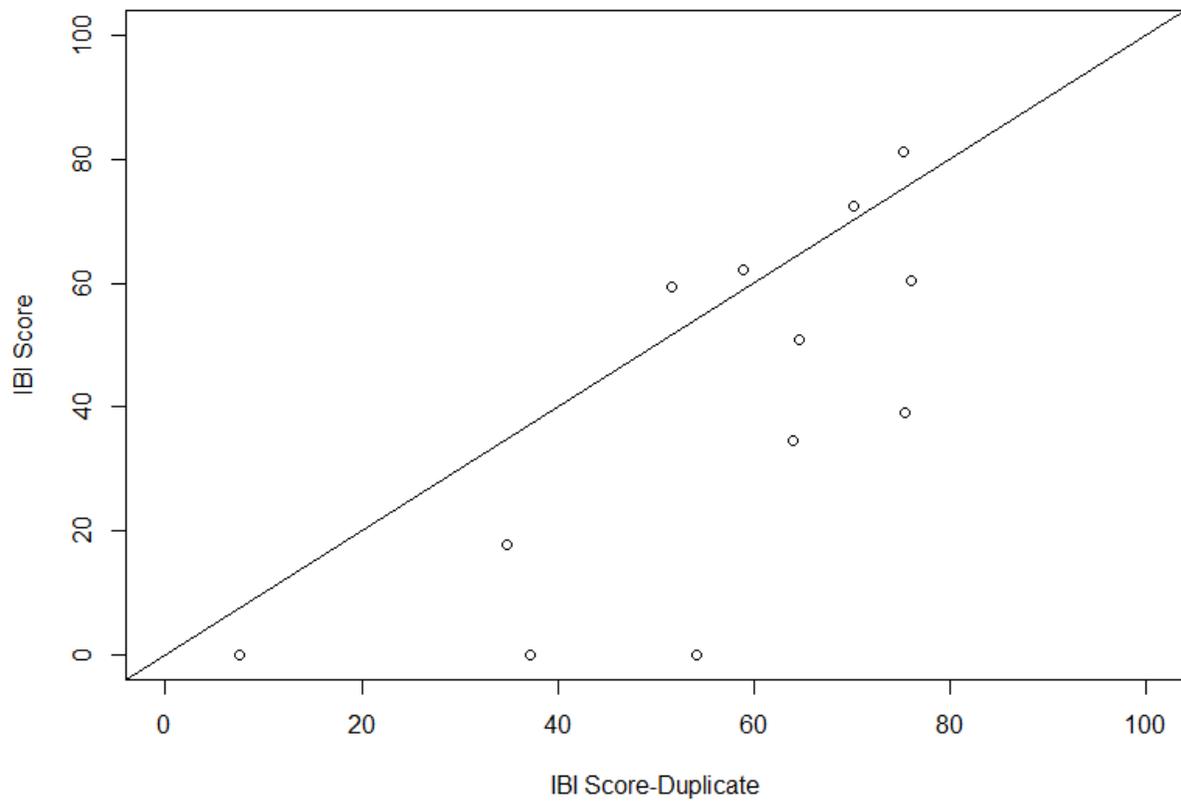


Figure 3.7-1: Final IBI scores for original (y-axis) and duplicate (x-axis) samples within the Mon CA-RV biomonitoring region. The solid line represents a 1:1 relationship.

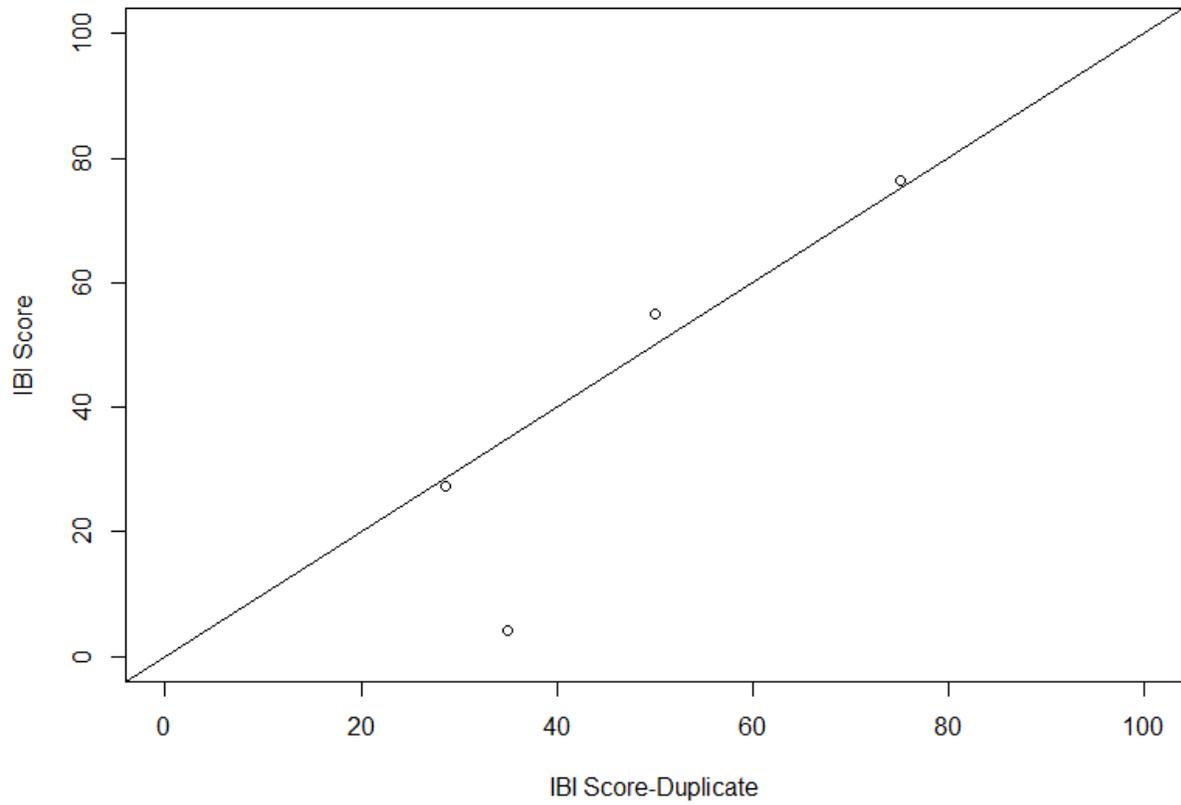


Figure 3.7-2: Final IBI scores for original (y-axis) and duplicate (x-axis) samples within the Ohio CA biomonitoring region. The solid line represents a 1:1 relationship.

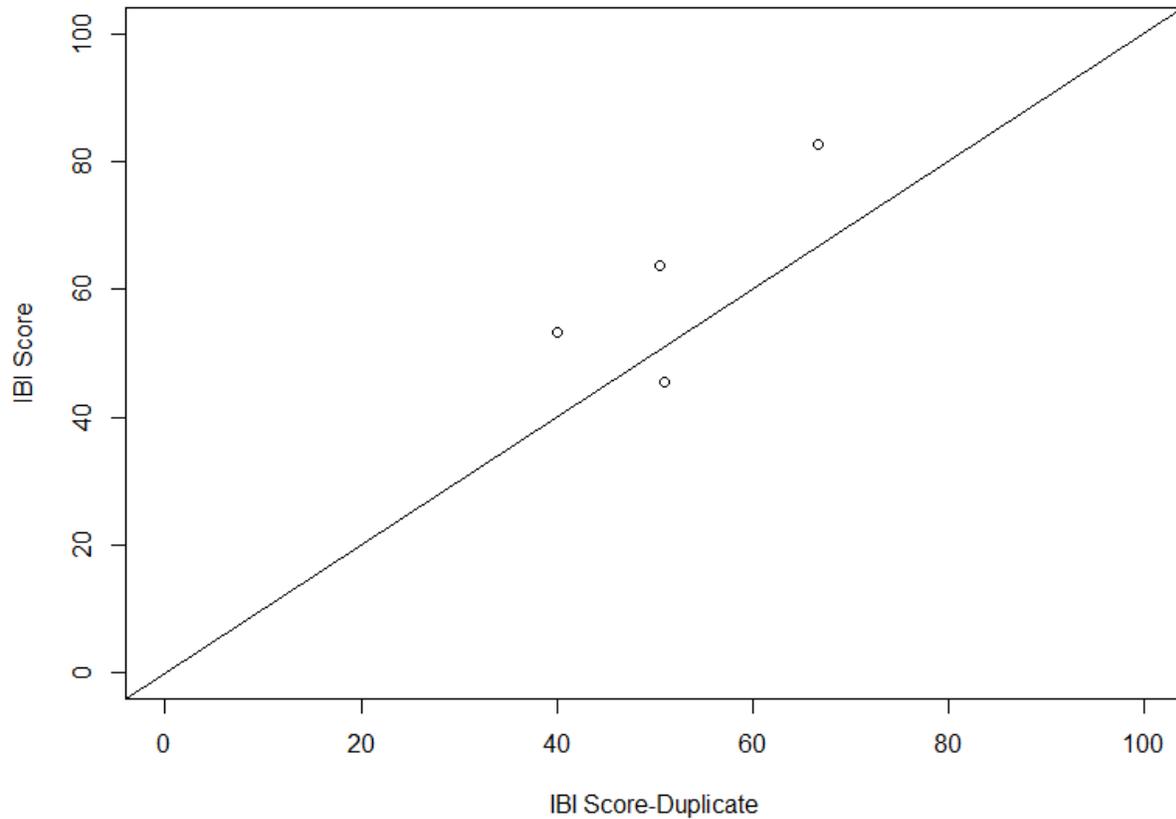


Figure 3.7-3: Final IBI scores for original (y-axis) and duplicate (x-axis) samples within the Ohio and Mon WAP biomonitoring region. The solid line represents a 1:1 relationship.

3.7.3 Assessment of Stream Condition

IBI scores exceeding the 75th percentile of the reference distribution in the Ohio CA (IBI \geq 76.82), Ohio and Mon WAP (IBI \geq 64.44), and Upper Kanawha (IBI \geq 67.89) were classified as having “Excellent” biotic integrity (Table 3.7-2). Scores between the 75th and 25th percentiles for the Ohio CA (76.82 – 46.12), Ohio and Mon WAP (64.44 – 50.92), and Upper Kanawha (67.89 – 39.79) were identified as having “Good” biotic integrity (Table 3.7-2). For the Ohio CA, Ohio and Mon WAP, and Upper Kanawha, any site exceeding the 25th percentile of the reference distribution was considered not impaired. Any IBI score below the 25th, for each region, was considered impaired. Impaired sites were divided into

two categories, “Degraded” and “Severely Degraded” (Table 3.7-2) based on the 5th percentile of the reference distribution within each biomonitoring region. The Mon CA-RV had a larger sample size of high quality reference sites than the other region, due to increased sampling efforts in 2013, therefore the 10th percentile of the reference distribution was used as the impairment threshold for this region.

Table 3.7-1: Spearman correlation coefficients for final IBI scores within each biomonitoring region against stream characteristics. Biotic variables include the West Virginia Stream Condition Index (WVSCI), Genus-Level Index of Most Probable Stream Status (GLIMPSS), total fish abundance, and the Mid-Atlantic Highlands Index of Biotic Integrity scores (MAH IBI). In stream characteristics compared were specific conductance (SPC) and pH, along with cumulative percentages of surface mining (C. Surface Mining), development (C. Development), agriculture (C. Agriculture), and total forest (C. Forest). Relationships with cumulative densities of structures (C. Structure Density) and National Pollution Discharge Elimination System Permits (C. NPDES Permit Density) were also evaluated.

Variable	Mon CA-RV	Ohio CA	Ohio-Mon WAP	Upper Kanawha
WVSCI	0.7922	-0.0793	0.0721	0.3980
GLIMPSS	0.7746	-0.1078	-0.053	0.3186
Fish Abundance	0.7707	0.5269	0.3174	0.5155
MAH IBI	0.7859	0.6866	0.6434	0.6256
SPC	-0.6640	-0.2736	-0.1983	-0.2949
pH	0.1110	-0.1065	-0.1842	0.1355
Drainage Area	0.2617	0.2372	-0.0995	0.0968
Elevation	0.1111	-0.3009	0.1342	0.1976
C. Surface Mining	-0.2026	-0.4148	-0.1348	-0.2585
C. Development	-0.2656	-0.1187	-0.3558	-0.3463
C. Agriculture	-0.3110	0.2083	-0.2003	0.0007
C. Structure Density	-0.0007	0.4265	-0.2103	-0.2628
C. Forest	0.3374	0.3326	0.3149	0.1816
C. NPDES Permit Density	-0.0412	0.1394	-0.1968	-0.2687

Table 3.7-2: Impairment category thresholds for each biomonitoring region based on the distribution of the reference sites. The 25th percentile within the Ohio CA, Ohio-Mon WAP, and Upper Kanawha and the 10th percentile in the Mon CA-RV were used to make the distinction between impaired (i.e. Degraded and Severely Degraded) and non-impaired (Good and Excellent) streams.

Condition	Mon CA-RV	Ohio CA	Ohio-Mon WAP	Upper Kanawha
Excellent	≥ 62.36	≥ 77.92	≥ 66.63	≥ 69.30
Good	62.36 – 56.15	77.92– 48.13	66.63 – 53.16	69.30 – 47.48
Degraded	56.15 – 45.38	48.13 – 23.64	53.16 – 43.21	47.48– 39.34
Severely Degraded	< 45.38	< 23.64	< 43.21	< 39.34

4.0 DISCUSSION

The development of Indices of Biotic Integrity for West Virginia warm water, wadeable streams followed common standardized techniques for selection fish community metrics (Stoddard et al. 2008) so that the final index in most of the biomonitoring regions were sensitive and responsive to anthropogenic impacts. Attempts were made to select metrics from key ecological categories (i.e. trophic, reproduction, and tolerance) in order to generate IBIs that give an overall view of stream condition.

4.1 NATURAL VARIATION

4.1.1 Regionalization of IBI

The evaluation of species presence and abundance of the original set of WVDEP classified reference sites allowed the first analysis of regionalization of West Virginia streams based on fish distributions. The further refinement of the spatial classification to include distinct watershed boundaries allows the IBI to be more biologically relevant and leaves little room for interpretation of which index to use based on sampling location. The analysis and production of IBI's for each biomonitoring region reduced the total number of reference sites available forcing the selection of supplemental reference sites from the pool of previously sampled sites. The implementation of these methods allowed for the development of regionally appropriate reference sites to accurately compare all other sites against. Very few other fish

based IBI's developed have the sample sizes to evaluate and produce meaningful biomonitoring regions over the extent of the study area.

Pond et al. (2012) classified West Virginia into two distinct regions for the Genus Level Index of Most Probable Stream Status (GLIMPSS) benthic macroinvertebrate multimetric index. The Mountain region consisted of the following ecoregions: Blue Ridge Mountain (not included in WV IBI), Ridge and Valley, and Central Appalachians. The Plateau region only contained the Western Allegheny Plateau. Even though fish distribution can relate to these ecoregions, they can be further confined by major watershed boundaries due to restricted in-stream movement, unlike macroinvertebrates. The combinations of both ecoregion and major basin allowed for some of the natural variation that is exhibited between fish communities to be controlled.

Neighboring states, such as Kentucky, Maryland, and Pennsylvania, have followed similar protocols by regionalizing developing Indices of Biotic Integrity. The Kentucky IBI utilizes both drainage basin (N=11) and ecoregion (N=7; Omernik 1987) to produce final ichthyoregions (N=6) in which separate IBI's were generated (Compton et al. 2003). Similar to the analysis presented here, the development of these ichthyoregions were determined using exploratory multivariate analyses suggesting several distinct fish faunal groups. Compton et al. (2003) also demonstrated that using ecological attributes in conjunction with taxonomic differences helped determine the final combinations of physiogeographic regions and river basins. Kentucky also has a distinct barrier to fish movement (Cumberland Falls) which results in highly dissimilar fish community structure above and below the falls which follows similar patterns seen in West Virginia (Kanawha Falls).

Similarly, Maryland IBI (Roth et al. 2000) has defined distinct geographic strata (N=3) corresponding to both physiogeographic region and river basin boundaries using cluster analysis and MANOVA with species assemblages. Pennsylvania Department of Environmental Protection determined prior to developing an IBI that there were distinct differences between Atlantic Slope and Ohio drainages in terms

of fish assemblages, so their development of an IBI was originally restricted to Atlantic Slope drainages only. However, after further analysis, it was determined that the Delaware River drainages lacked appropriate sample sizes in order to include in a final IBI, so it was subsequently removed from analysis (PA DEP 2012). Similar circumstances prevented the inclusion of Potomac drainages in IBI generation for West Virginia streams. It should be noted that further research in this region is needed in order to produce the sample sizes of reference sites and all sites will be needed in order to determine the feasibility of a cohesive IBI for the Potomac region.

4.1.2 Drainage Area Controls

The relationship between stream size (i.e. discharge or volume) has been well established (Angermeier and Schlosser 1989; Matthews and Robison 1998) and the majority of fish based IBIs account for this natural variation among sites. For the WV IBI's, the relationships between all fish community metrics and drainage area within the reference sites of each biomonitoring region was evaluated. If a significant relationship ($P < 0.05$) between drainage area (log base 10) and a metric was determined, then that equation to predict that metric value for all sites was used. This generated an expected metric value for a specific stream size. Rather than using the residuals of the prediction or standardizing all stream sizes (McCormick et al. 2004), an observed versus expected approach was utilized. This approach is commonly used in RIVPACs type designs and allows one to evaluate the deviations from what is expected at a given stream based on its size. The approach presented here was modeled after the Maryland IBI (Roth et al. 2000) with the exception that high quality sites not counted as reference were not included in our evaluations of relationships between individual metrics and drainage area.

4.1.3 Stream Temperature Classes

Segregation of stream types into temperature classes are common in IBIs that are developed in regions with high geographic variation and stream temperature regimes (see Lyons et al. 1996 and Lyons 2012). Models predicting in-stream temperatures have found that there are several local and regional

environmental factors controlling stream temperature. These variables can include elevation, watershed precipitation, slope, riparian cover, aspect, and air temperature (Segura et al. 2015). These measurements, in conjunction with continuous stream temperature data, could be used to generate predictions of maximum daily mean water temperature for all wadeable streams in West Virginia.

The immediate need for stream temperature modeling becomes important when evaluating the current classification of stream temperature classes using species assemblages. There were 2 duplicate stream samples that shifted from cold water streams in one year to a warm water stream in the following years (Mon CA-RV biomonitoring region). One of these shifts has been attributed to the addition of a top-release dam upstream of the sampling location. However, the reason for the shift of the second site from a cold water species assemblage to a warm water assemblage is unknown. These shifts demonstrate the need to develop non-fish based classification criteria or *a priori* expectations of stream temperature regimes prior to sampling in order to evaluate temperature impaired cold water streams that can no longer support a cold water fish assemblage. In addition, the biological status and locations of current cold water streams is unknown and monitoring these locations for economically valuable natural resources (i.e. trout fishing) in the face of increased anthropogenic and climate changes.

4.4 TEMPORAL VARIABILITY

Among year variability between IBI scores within each biomonitoring region should continue to be evaluated as duplicate samples are generated. Even though there were duplicate samples within each biomonitoring region, the temporal variability among years, or within a year, with these low numbers of duplicate samples, cannot be accurately determined. The Mon CA-RV biomonitoring region had the most duplicate samples due to increased sampling efforts by the West Virginia DEP in 2013. However, this region also demonstrated the highest variation between duplicate samples due to chemical stream restoration efforts. Even though some of this variation is attributed to acid mine drainage remediation in

the Three Forks watershed, this still shows the importance of long term monitoring of sites within each biomonitoring region.

4.5 UNCERTAINTY OF THE IBI

As would be expected, the least-impacted, or reference, sites had the higher IBI scores within each region. However, when sites types were compared within each region, some questions about the uncertainty of the IBI were formed. For example, the Mon CA-RV biomonitoring region did not exhibit a significant difference between stressed and other sites. This lack of a significant difference did not come as a surprise due to the definition of the “other” site type. Sites within the “other” category were placed there because there was either insufficient data to elevate the site to reference or to classify it as a stressed site, or the site was of intermediate quality and did not meet the standards of reference or stressed sites. Either outcome should produce a category of sites that span a wide range of environmental conditions and may not be statistically different from either reference or stressed sites, because it may contain both.

The Upper Kanawha region however, demonstrates no significant difference between any of the site types. The exact cause for the lack of difference is unknown. This region is known for its high quality streams and the majority of the streams in this region are historically cold water streams. The criteria used to classify sites into reference/stressed and cold/warm should be closely evaluated for this region in order to ensure streams are being placed into appropriate categories, which may not be the same criteria used for the other regions. Additionally, the high landscape heterogeneity in this region may indicate that the recommended reference site sample size ($N=34 - 40$; Yoder and Rankin 1995) be met for IBI development in this region.

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APPENDIX A

All 171 species occurring in the dataset were classified based on native status, spawning, feeding, tolerance values, and other data. Native status consists of only species native to the Ohio, Monongahela (Mon), Potomac, and Upper Kanawha (UK) drainages or all drainages (WV). Spawning consisted of rock-gravel spawners (RG), gravel-sand spawners (GS), non-guarding lithophils (NGL), cavity spawners (CAV), and clean gravel spawners (CGS). The feeding (trophic) category consisted of invertivore-piscivore (IP), invertivore (IN), macro-omnivore (MO), and omnivore-herbivore (OH). Tolerance values ranged from intolerant (I), moderate tolerance (M), and tolerant (T). Other classifications included benthic species (B), game species (G), and cold water species (C). Lithophilic spawners in sand to rock (LSR) is consisted of any species that was classified as either RG or GS, or showing no substrate preference. Species are listed in descending order according to their scientific name.

Common	Scientific	Code	Family	Native	Spawning	Trophic	Tolerance	Other
Skipjack herring	<i>Alosa chrysochloris</i>	ALCH	Clupeidae	Ohio		IP	M	
Rock bass	<i>Ambloplites rupestris</i>	AMRU	Centrarchidae	Mon, Ohio		IP	M	G, B
Black bullhead	<i>Ameiurus melas</i>	AMME	Ictaluridae	Ohio		MO, OH	M	G, B
Yellow bullhead	<i>Ameiurus natalis</i>	AMNA	Ictaluridae	WV		MO, OH	T	G, B
Brown bullhead	<i>Ameiurus nebulosus</i>	AMNE	Ictaluridae	WV		MO, OH	T	G, B
Western sand darter	<i>Ammocrypta clara</i>	AMCL	Percidae	Ohio	LSR	IN, IP	I	B
Eastern sand darter	<i>Ammocrypta pellucida</i>	AMPE	Percidae	Ohio	LSR	IN, IP	I	B
American eel	<i>Anguilla rostrata</i>	ANRO	Anguillidae	Potomac, Ohio, UK		IP	T	
Freshwater drum	<i>Aplodinotus grunniens</i>	APGR	Sciaenidae	Ohio		IN, IP	M	G, B
Central Stoneroller	<i>Campostoma anomalum</i>	CAAN	Cyprinidae	WV	RG, CGS	MO, OH	T	B
Goldfish	<i>Carassius auratus</i>	CAAU	Cyprinidae	None		MO, OH	T	
River carpsucker	<i>Carpiodes carpio</i>	CACA	Catostomidae	Ohio, Mon		MO, OH	M	B
Quillback	<i>Carpiodes cyprinus</i>	CACY	Catostomidae	Mon, Ohio		MO, OH	T	B
Highfin carpsucker	<i>Carpiodes velifer</i>	CAVE	Catostomidae	Ohio		MO, OH	I	B
Longnose sucker	<i>Catostomus catostomus</i>	CACT	Catostomidae	Mon	RG, NGL	MO, OH	I	B, C
White sucker	<i>Catostomus commersonii</i>	CACO	Catostomidae	WV	GS, NGL	MO, OH	T	B

Common	Scientific	Code	Family	Native	Spawning	Trophic	Tolerance	Other
Southern redbelly dace	<i>Chrosomus erythrogaster</i>	PHER	Cyprinidae	Ohio		MO, OH	M	
Mountain redbelly dace	<i>Chrosomus oreas</i>	PHOR	Cyprinidae	UK	RG	MO, OH	I	C
Redside dace	<i>Clinostomus elongatus</i>	CLEL	Cyprinidae	Mon, Ohio	RG	IN, IP	I	
Rosyside dace	<i>Clinostomus funduloides</i>	CLFU	Cyprinidae	WV	RG	IN, IP	I	
Mottled sculpin	<i>Cottus bairdii</i>	COBA	Cottidae	Mon, Ohio, UK	CAV	IN, IP	M	B
Blue Ridge sculpin	<i>Cottus caeruleomentum</i>	COCA	Cottidae	Potomac		IN, IP	M	B
Banded sculpin	<i>Cottus carolinae</i>	COCR	Cottidae	UK	CAV	IN, IP	M	B
Slimy sculpin	<i>Cottus cognatus</i>	COCO	Cottidae	Potomac	CAV	IN, IP	I	B, C
Potomac sculpin	<i>Cottus girardi</i>	COGI	Cottidae	Potomac	CAV	IN, IP	M	B
Kanawha sculpin	<i>Cottus kanawhae</i>	COKA	Cottidae	UK		IN, IP	I	B, C
Checkered Sculpin	<i>Cottus n.sp.</i>	CORO	Cottidae	Potomac		IN, IP	M	B, C
Bluestone sculpin	<i>Cottus sp.</i>	COBL	Cottidae	UK		IN, IP	M	B
Diamond darter	<i>Crystallaria cincotta</i>	CRCI	Percidae	Ohio		IN, IP	I	B
Grass carp	<i>Ctenopharyngodon idella</i>	CTID	Cyprinidae	None		MO, OH	M	B
Satinfin shiner	<i>Cyprinella analostana</i>	CYAN	Cyprinidae	Potomac	CAV	OH	T	
Whitetail shiner	<i>Cyprinella galactura</i>	CYGA	Cyprinidae	UK	CAV	IN, IP	M	
Spotfin shiner	<i>Cyprinella spiloptera</i>	CYSP	Cyprinidae	WV	CAV	IN, IP	T	
Steelcolor shiner	<i>Cyprinella whipplei</i>	CYWH	Cyprinidae	Mon, Ohio	CAV	IN, IP	M	
Common carp	<i>Cyprinus carpio</i>	CYCA	Cyprinidae	None		MO, OH	T	G
Gizzard shad	<i>Dorosoma cepedianum</i>	DOCE	Clupeidae	Mon, Ohio		MO, OH	T	
Threadfin shad	<i>Dorosoma petenense</i>	DOPE	Clupeidae	Ohio		MO, OH	M	
Appalachia darter	<i>ercina gymnocephala</i>	PEGY	Percidae	UK	GS, CGS	IN, IP	I	B
Streamline chub	<i>Erimystax dissimilis</i>	ERDI	Cyprinidae	Ohio, UK	RG, NGL	OH	I	B
Creek chubsucker	<i>Erimyzon oblongus</i>	EROB	Catostomidae	Potomac	GS, NGL	MO, OH	I	B
Grass pickerel	<i>Esox americanus</i>	ESAM	Esocidae	Potomac,		IP	M	

Common	Scientific	Code	Family	Native	Spawning	Trophic	Tolerance	Other
				Ohio				
Northern Pike	<i>Esox lucius</i>	ESLU	Esocidae	None	NGL	IP	I	G
Muskellunge	<i>Esox masquinongy</i>	ESMA	Esocidae	Mon, Ohio		IP	I	G
Chain pickerel	<i>Esox niger</i>	ESNI	Esocidae	Potomac		IP	M	G
Greenside darter	<i>Etheostoma blennioides</i>	ETBL	Percidae	Mon, Ohio, UK	RG, NGL	IN, IP	I	B
Rainbow darter	<i>Etheostoma caeruleum</i>	ETCA	Percidae	Mon, Ohio, UK	RG, CGS	IN, IP	M	B
Bluebreast darter	<i>Etheostoma camurum</i>	ETCM	Percidae	Mon, Ohio, UK	GS	IN, IP	I	B
Fantail darter	<i>Etheostoma flabellare</i>	ETFL	Percidae	WV	RG, CAV	IN, IP	M	B
Longfin darter	<i>Etheostoma longimanum</i>	ETLO	Percidae	None		IN, IP	I	B
Spotted darter	<i>Etheostoma maculatum</i>	ETMA	Percidae	Ohio	CAV	IN, IP	I	B
Johnny darter	<i>Etheostoma nigrum</i>	ETNI	Percidae	Mon, Ohio, UK	RG, CAV	IN, IP	M	B
Tessellated darter	<i>Etheostoma olmstedii</i>	ETOL	Percidae	Potomac	CAV	IN, IP	M	B
Candy darter	<i>Etheostoma osburni</i>	ETOS	Percidae	UK	GS	IN, IP	I	B
Snubnose darter	<i>Etheostoma simoterum</i>	ETSI	Percidae	None	RG	IN, IP	M	B
Tippecanoe darter	<i>Etheostoma tippecanoe</i>	ETTI	Percidae	Ohio	RG	IN, IP	I	B
Variegated darter	<i>Etheostoma variatum</i>	ETVA	Percidae	Mon, Ohio	GS, NGL	IN, IP	M	B
Banded darter	<i>Etheostoma zonale</i>	ETZO	Percidae	Mon, Ohio	NGL	IN, IP	I	B
Tonguetied minnow	<i>Exoglossum laurae</i>	EXLA	Cyprinidae	Mon, UK	RG, CGS	IN, IP	M	
Cutlips minnow	<i>Exoglossum maxillingua</i>	EXMA	Cyprinidae	Potomac	RG, CGS	IN, IP	I	
Northern studfish	<i>Fundulus catenatus</i>	FUCA	Fundulidae	None		IN, IP	I	
Banded killifish	<i>Fundulus diaphanus</i>	FUDI	Fundulidae	Potomac, Mon, Ohio		IN, IP	T	
Mosquitofish	<i>Gambusia affinis</i>	GAAF	Poeciliidae	None		IN, IP	T	
Eastern mosquitofish	<i>Gambusia holbrooki</i>	GAHO	Poeciliidae	None	CGS	IN, IP	T	
Goldeye	<i>Hiodon alosoides</i>	HIAL	Hiodontidae	Ohio	NGL	IN, IP	I	B
Mooneye	<i>Hiodon tergisus</i>	HITE	Hiodontidae	Mon, Ohio		IN, IP	I	

Common	Scientific	Code	Family	Native	Spawning	Trophic	Tolerance	Other
Eastern silvery minnow	<i>Hybognathus regius</i>	HYRE	Cyprinidae	Potomac		MO, OH	I	B
Bigeye chub	<i>Hybopsis amblops</i>	HYAM	Cyprinidae	Mon, Ohio	GS, NGL	IN, IP	M	
Northern hogsucker	<i>Hypentelium nigricans</i>	HYNI	Catostomidae	WV	RG, CGS, NGL	IN, IP	M	B
Ohio lamprey	<i>Ichthyomyzon bdellium</i>	ICBD	Petromyzontidae	Ohio	RG	MO, OH	M	B
Northern Brook lamprey	<i>Ichthyomyzon fossor</i>	ICFO	Petromyzontidae	Ohio	RG	MO, OH	I	B
Mountain brook lamprey	<i>Ichthyomyzon greeleyi</i>	ICGR	Petromyzontidae	Ohio	GS, CGS	MO, OH	I	B
Silver lamprey	<i>Ichthyomyzon unicuspis</i>	ICUN	Petromyzontidae	Ohio	GS	MO, OH	M	B
Channel catfish	<i>Ictalurus punctatus</i>	ICPU	Ictaluridae	Mon, Ohio, UK		MO, OH	T	B, G
Smallmouth Buffalo	<i>Ictiobus bubalus</i>	ICBU	Catostomidae	Ohio		OH	M	B
Bigmouth buffalo	<i>Ictiobus cyprinellus</i>	ICCY	Catostomidae	Ohio		OH	M	B
Black Buffalo	<i>Ictiobus niger</i>	ICNI	Catostomidae	Ohio		MO, OH	M	B
Brook silverside	<i>Labidesthes sicculus</i>	LASI	Atherinopsidae	Mon, Ohio		IN, IP	I	
Least brook lamprey	<i>Lampetra aepyptera</i>	LAAE	Petromyzontidae	Mon, Ohio	GS, CGS	MO, OH	I	B
Longnose gar	<i>Lepisosteus osseus</i>	LEOS	Lepisosteidae	Mon, Ohio		IP	M	G
Redbreast sunfish	<i>Lepomis auritus</i>	LEAU	Centrarchidae	Potomac	GS	IP	M	G
Green sunfish	<i>Lepomis cyanellus</i>	LECY	Centrarchidae	Mon, Ohio		IP	T	G
Pumpkinseed	<i>Lepomis gibbosus</i>	LEGI	Centrarchidae	Potomac, Mon, Ohio		IN, IP	M	
Warmouth	<i>Lepomis gulosus</i>	LEGU	Centrarchidae	Ohio		IP	M	
Orangespotted Sunfish	<i>Lepomis humilis</i>	LEHU	Centrarchidae	Ohio	GS	IP	M	
Bluegill	<i>Lepomis macrochirus</i>	LEMA	Centrarchidae	Potomac, Mon, Ohio		IN, IP	T	G
Longear sunfish	<i>Lepomis megalotis</i>	LEME	Centrarchidae	Potomac, Mon, Ohio		IN, IP	M	G
Redear sunfish	<i>Lepomis microlophus</i>	LEMI	Centrarchidae	None		IN, IP	M	

Common	Scientific	Code	Family	Native	Spawning	Trophic	Tolerance	Other
American brook lamprey	<i>Lethenteron appendix</i>	LAAP	Petromyzontidae	Ohio	GS, CGS	MO, OH	I	B
White shiner	<i>Luxilus albeolus</i>	LUAL	Cyprinidae	UK	RG	IN, IP	M	
Striped shiner	<i>Luxilus chrysocephalus</i>	LUCH	Cyprinidae	Mon, Ohio	RG	OH	T	
Common shiner	<i>Luxilus cornutus</i>	LUCO	Cyprinidae	Potomac	GS	OH	M	
Rosefin shiner	<i>Lythrurus ardens</i>	LYAR	Cyprinidae	UK	RG	IN, IP	M	
Redfin shiner	<i>Lythrurus umbratilis</i>	LYUM	Cyprinidae	Mon, Ohio		IN, IP	T	
Speckled chub	<i>Macrhybopsis aestivalis</i>	MAAE	Cyprinidae	Ohio		IP	I	B
Shoal chub	<i>Macrhybopsis hyostoma</i>	MAHY	Cyprinidae	Ohio		IN, IP	I	
Silver chub	<i>Macrhybopsis storeriana</i>	MAST	Cyprinidae	Mon, Ohio		IN, IP	I	B
Pearl dace	<i>Margariscus margarita</i>	MAMA	Cyprinidae	Potomac, Mon	GS, NGL	IN, IP	M	
Smallmouth bass	<i>Micropterus dolomieu</i>	MIDO	Centrarchidae	Potomac, Mon, Ohio		IP	M	G
Spotted bass	<i>Micropterus punctulatus</i>	MIPU	Centrarchidae	Mon, Ohio, UK		IP	M	G
Largemouth bass	<i>Micropterus salmoides</i>	MISA	Centrarchidae	Potomac, Mon, Ohio		IP	M	G
Spotted sucker	<i>Minytrema melanops</i>	MIME	Catostomidae	Ohio	RG, NGL	OH	M	B
White Perch	<i>Morone americana</i>	MOAM	Moronidae	None		IP	M	G
White bass	<i>Morone chrysops</i>	MOCH	Moronidae	Ohio		IP	T	G
Striped bass	<i>Morone saxatilis</i>	MOSA	Moronidae	None		IP	I	G
Silver redhorse	<i>Moxostoma anisurum</i>	MOAN	Catostomidae	Mon, Ohio	RG, NGL	IN, IP	M	B
Smallmouth redhorse	<i>Moxostoma breviceps</i>	MOBR	Catostomidae	Ohio		IN, IP	M	B
River redhorse	<i>Moxostoma carinatum</i>	MOCA	Catostomidae	Ohio	RG, NGL	IN, IP	I	B
Black redhorse	<i>Moxostoma duquesni</i>	MODU	Catostomidae	Ohio	RG, NGL	IN, IP	I	B
Golden redhorse	<i>Moxostoma erythrurum</i>	MOER	Catostomidae	Potomac, Mon, Ohio	GS, CGS, NGL	IN, IP	I	B
Shorthead redhorse	<i>Moxostoma macrolepidotum</i>	MOMA	Catostomidae	Potomac	RG, NGL	IN, IP	M	B
Bluehead chub	<i>Nocomis leptocephalus</i>	NOLE	Cyprinidae	UK	RG, CGS	MO, OH	M	
River chub	<i>Nocomis micropogon</i>	NOMI	Cyprinidae	Potomac, Mon, Ohio	RG, CGS	IN, IP	M	
Bigmouth chub	<i>Nocomis platyrhynchus</i>	NOPL	Cyprinidae	UK	RG	IN, IP	M	

Common	Scientific	Code	Family	Native	Spawning	Trophic	Tolerance	Other
Golden shiner	<i>Notemigonus crysoleucas</i>	NOCY	Cyprinidae	Potomac, Mon, Ohio		MO, OH	T	
Comely shiner	<i>Notropis amoenus</i>	NOAM	Cyprinidae	Potomac	RG	IN, IP	T	
Popeye shiner	<i>Notropis ariommus</i>	NOAR	Cyprinidae	Mon, Ohio	RG, NGL	IN, IP	I	
Emerald shiner	<i>Notropis atherinoides</i>	NOAT	Cyprinidae	Mon, Ohio		MO, OH	M	
River shiner	<i>Notropis blennius</i>	NOBL	Cyprinidae	Ohio	GS, NGL	IN, IP	M	
Bigeye shiner	<i>Notropis boops</i>	NOBO	Cyprinidae	Ohio	GS, NGL	IN, IP	I	
Silverjaw minnow	<i>Notropis buccatus</i>	NOBU	Cyprinidae	WV	GS, NGL	IN, IP	T	
Ghost shiner	<i>Notropis buchanani</i>	NOBC	Cyprinidae	Mon, Ohio	GS, NGL	IN, IP	M	
Spottail shiner	<i>Notropis hudsonius</i>	NOHU	Cyprinidae	Potomac	GS, NGL	OH	M	
Silver shiner	<i>Notropis photogenis</i>	NOPH	Cyprinidae	Mon, Ohio, UK		IN, IP	T	
Swallowtail shiner	<i>Notropis procne</i>	NOPR	Cyprinidae	Potomac	GS, NGL	IN, IP	M	
Rosyface shiner	<i>Notropis rubellus</i>	NORU	Cyprinidae	WV	RG, NGL	IN, IP	I	
New River shiner	<i>Notropis scabriceps</i>	NOSC	Cyprinidae	UK	GS, NGL	IN, IP	I	
Sand shiner	<i>Notropis stramineus</i>	NOST	Cyprinidae	Ohio, UK	LSR	OH	M	
Telescope shiner	<i>Notropis telescopus</i>	NOTE	Cyprinidae	None	GS, NGL	IN, IP	M	
Mimic shiner	<i>Notropis volucellus</i>	NOVO	Cyprinidae	Mon, Ohio, UK		IN, IP	M	
Channel shiner	<i>Notropis wickliffi</i>	NOWI	Cyprinidae	Ohio		IN, IP	M	
Mountain madtom	<i>Noturus eleutherus</i>	NOEL	Ictaluridae	Ohio	CAV	IN, IP	I	B
Yellowfin madtom	<i>Noturus flavipinnis</i>	NOFL	Ictaluridae	None	CAV	IN, IP	I	
Stonecat	<i>Noturus flavus</i>	NOFU	Ictaluridae	Mon, Ohio, UK	CAV	IN, IP	M	B
Margined madtom	<i>Noturus insignis</i>	NOIN	Ictaluridae	Potomac, UK	CAV	IN, IP	M	B
Brindled madtom	<i>Noturus miurus</i>	NOMU	Ictaluridae	Ohio, UK	CAV	IN, IP	M	B
Northern madtom	<i>Noturus stigmosus</i>	NOSG	Ictaluridae	Ohio	CAV	IN, IP	I	B

Common	Scientific	Code	Family	Native	Spawning	Trophic	Tolerance	Other
Rainbow trout	<i>Oncorhynchus mykiss</i>	ONMY	Salmonidae	None		IP	I	G, C
Cheat minnow	<i>Pararhinichthys bowersi</i>	PABO	Cyprinidae	Mon		IN, IP	M	B
Yellow perch	<i>Perca flavescens</i>	PEFL	Percidae	None		IP	M	G
Logperch	<i>Percina caprodes</i>	PECA	Percidae	Mon, Ohio	GS, CGS	IN, IP	M	B
Channel darter	<i>Percina copelandi</i>	PECO	Percidae	Ohio	RG	IN, IP	I	B
Gilt darter	<i>Percina evides</i>	PEEV	Percidae	Ohio	GS, CGS	IN, IP	I	B
Longhead darter	<i>Percina macrocephala</i>	PEMA	Percidae	Ohio	RG, NGL	IN, IP	I	B
Blackside darter	<i>Percina maculata</i>	PEMC	Percidae	Mon, Ohio	GS, CGS	IN, IP	M	B
Stripeback darter	<i>Percina notogramma</i>	PENO	Percidae	None	CGS	IN, IP	I	B
Sharpnose darter	<i>Percina oxyrhynchus</i>	PEOX	Percidae	Mon, Ohio, UK	GS	IN, IP	I	B
Slenderhead darter	<i>Percina phoxocephala</i>	PEPH	Percidae	Ohio	GS	IN, IP	I	B
Roanoke darter	<i>Percina roanoka</i>	PERO	Percidae	None	GS, CGS	IN, IP	M	B
Dusky darter	<i>Percina sciera</i>	PESC	Percidae	Ohio	GS	IN, IP	M	B
River darter	<i>Percina shumardi</i>	PESH	Percidae	Ohio	GS, CGS	IN, IP	M	B
Trout-perch	<i>Percopsis omiscomaycus</i>	PEOM	Percopsidae	Ohio		IN, IP	M	B
Suckermouth minnow	<i>Phenacobius mirabilis</i>	PHMI	Cyprinidae	Ohio	GS, NGL	OH	M	B
Kanawha minnow	<i>Phenacobius teretulus</i>	PHTE	Cyprinidae	UK	RG, NGL	OH	I	B
Bluntnose minnow	<i>Pimephales notatus</i>	PINO	Cyprinidae	WV	CAV	MO, OH	T	
Fathead minnow	<i>Pimephales promelas</i>	PIPR	Cyprinidae	Ohio	CAV	MO, OH	T	
Bullhead minnow	<i>Pimephales vigilax</i>	PIVI	Cyprinidae	Ohio	CAV	MO, OH	M	
Paddlefish	<i>Polyodon spathula</i>	POSP	Polydontidae	Ohio	NGL	MO, OH	I	G
White crappie	<i>Pomoxis annularis</i>	POAN	Centrarchidae	Mon, Ohio		IP	T	G
Black crappie	<i>Pomoxis nigromaculatus</i>	PONI	Centrarchidae	Mon, Ohio		IP	M	G
Flathead catfish	<i>Pylodictis olivaris</i>	PYOL	Ictaluridae	Mon, Ohio, UK	CAV	IP	M	G, B
Blacknose dace	<i>Rhinichthys atratulus</i>	RHAT	Cyprinidae	WV	GS, CGS	MO, OH	T	B
Longnose dace	<i>Rhinichthys cataractae</i>	RHCA	Cyprinidae	WV	CGS	IN, IP	M	B, C
Western	<i>Rhinichthys obtusus</i>	RHOB	Cyprinidae	Ohio	CGS	MO, OH	T	B, C

Common	Scientific	Code	Family	Native	Spawning	Trophic	Tolerance	Other
blacknose dace								
Brown trout	<i>Salmo trutta</i>	SATR	Salmonidae	None	CGS	IP	I	G, C
Brook trout	<i>Salvelinus fontinalis</i>	SAFO	Salmonidae	WV	CGS	IP	I	G, C
Sauger	<i>Sander canadensis</i>	SACA	Percidae	Mon, Ohio		IP	M	G, B
Walleye	<i>Sander vitreus</i>	SAVI	Percidae	Mon, Ohio		IP	M	G, B
Creek chub	<i>Semotilus atromaculatus</i>	SEAT	Cyprinidae	WV	GS	IP	T	
Fallfish	<i>Semotilus corporalis</i>	SECO	Cyprinidae	Potomac	RG	IP	M	G
Torrent sucker	<i>Thoburnia rathoeca</i>	THRH	Catostomidae	Potomac, UK	RG, CGS, NGL	MO, OH	I	B