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***Aquatic Life Stressor Identification: Preliminary  
Data for Southeast Ohio***



Midwest Biodiversity

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## ***Preliminary Aquatic Life Stressor Identification Data for Southeast Ohio***

### **Introduction**

Aquatic Life uses are the narrative ecological goals that states set for each of their waterbodies. Ohio is unique in having numeric biocriteria for each of the tiered stream uses in its Water Quality Standards (WQS) regulations. Impairment of these aquatic life uses as measured by biological indices usually drives the listing of impaired waters as required by section 304(l) of the Clean Water Act (CWA). The listing of such “impaired” waters drives the development of total maximum daily loads (TMDLs) of pollutants and other management activities necessary to restore these waters. Scientifically sound identification of contributing stressors is integral to this process.

In Southeast Ohio, because of extensive mining, acid mine abatement plans (AMDATs), developed by Ohio DNR and partners are an important tool to restore acid-impaired waters. It is important to both the TMDL and AMDAT programs that impaired waters and watersheds are accurately identified and that the relative contribution of each stressor to this impairment is quantified. While biological data excels in integrating the impacts of multiple stressors, it is difficult to use this data *by itself* to completely characterize stressors responsible for impairment. Similarly, data on stressor data can be invaluable for identifying with some precision the relative contribution of individual stressors, but by themselves can be poor predictors of biological conditions.

The data included in this report was derived as part of the basis for developing a stressor identification guide for Southeast Ohio. The complete manual will provide step-by-step guidance to help scientists identify the key stressor impairing aquatic life in Southeast Ohio and should include information on fish assemblages, macroinvertebrates (at multiple scales of taxonomic resolution), and on algal assemblages. This effort is a step towards that manual and provides some initial baseline data and analytic approaches and tools that will eventually comprise an approach and tool box for a comprehensive assessment of factors limiting streams and rivers in Southeast Ohio.

This effort provides the following products:

- 1) weighted stressor and/or average stressor values (WSVs or ASVs) for fish species and macroinvertebrate genera using Statewide data and separately for the Western Allegheny Plateau (WAP) level III ecoregion of Southeast Ohio;
- 2) tolerance indicator values (TIVs) calculated using WSVs and/or ASVs for fish species and macroinvertebrate genera using Statewide and separately for the WAP ecoregion;
- 3) data on fish species and macroinvertebrate genera, for each key stressor, Statewide and in Southeast Ohio, paired with presence or absence of each fish species and macroinvertebrate genus than can be used to generate logistic regression models;

- 4) data on fish species and macroinvertebrate genera, in approximately equal-sized bins (20) for each key stressor, Statewide and in Southeast Ohio, paired with percent of sites with the presence of each fish species and macroinvertebrate genus than can be used to generate smoothing curves to visually classify the shape and form of the response to each stressor; and
- 5) an estimate of approximately 20 or so of the most sensitive and tolerant fish species and key stressors important to the mined areas of the WAP ecoregion that can form the basis for an atlas of stressor-response relationships between these species and key stressors.

This document provides descriptions of the data in each of these tables and a description of how they can be used in stressor identification efforts. It also describes some of the limitations of the data and analytical approaches. Stressor identification efforts improve over time as stressor identification studies supplement the knowledge base on site specific stressor-response relationships. The accrual of data and analyses are important because they provide a template for understanding biological responses in characteristically complex multi-stressor watersheds. Because stressors rarely occur alone in nature and because natural underlying environmental conditions vary spatially with geology, land use, stream size, gradient, groundwater and surface water regimes, soils, elevation, latitude, and subecoregion, the relative contribution of stressors may be similar in general effect, but moderated or exacerbated by one or more natural factors. Similarly, anthropogenic effects can change over time with a lessening of some stressors (e.g., point sources), but changes in other factors such as the extent and nature of land development. The potential effects of some of these anthropogenic changes are relatively well known (e.g., urbanization, mining) while others have little data available (e.g., effects from fracking).

### **Background**

The absence of impact cannot be readily inferred from a lack of elevated stressor data, especially where impacts may be episodic (e.g., Belluci et al. 2010). Biological data as response indicators, combined with stressor data as indicators of causative agents, can be used in a complementary fashion to characterize aquatic life impairments using a weight of evidence approach. An analogy for this approach is one of a coarse vs. fine focus on a microscope. Biology is the coarse focus that lets you identify the location and magnitude of impact and stressor data is the fine focus that allows one to get a clear picture of the object. Biological data without associated stressor data would leave you with a rather unfocused picture of the object of interest; stressor data without biological data would make it difficult to find and feel confident that you are focused on the object of importance.

Ohio has one of the most extensive biological, habitat, and chemical monitoring data sets for streams and rivers with over 25,000 data collection events statewide. Much of this data reflects co-current collection of fish and macroinvertebrate data, habitat data as measured by the QHEI and water chemistry data that includes nutrient parameters and dissolved oxygen, dissolved and suspended materials parameters and toxicants including metals and ammonia. Our initial focus in this study is on providing species and taxa based stressor-response data as a basis for future analyses and expansion of

this effort. A long-term goal would be an atlas of species/taxa responses to stressors that can be delivered in both report form and on the internet.

More detailed and formal stressor identification methods have been developed by U.S. EPA (USEPA 1999) and are a great resource for conducting stressor identifications. The web site supporting this process, which we have borrowed from for this document is also a great resource: [http://www.epa.gov/caddis/si\\_step1\\_overview.html](http://www.epa.gov/caddis/si_step1_overview.html). Their process is outlined in Figure 1. The data we provide here can be used in the Caddis process or in other less formal approaches to stressor

identification. The key data products of this study will be provided as Excel data files by species or taxon of interest and by stressor with the following sections providing descriptions of the data and examples of how the results can be used. For fish and macroinvertebrates we have also summarized information on the most useful indicator fish species and macroinvertebrate taxa for understanding typical mining and mining-related impacts. Habitat data is also provided because it reflects the template within which species live. The frequent modification of habitat associated with mining and other human activities make it an important contributing factor to understanding direct and indirect mining impacts. Our goal is for investigators to be able to apply these data to watershed study results in the WAP ecoregion of Southeast Ohio.

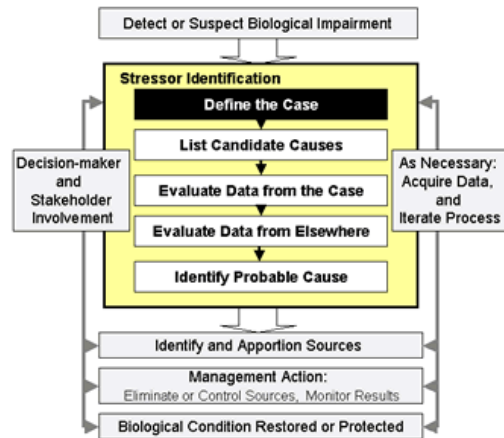


Figure 1. Summary flow chart of the stressor identification process from US EPA (1999).

**Biology as Stressor Indicators**

The initial role of biological data is as a *response* indicator that integrates the effects of all of the stressors that influence the biota in a stream reach. Ohio has institutionalized the condition of the assemblage considered impaired in its biocriteria, which vary with aquatic life use tier (EWH, CWH, WWH, MWH, LRW), ecoregion, and stream size (Ohio EPA 1989, 1991). It is the impairment of one or more biological measures (e.g., ICI, IBI, MIwb) that triggers a causal analysis to identify the causes of biological impairment in Ohio.

*Biological Impairment*

It is the impairment of biological criteria in Ohio that triggers a causal analysis. The biocriteria as derived a baseline benchmarks for different ecoregions and stratifications (e.g., stream size) that have been derived to account for natural and widespread baseline anthropogenic differences across Ohio (see later section on WQS). The biocriteria account for most, but not all “natural” differences across the State, so there are a small subset of sites that may be impaired by “natural” limitations not accounted for in the derivation of the biocriteria. One common natural limitation is what can be termed “wetland” streams. These are where wetland habitats are extensive and they may limit attainment of the WWH biocriteria



in certain ecoregions. This does not include short low gradient reaches interspersed with more typical habitats which should be able to attain regional benchmarks. Similarly, small streams with falls or dams that preclude recolonization by fish populations may not be able to attain a WWH aquatic life use. In these cases more weight would be placed on macroinvertebrate assemblages. The proper placement of a stream in an aquatic life tier through a use attainability analysis (UAA) is a routine, but essential part of the process for determining biological attainment and must be done prior to establishing impairment or stressor identification efforts.

The Technical Support Documents (TSDs) of Ohio EPA are reports that summarize this process for Ohio streams, rivers and watersheds studied by Ohio EPA. In fact the existence of a TSD for a watershed is a typical first step in examining data collected by an entity such as a watershed group, consultant, or researcher. Most TSDs can be found online at the Ohio EPA, Division of Surface Water web site: [http://www.epa.state.oh.us/dsw/document\\_index/psdindx.aspx](http://www.epa.state.oh.us/dsw/document_index/psdindx.aspx). This web site also contains other technical resource documents that can be useful to identifying stressors in Ohio waters: [http://www.epa.state.oh.us/dsw/document\\_index/docindx.aspx](http://www.epa.state.oh.us/dsw/document_index/docindx.aspx).

Stressor identification analyses can be very detailed and include information derived from a wide range of disciplines including aquatic ecology, biology, hydrology, geology, geomorphology, statistics, chemistry, and toxicology. The level of detail is typically related to the complexity of the impacts. For many impacts however, the stressor identification process can be used with very general tools (e.g., fish or macroinvertebrate assemblage data, habitat and simple chemical results). Recently, the inclusion of diatom and algal assemblage data has further advanced stressor identification, particularly for mine drainage scenarios (Zalack et al., 2010). In this report we will not discuss the specific steps of a stressor identification effort which will be an integral part of a more extensive stressor identification guide.

### ***Ohio Data***

The data used in generating the species and taxon-specific measures of sensitivity was taken from a 30-year+ data set from Ohio. Although data was primarily collected by Ohio EPA it also contains data collected by Ohio DNR, Ohio DOT, Universities (e.g., Ohio University), the Midwest Biodiversity Institute, and some consulting company data. Ohio data consists of fish and macroinvertebrate assemblage data collected with standard Ohio EPA methods (Ohio EPA 1989a) and includes both “raw” data (species occurrence and counts or presence/absence data) and “summarized” data (Index on Biotic Integrity [IBI] and metrics for fish; Invertebrate Community Index [ICI] and metrics for macroinvertebrates). Habitat data is based on the Qualitative Habitat Evaluation Index (QHEI)(Rankin 1995; Ohio EPA 2006) and a subset of variables designed to measure habitat metrics influenced by flow (Hydro-QHEI; Rankin and Yoder 2011). Water chemistry data includes parameters collected during intensive watershed surveys and includes ionic parameters (e.g., conductivity, total sulfate, total, chloride, etc.), pH, dissolved oxygen and BOD, temperature, hardness, alkalinity, nutrients (total phosphorus, nitrate, total ammonia, organic nitrogen) and metals (most measured in their total form). This data was collected during summer grab samples for a station with frequency of collection ranging from 1-10 samples depending on parameter and study design. For a subset of sites, stream flow statistics (e.g., mean annual, mean September, etc) were estimated from USGS regression models (Koltun et al. 2006). The above stressors include most of

the key stressor responsible for the majority of aquatic life impairments in Ohio. Other stressors can cause impairment to aquatic life that would not necessarily be found in routine monitoring data. Examples of these are more exotic toxicants (e.g., organic chemicals). Our analyses here use water column chemistry data, but fairly extensive sediment chemistry data also exists. This data is important in mined areas, and should be included in future data mining efforts for more complete stressor identification efforts. These should be considered when performing a stressor identification study. The nature of the biological response can provide some insight into the nature of these other stressors (e.g., toxic responses such as increase deformities, lesions, tumors and another anomalies on fish and invertebrates).

It is quite likely that one or more of the above chemical stressors may be responsible for impairment, but not be reflected in the summer grab samples, usually due to the episodic nature of chemical events. Industries and waste water plants can have upsets and spills, and delivery of pollutants from agricultural and mining sources may be weather related (e.g., storm, snow melt). As will be discussed later, habitat impacts can occur at multiple scales and good local habitat may be not sufficient to support sensitive species when other sites in the watershed are severely degraded. Impacts in urban areas can be particularly complex with mixes of stressors include flashy flows, accumulations of metals and other toxicants in sediments, and more exotic chemicals that may run off of industrial and other impervious sites (e.g., PCBs, PAHs). In most urban areas of Ohio, combined sewer overflows (CSOs) can deliver a complex mixture of nutrients, metals, bacteriological, and other constituents that can have serious impacts on aquatic life. In urban areas, other sources of information (e.g., in situ studies) may be important to nail down responsible stressors prior to requiring as costly mitigation approach (Crane et al. 2007). The remainder of this documents summarizes the development of species- and taxon-specific data that can be used in a stressor identification effort.

### ***Species and Taxon-Based Stressor Tolerance Measures***

A variety of methods have been used to measure the sensitivity of individual fish species or macroinvertebrate taxa to stressors in streams. Most multimetric indices use metrics that reflect the general intolerance or tolerance of species and taxa to “general” environmental stress. “General” or “cumulative” environmental stressor is conceptually considered to be the sum of the non-natural stressors that might occur at a site or in a watershed and the assumption is that most “intolerant” or “tolerant” species respond in a similar manner to multiple stressors that make up this cumulative “stressor load” on the assemblages. Although this concept has proved accurate and useful under real world conditions and for measuring attainment of aquatic life use goals, the relative magnitude of response of different taxa and species to specific stressors does vary. Species vary in response due to differing life history attributes and evolutionary exposures to similar categories of stressors under natural conditions. Differing diets, for example (insectivore vs. herbivore vs. omnivore) have resulted in differing natural exposures to types of chemicals (e.g., natural toxicants) that occur in natural prey items and may pre-adapt a species to be more or less sensitive to these compounds. Tolerance to these natural “toxic” compounds may have made these species more tolerant to synthetic or extracted toxicants discharged as components of industrial wastes. Similarly, species adapted to high oxygen mesohabitats (e.g., riffles, runs) may be more susceptible to pollutants that reduce these pollutants, or

to nutrient parameters that can lead to reductions of oxygen as micro-organisms assimilate these compounds. Such species are often considered to be “generally” sensitive. In this effort we are focusing on species-specific (fish) or genus-specific (macroinvertebrates) data.

### ***Weighted Stressor Values (WSVs) and Average Stressor Values (ASVs)***

In this document we provide data on tolerance using what are called “Weighed Stressor Values” (WSVs) and Average Stressor Values (ASVs). These are included as Excel files of WSVs for fish species (Appendix 1) and Excel files of ASVs for macroinvertebrates at the genus level (QUAL samples, Appendix 2). For macroinvertebrates the lowest practicable is the level of taxonomic resolution used by Ohio EPA and for the Invertebrate Community Index (ICI) which form part of the Ohio biocriteria. The genus taxonomic level is used in a wide variety of indices including the MAIS index (Smith and Voshell 1997), also used in the WAP ecoregion of Ohio. We provide genus level results for this study, but future work will include Excel files of WSVs for macroinvertebrates from the HD sample data at the Ohio EPA level of taxonomic resolution.

### ***Tolerance Indicator Values (TIVs)***

Although WSVs and ASVs are important for examining the influence of individual stressors on tolerance/intolerance, differing measurement scales among variables makes it difficult to compare WSVs among stressors. For example, QHEI ranges from 10-100, D.O. from 0 to approximately 20 mg/l and pH typically ranges from <1-9 SU. To improve the ability to compare stressors or groups of sensitive or tolerant species we also standardized WSVs and ASVs by converting them to an ordinal ranking scale of 1-10 for each taxa and stressor, where 1 indicates the upper or lower 10<sup>th</sup> percentile that reflects the most sensitive conditions and 10 the lower or upper 10<sup>th</sup> percentile rank that represents the most tolerant conditions for each species or taxon. These values can be used to compare sensitivities to different stressors and to create stressor indices for categories of stressors along a standard scale of values (1-10). In addition, we generated TIVs values for other statistics including the median, and the 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup> percentiles and an average of all the TIVs (weighted mean, median and percentiles). This “average” TIV measure incorporates variation by ranking and integrating multiple statistics that reflect differing magnitudes or variation in the sensitivity to a stressor. For example, two species can have a similar TIV for a stressor based on a WSV, but one may have a greater range of sensitivity reflected in a higher TIV for the 25<sup>th</sup> percentile statistic. In this document we are focused on the Western Allegheny Plateau ecoregion, but also compiled statistics separately at a statewide level because some species and taxa have too few collections in the WAP for stable WSV or ASVs.

### **Using the Excel WSV/TIV Files**

Included with this report are a series of Excel files that contain WSV and TIV files for Ohio fish species (Appendix 1) and macroinvertebrate taxa (Appendix 2). These files include data for combinations of species or taxa and each stressor variable we analyzed. We conducted these analyses at two spatial scales: 1) statewide, and 2) separately for sites in the WAP ecoregion. Data for each row in the tables includes species/taxa codes and names, parameter name, sample size, and statistics including weighted means (for data with abundance data), mean, median, and percentiles (10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, median) and

minimum and maximum values for both statewide and WAP ecoregion data. These files also include TIV values separately for each statistic and an average of the weighted mean, mean and percentile statistic TIVs. Weighted stressor values (WSVs) or average stressor values (ASVs) are the “raw” measure of association with a stressors, whereas the TIVs are the ordinal ranking of the WSVs on a scale of 1-10 with 1 being most sensitive and 10 most tolerant.

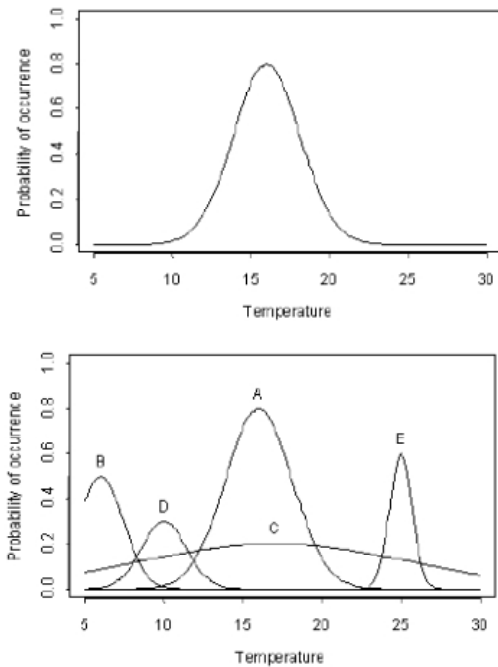
***Presence/Absence vs. Relative Abundance Measures in Species Tolerance Analyses*** We are using two types of ambient data to assess species tolerance or sensitivity to environmental factors and stressors: 1) taxon or species abundance estimates (e.g., WSVs), and 2) taxon or species presence or absence data (e.g., logistic regression approaches). In Ohio we have relative abundance data on fish species. For macroinvertebrates we have relative abundance data to lowest practicable taxonomic resolution for data collected with Hester Dendy (HD) artificial samplers, but also “qualitative” presence-absence data from all sites with HD samplers and also for streams too small to sample with HD samplers (e.g., generally less than 10 sq mi) or where a more qualitative assessment was deemed sufficient. In addition, all data with relative abundance data can be analyzed as presence-absence data. For this effort we focused on qualitative samples (“QUALS”) data because they are collected at all sites and are used in smaller streams too shallow to place HD-samplers. These small stream sites (i.e., generally less than 10 sq mi) are abundant and are often sites critical to identifying and understanding the contribution of mine impacts in a watershed.

Species abundance estimates can vary substantially due to both stressor and natural variation in the environment, but also as a result of sampling issues. Seining for fish can be very effective in shallow, low cover pools and gravelly riffles, but can be wildly ineffective in deep pools or pools with complex cover or undercut banks. The majority of the data used here was collected with standardized pulsed-DC methods as standardized by Ohio EPA (1987), methods that have reduced inefficiency. Presence/absence data is typically less variable; however, species that occur in low numbers because of natural rarity or because they are made rare by stressor effects may be considered absent at a site, but actually be present in low numbers. Multimetric indices such as the IBI minimize some of this variation by using combinations of species (i.e., metrics or guilds) rather than rely on single species.

One obvious difference between these types of the data is the inclusion of “absence” data. Weighted stressor averages only use data from sites where a taxa or species was collected. Sites with zero abundances are not represented in a weighted average value. The inclusions of zeros (absence) data in presence-absence data assumes that if a species was not collected at a site it does not exist there, but should. For our analyses here we refined this a bit by only including absence sites if: 1) the taxa/species was collected elsewhere in the same Huc-11 watershed and 2) the site was within the 10<sup>th</sup>-90<sup>th</sup> percent of sites where the species has been collected by drainage area size. Thus we exclude absences where the taxa/species may not occur because of biogeography (outside of the normal species range distribution) and exclude absences because streams are smaller or larger than the waters it typically inhabits. Because we are interested in being able to rank species relative sensitivities, and we are using the same universe of sites to calculate weighted averages or other measures, we are assuming errors or bias in this approach would be relatively similar across species (Yuan 2006), allowing robust rankings of sensitivity and tolerance to individual stressors.

**Logistic Regression Analyses & Smoothing Curve Fits**

In addition to weighed averages or WSVs, species sensitivities and tolerances have been analyzed with other approaches that define statistics such as environmental optima, tolerance and environmental breadth using various regression approaches that have been called indirect measures (Yuan 2006). Ecological theory predicts a typical response to a common natural environmental variable such a temperature or stream size to be a unimodal distribution with a maximum value of frequency of occurrence (presence/absence) or abundance along the environmental gradient (Yuan 2006; Figure 2, top). Different species may have differing sensitivities along such a gradient (Figure 2, bottom) and it is these differences that we are “exploiting” to understand biological responses to stressors and for conducting SI analyses. Figure 2 (bottom) portrays responses to a typical natural variables as unimodal; however, species may respond in other ways to anthropogenic stressors for example by decreasing linearly or curvilinearly (monotonically) responding to toxicants or increasing linearly or curvilinear to certain habitat features. Yuan (2006) points out that a monotonic response may be evident when an incomplete stressor gradient is examined (e.g., the tails of Figure 2, top). We employed two main “indirect” methods to characterize species responses to environmental variables: 1) smoothing curves using binned stressor-response data and 2) logistic regression techniques.



**Figure 2. Theoretical unimodal distribution of a species along a temperature gradient (top) and hypothetical distributions of species with different sensitivities (bottom) (taken from Yuan 2006).**

**Smoothing Curves**

Smoothing curves using binned data provide a tool to illustrate species responses to environmental and stressor variables. To construct these plots data is binned to 20 approximately *equally-sized* bins (based on sample size) and the average stressor value is calculated for each bin. These stressor values (midpoint of each bin) are then plotted vs. the proportion of sites in which the species or taxa is present within each bin (Figure 3). For fish (Appendix 3) and macroinvertebrate data (genus-level, Appendix 4) we provide Excel files for each stressor that contains: the stressor value midpoint of each bin and the proportion of sites where the taxa is present for each bin. Each Appendix contains two Excel files, one for statewide results and other for the WAP ecoregion separately. Figure 3 (top) illustrates smoothing curves for a habitat sensitive fish species (black redhorse) and a habitat tolerant fish species (fathead minnow) in response to the overall QHEI score. Note that we derived bins based on approximately equal sample sizes rather than using equal divisions of bins based on the stressor variable. The frequency of

points along the stressor axis, thus also provides some information relative to a species' sensitivity. If relatively few of the points fall in a certain portion of the curve it may indicate that species is sensitive to that level of stressor. For example in Figure 3 (top), only two of the twenty bins (points) for black redhorse occur below a QHEI of 50 and these represent mostly "absences" (presences < 10%). Conversely, for fathead minnow six bins occur below QHEIs for 50 and represent where fathead is more frequently present (25-55%; Figure 3, top).

Although we provide data for each taxa statewide and separately for the WAP ecoregion, many of the taxa we examined have similar responses using each data set as in illustrated for *Stenacron* (mayfly genus) and *Laccophilus* (predacious diving beetle genus) responses to habitat (Figure 3, bottom). Given the larger sample sizes, for some genera it may be preferable to use statewide data where data are sparse in the WAP ecoregion in the binning dataset.

*Using the Excel Smoothing Curve Data Table*

Because of the large number of possible smoothing plots than can be done with this dataset (# of stressors x number of species+taxa) we are including the data in Excel format so that a user can select and plot any taxa or species of interest (Appendices 3 and 4). Files in this iteration of the report are included for fish data at the species level and macroinvertebrate data at the genus level from qualitative samples. There are files for statewide results and for the WAP ecoregion separately. Data includes columns that identify the stressor, the species or taxon, and columns that contain the midpoint value of the stressor bin, the percent of sites within this range where the species or taxon was present. It also includes a column with a count of the total sites where the species was present, total

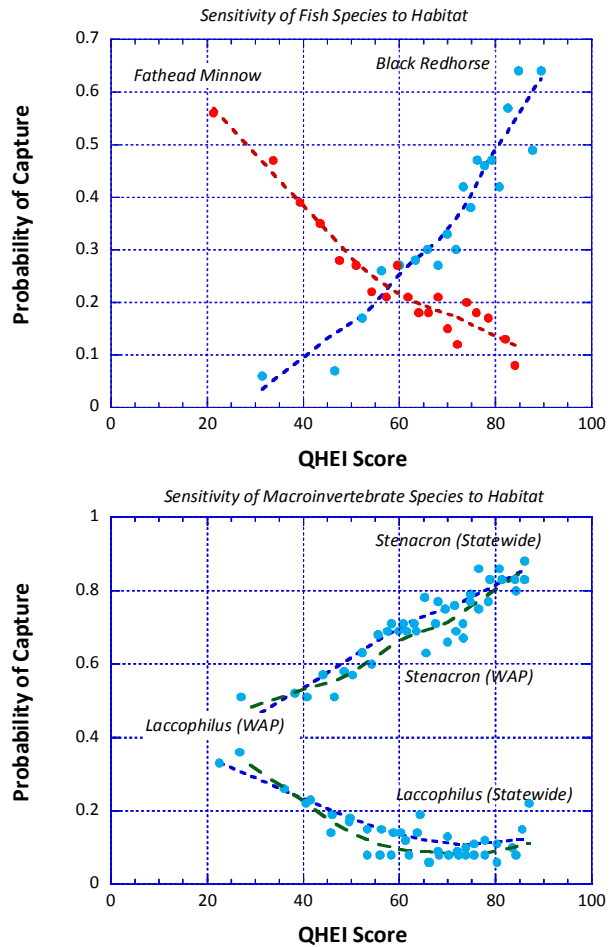


Figure 3. Smoothing curves fit to binned data of QHEI score vs. probability of capture of a fathead minnow and black redhorse (statewide data, top) and for two macro genera (bottom).

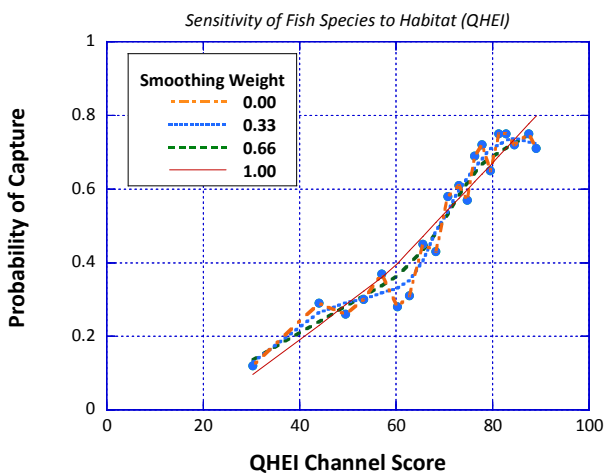


Figure 4. Smoothing curve fit to binned data of QHEI channel score vs. probability of capture of a fish species. Lines demonstrate forms of curves using differing averaging or smoothing weights.

sites (absent + present), and the number of sites represented in each of the twenty intervals or bins. There are twenty rows of data for each species/taxon and stressor combination. A species or taxon was included only where there were at least 50 presences at the statewide or WAP geographic scale. Note this is a larger sample-size threshold than we used for WSV or ASV analysis, thus some species may be included in the WSV tables, but not included in the binned dataset.

*Constructing a Curve*

There are a number of software packages that will fit a smoothed curve to a set of x-y data. In addition one can use nonlinear regression tools to fit curves to such data. We will demonstrate plotting a smoothing curve using the KaleidaGraph v4.1 (copyright 2009, Synergy Software). There are a number of smoothing functions that can be applied to the data using this tool, but we used a weighted smoothing curve. It allows you to modify what is called the smoothing factor, which controls the fraction of the data considered during smoothing. The smaller the value, the more individual points affect the final curve. Typical values are between 33 and 66. The minimum value is zero and the maximum value is 100. Figure 4 illustrates the application of a smoothing curve to binned data on shorthead redhorse and QHEI channel score. We applied smoothing curves with each of the four weights mentioned above. A weight of zero typically provides a line that passes through each point. The graphs in this effort generally were done with a weight of 0.66 which illustrates the curvilinear shape of the response, but reduces the effects of individual outliers.

*Non-Linear Regressions*

Where some specific non-linear function is hypothesized for a stressor-response relationship there are software products that will apply a wide variety of non-linear functions to the data and provide output to measure goodness of fit of these curves. We used a software package called XLSTAT (XLSTAT Version 2012.5.01, Copyright Addinsoft 1995-2012) to fit a non-linear function to data for smallmouth bass collection probability with QHEI (habitat) scores (Figure 5). The equation of this regression is: Pct Occurrence =  $173.6 * \text{QHEI Midpoint} / (18446.7 + \text{QHEI Midpoint})$  and the  $r^2$  for this regression model is high at 0.97. There are a nearly unlimited number of mathematic functions that could be fit to the data and a large literature on selecting curves that provide the best fit. Because our data for these analyses is binomial (presence/absence) we chose to use logistic regression rather select from a number of non-linear models. Where there is some theoretical response curve that is expected, non-linear regression may be appropriate; however given our data we are relying

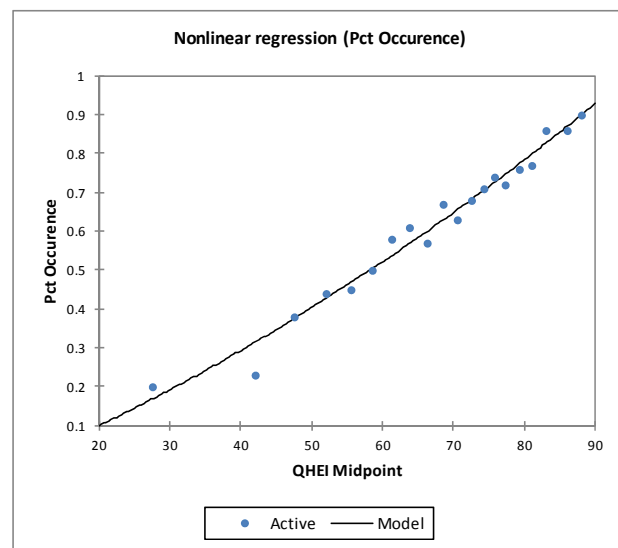


Figure 5. Non-linear curve fit to a plot of binned QHEI data vs. percent occurrence for smallmouth bass in Ohio. Statewide data.

primarily on the logistic approach for quantifying probabilities and smoothing curves for visualizing the shape of the biological response to stressors.

### *Logistic Regression Techniques*

Logistic regression is typically used when the response variable of interest is in binary form, in our case the presence (1) or absence (0) of a species or taxon. Presence data is readily obtained because of the presence of a species or taxon in a collection at a site. Identification of the *absence* of a species in a collection is more problematic. A species may be absent because the stressor of interest has reduced or eliminated the species; this is the pattern we are attempting to extract out of this data. Conversely there is some probability that a species is actually present, but not collected because of chance or low population numbers at a site result in low collection probabilities. As long as these probabilities are similar along the gradient of the stressor or represent a response to the stressor (probability of capture is lower because the stressor magnitude is greater) then the logistic regression should provide a useful description of the response. The value of the logistic regression is that it provides an explicit equation related to the probability or odds of capture along a given stressor gradient. Thus the equation can be used to predict or explain the absence or presence of a species given a specific level of a stressor in the environment. It can be interpreted as the how the odds or probability of occurrence changes with increases in the stressor.

Figure 6 illustrates logistic regressions for smallmouth bass vs. QHEI (habitat) statewide (top) and separately for the WAP ecoregion (bottom). One way to ascertain the accuracy of the regression is to take a subsample of the data and examine how accurately the model classifies the presence or absence given a random model (Table 1). We used a 40% correct classification as a cutoff for a “good” regression. Confidence bands can also be derived around the logistic curve (Figure 6). Thus for smallmouth bass, both statewide and in the WAP ecoregion, increases in QHEI scores increase the probability of capture of this species. For QHEI scores in the 20s (very poor habitat) there is less than a 10% chance of capturing a smallmouth bass. Although it is not reflected in this data, our experience and knowledge of the data indicate that smallmouth bass collected at sites with very poor habitat tend to be young-of-the-year. Conversely, sites with QHEI scores of 90 or higher (best habitat) have smallmouth bass present in about 90% or more of the collections. Thus smallmouth bass are strongly habitat dependent which agrees with our biological judgment and the extensive literature on this species.



**Table 1. Classification table for the estimation sample for the logistic regression of smallmouth bass presence (1) or absence (0) vs. QHEI score for Statewide data (top) or from the WAP ecoregion (bottom).**

from \ to	0	1	Total	% correct
0	1228	1406	2634	46.62%
1	619	3526	4145	85.07%
Total	1847	4932	6779	70.13%
from \ to	0	1	Total	% correct
0	174	207	381	45.67%
1	76	521	597	87.27%
Total	250	728	978	71.06%

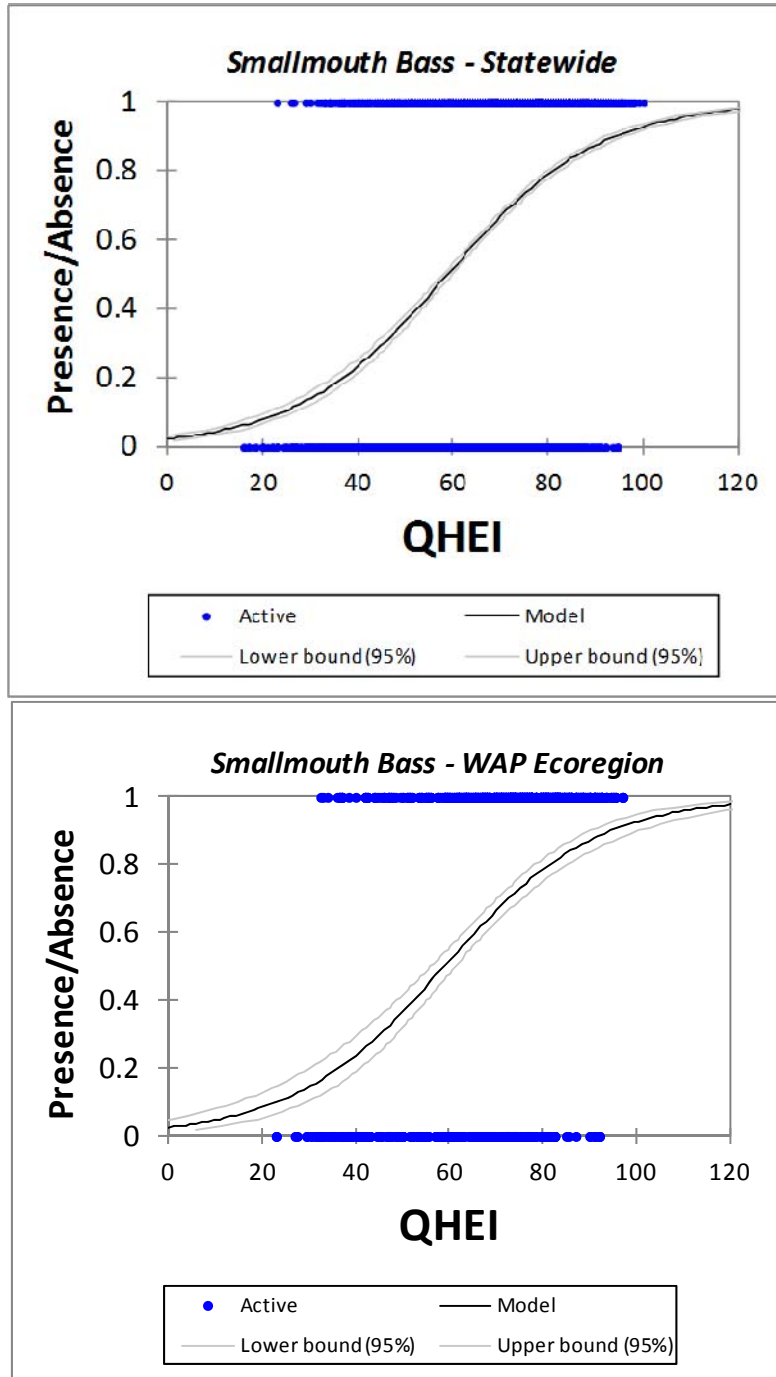


Figure 6. Logistic regression plots of QHEI vs. presence/absence probability for smallmouth bass at a statewide level (top) and for the WAP ecoregion (bottom). Lines reflect the logistic model and 95% confidence boundaries

### *Problems with Logistic Regression Approach*

One problem that arose with the logistic regression approach vs. the weighted stressor data is that some of the species ranked as sensitive or tolerant with the weighted stressor approach did not have a classification correction frequency of greater than 40%, particularly when classifying a species as being correctly present. This occurred despite a species being considered very sensitive on the basis of weighted stressor results and other observations (e.g., distributional studies). For example, with the shorthead redhorse (Figure 7, top) there was a strong classification estimate with the likelihood of being present correctly predicted 79.5% of the time in test data. In contrast, black redhorse, considered a very habitat sensitive sucker using weighted means only had a correct test classification estimate of 37.6%, slightly under our cutoff of 40% even though confidence bands were narrow (Figure 7, bottom). Other tests of significance; however, related to the logistic regression including the log-likelihood, Wald and Hosmer- Lemeshow estimates showed significant results (P-values <0.001).

Another example is provided by examining creek chub and fathead minnow response to habitat. While sensitive species show a strong increase in collection frequency, several habitat tolerant species show a much less steep or rather a flat response to habitat stressors (Figure 8, top). In other words the probability of capture of creek chubs is high in poor habitat (minimum prob. ~ 0.60), but only slightly better in streams with good habitat (maximum prob. ~ 0.9) than observed with the sensitive shorthead redhorse species (minimum prob. ~ 0.05% -> maximum prob. ~ 0.90%, Figure 7, left). Fathead minnow was one of the few species where the probability of capture was actually higher in poor habitat (maximum prob. ~0.70%) than in the best habitat (minimum prob. ~ <0.10%; Figure 8, bottom).

An issue with the use of the 40% classification cutoff for a significant model is that species such as the fathead minnow and black redhorse were below this cutoff. Unfortunately the cause of this test “failure” does not appear to be associated with the trend along the stressor gradient (note the tight 95% confidence intervals for the models), but rather with our original method of predicting the presence or absence of a species for use in our analyses. Classification test success (>40 correct cutoff) appears to be related to our ability to have “base” models where prediction of presence is greater than 70% at some point along a stressor gradient. Where we have a high frequency of absences even with a strong gradient in these curves (again reflected in the tight error bands) it is difficult to reach the 40% correct classification cutoff.

We used two factors in creating our base prediction for computing absences from Ohio data collections: 1) we censored sites below the 10<sup>th</sup> or above the 90<sup>th</sup> percentile of the species distribution with stream size (i.e., drainage area, sq mi), and 2) censored data (absences) based on biogeography by excluding sites where the species was not collected elsewhere in the same Huc11 watershed. For certain species that are patchy, but have a wide geographic distribution it can be difficult to predict their absence or presence at all levels of the stressor. For example, fathead minnow can be predominant in some regions (e.g., HELP ecoregion) in streams with poor habitat, but absent sporadically enough in poor habitat in other ecoregions (where they occur, but more sporadically), so they do not exceed 90% occurrence levels anywhere along the gradient. Conversely, shorthead redhorse and smallmouth bass predictably occur at near 90% frequency as streams with the highest QHEI scores throughout their ranges. Based on

the way the classification test works smallmouth bass and shorthead redhorse meet the 40% cutoff, but fathead minnow, black redhorse and creek chub do not. A more refined distributional model would likely include other factors (e.g., gradient, scale of habitat loss) that might result in more accurate predictions of presence/absence. In a relative sense; however, as long as presence and absence of a species is not biased along the gradient, other tests of logistic model significance may be more meaningful.

Classification tests have been suggested as being most appropriate when classification is the objective of a study (Homer and Lemeshow 2000). Our goal with the logistic regression; however, was not necessarily the ability to predict a classification, but rather to quantify the shape, magnitude and direction of a species or taxon's response to a stressor along a stressor gradient in order to identify and distinguish sensitive and tolerant species. We know for example that in regions such as the WAP, fathead minnow are present, but more sporadic in distribution. In the HELP ecoregion lack of fathead minnow in channelized streams would be surprising. In the WAP ecoregion they are more commonly found in habitat degraded streams, but not universally so. The Hosmer-Lemeshow test may be a more appropriate test of the response of a species along a stressor gradient. This test divides the stressor gradient into 10 groups and compares the goodness of fit between the predicted (no trend) and observed values along the stressor gradient. In the absence of a more refined species or taxonomic model, and an assumption that absences not related to the stressor do not change substantially along the stressor gradient, this test may be more meaningful. These other test of significance; however, also need to be tempered because the large sample sizes for some species could result in patterns that are statistically, but not biologically meaningful. Creek chub, for example show a significant model with habitat (positive trend); however the high occurrence at sites with the very poor habitat (QHEI scores less than 20) indicates that these are tolerant to poor habitat. The presence of tolerant species at very good sites is not unexpected, because they tend to inhabit small patches of marginal or poor habitat with a template of rich habitat features. Those species that show classification tests greater than 40% may still be useful as key ubiquitous species in the sense that even a rather simple distributional model can provide reliable stressor-response signals under a variety of environmental conditions. We suggest that the identification of sensitive or tolerant specie and taxa may be best done using WSVs or ASVs and tempered with logistic regression results and knowledge of life history attributes and site-specific studies.

### **Biologically-based Stressor Metrics**

The identification of the relative sensitivity of various species or taxa can be used to derive stressor-specific biological metrics that should be particular sensitive to the stressor used to derive the relative sensitivity or tolerance to these stressors. We calculated species richness metrics by selecting cutoffs of TIV values to identify sensitive or tolerant species for each stressor. These metrics can then calculated for individual sites and compared to results from reference sites. Such metrics can be calculated for any stressor and organism group, but for our purposes here we calculated example stressor-specific taxa richness metrics (sensitive and tolerant) for several key mine drainage parameters including conductivity, pH, alkalinity, total manganese, and total aluminum and three habitat parameters: overall QHEI score, QHEI substrate and QHEI channel scores.

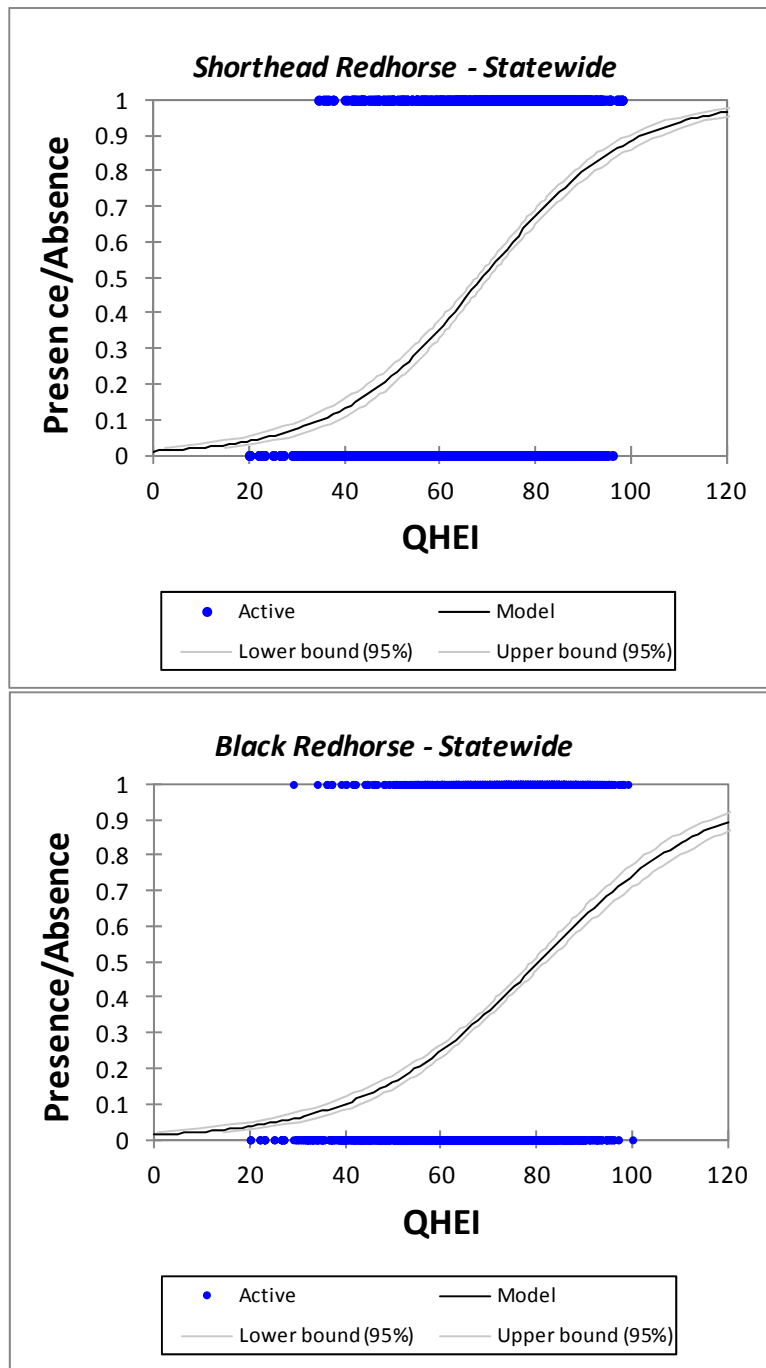


Figure 7. Logistic regression plots of QHEI vs. presence/absence probability for shorthead redhorse (top) and black redhorse (bottom). Lines reflect model and 95% confidence boundaries.

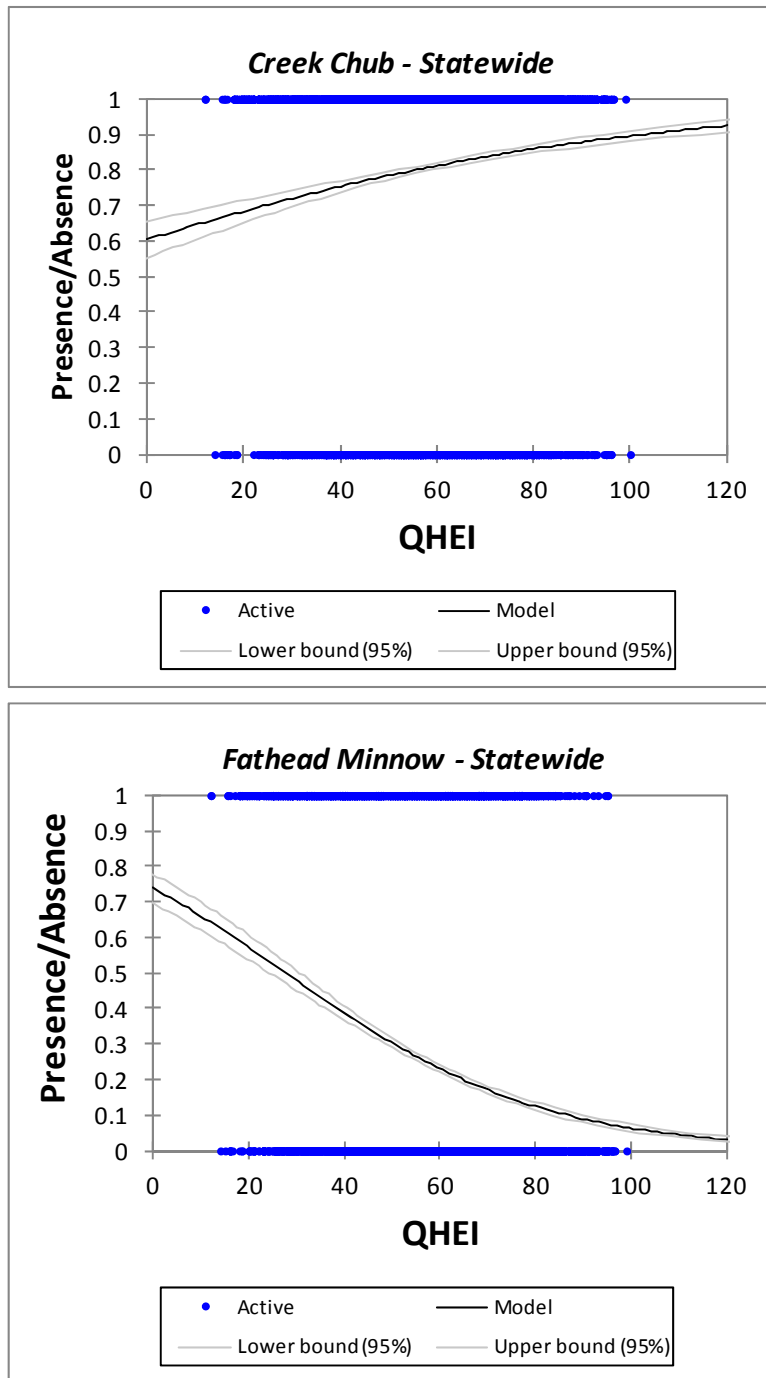


Figure 8. Logistic regression plots of QHEI vs. presence/absence probability for creek chub (top) and fathead minnow (bottom). Lines reflect model and 95% confidence boundaries.

We used the statewide data to select the cutoff values for the weighted mean (fish) or average (macroinvertebrates) TIV for each stressor and species (fish) or genus (macroinvertebrates) to identify taxon as intolerant, (TIV =1), sensitive (TIV >1 and ≤ 2), tolerant (≥ 9 and < 10) or very tolerant (TIV=10). We then calculated taxa/species richness for all sites in the Ohio database.

*Selecting an Array of Sensitive and Tolerant Fish Species for Mine-Affected Waters*

One objective of our work was to select approximate 20 fish species that reflect specific sensitivity or tolerance to mine-related stressors as useful indicators during stressor identification efforts. Our first plan was to use the results of logistic regression analyses to identify such species, but problems related the efficiency of the classification test suggested that we should not rely on that data alone. Instead we used the TIVs of species-specific weighted stressor values to a suite of common mine parameters and habitat variables and will use logistic regression results in a supporting role. Tables 2 and 3 list the fish species that were classified as sensitive or tolerant to a suite of commonly collected mine parameters (ph, chloride, sulfate, Mn, Al, conductivity and three habitat parameters that are often affected by modifications to streams in mined areas (total QHEI and QHEI channel score) or by high levels of exported sediments that affect the bedload (QHEI substrate score). We categorized sensitive species into two classes using the weighted mean TIV: intolerant (TIV=1, upper 10%), sensitive (TIV=2, 11-20%) and tolerant species into two classes: tolerant (TIV=9, 80-89%) or very tolerant (lower 10%).

Table 2. Species considered sensitive to typical mine parameters (ph, chloride, sulfate, managanese, aluminum and conductivity, along with key habitat parameters and a count for parameters to which a species is sensitive or tolerant. Data from Wadeable streams the WAP ecoregion.

Species Code	Species Name	IBI Tol.	Mine Parameters						Habitat Variables			Count		
			Ph	Chl	Sulf	Mn	Al	Cond	QHEI	Subs	Chan	# Sen	# Tol	
43 035	MIMIC SHINER	I	S	S	S	S	S	S	I	S	S	I	6	0
01 007	AMER BROOK LAMPREY	R	S	I	I	I			I				5	0
80 007	SLENDERHEAD DARTER	R		I	I	I	S	I	I	I	I	S	5	0
43 031	STEELCOLOR SHINER	P	I	I	I		I			I	I	I	4	0
47 008	STONECAT MADTOM	I	S	S		S		S	S	S	S	S	4	0
80 013	EASTERN SAND DARTER	R	T	I	I		I	I				S	4	1
80 017	VARIEGATE DARTER	I	I	S		I	S			I	I	I	4	0
01 006	LEAST BROOK LAMPREY		V	S		V	I	S					3	2
40 009	BLACK REDHORSE	I	S			S		S	I	I	I	I	3	0
43 005	RIVER CHUB	I	I			S		S	S	S	I	I	3	0
43 021	SILVER SHINER	I	S			S	S		S	S	S	I	3	0
43 022	ROSYFACE SHINER	I	S			I		S	I	S	I	I	3	0
47 012	BRINDLED MADTOM	I		I			S	S					3	0

To illustrate the usefulness of these “biological stressor” richness metrics we plotted the number of sensitive species at each site (intolerant + sensitive) for each stressor vs. 1) the original stressor value at each site, and 2) the IBI score at each site (Figures 9-11). We saw relatively sharp threshold relationships for each stressor metric when plotted against both the stressor and IBI. These species appear to be useful indicators that are also related well to the overall Ohio fish IBI index. The distinct limiting thresholds indicate that the presence of high numbers of these sensitive species for each stressor indicate concentrations or levels where the stressor is not limiting and associated with assemblages that meet Ohio WQS as measured by the biocriteria. Such thresholds can be defined using quantile regression through the 90<sup>th</sup> or 95<sup>th</sup> percentiles of the data (Cade and Noon 2003).

Table 3. Species considered tolerant to typical mine parameters (ph, chloride, sulfate, managanese, aluminum and conductivity, along with key habitat parameters and a count for parameters to which a species is sensitive or tolerant. Data from Wadeable streams the WAP ecoregion.

Species Code	Species Name	IBI Tol.	Mine Parameters						Habitat Variables			Count	
			Ph	Chl	Sulf	Mn	Al	Cond	QHEI	Subs	Chan	# Sen	# Tol
10	004	LONGNOSE GAR		T	V	V	T	V	S			0	5
20	003	GIZZARD SHAD		T	V	T	T	T				0	5
43	020	EMERALD SHINER	T	V	V	V		V				0	5
80	004	DUSKY DARTER	M	V	V	V	V	V				0	5
47	005	BROWN BULLHEAD	T		V	T	V	T	T	V		0	4
47	006	BLACK BULLHEAD	P	T		T	T	T	V	V	T	0	4
77	005	SPOTTED BASS		V		V	V		V			0	4
80	014	JOHNNY DARTER				V	T	T				0	4
43	002	GOLDFISH	T	V	V			T	V	V	V	0	3
43	003	GOLDEN SHINER	T	T	V			V	T	V	T	0	3
43	015	SUCKERMOUTH MINNOW			V	T			V		T	0	3
43	025	STRIPED SHINER				V	T		T			0	3
43	039	SILVERJAW MINNOW				V	V		T	T		0	3
43	042	FATHEAD MINNOW	T	T	V				T	V	V	0	3
80	001	SAUGER		T		T	T		S			0	3
85	001	FRESHWATER DRUM	P			V		V	V			0	3

Each of these species richness metrics (Figures 9-11) could be assigned score (e.g., 0-10, or 1-3) depending on responses and combined into a type of AMD-specific multimetric or IBI score. Prior to the creation of such a multimetric stressor index we would want to also explore tolerant (“negative”) metric responses and examine the response of proportional metrics (e.g, percent of sensitive or tolerant individuals). This could also be extended to the macroinvertebrate genus-level data. The files included as part of this product (TIV data in Appendix 2) would provide the tolerance designations for constructing these indices.



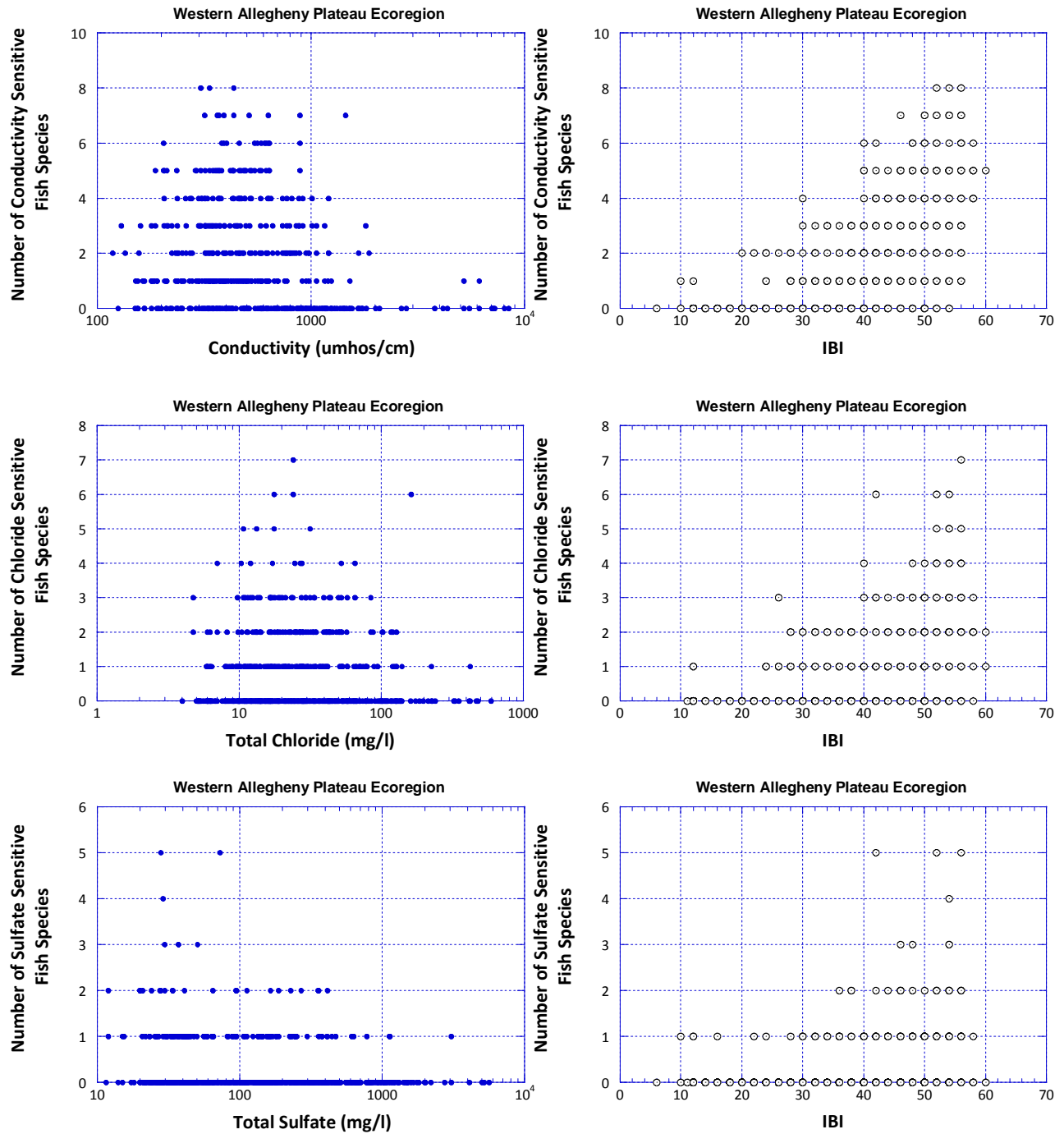


Figure 9. Plots of conductivity (top), chloride (middle) and sulfate (bottom) sensitive species vs. actual stressor variables (left) or fish IBI (right) for sites in the Western Allegheny Plateau ecoregion of Ohio; wadeable sites only.

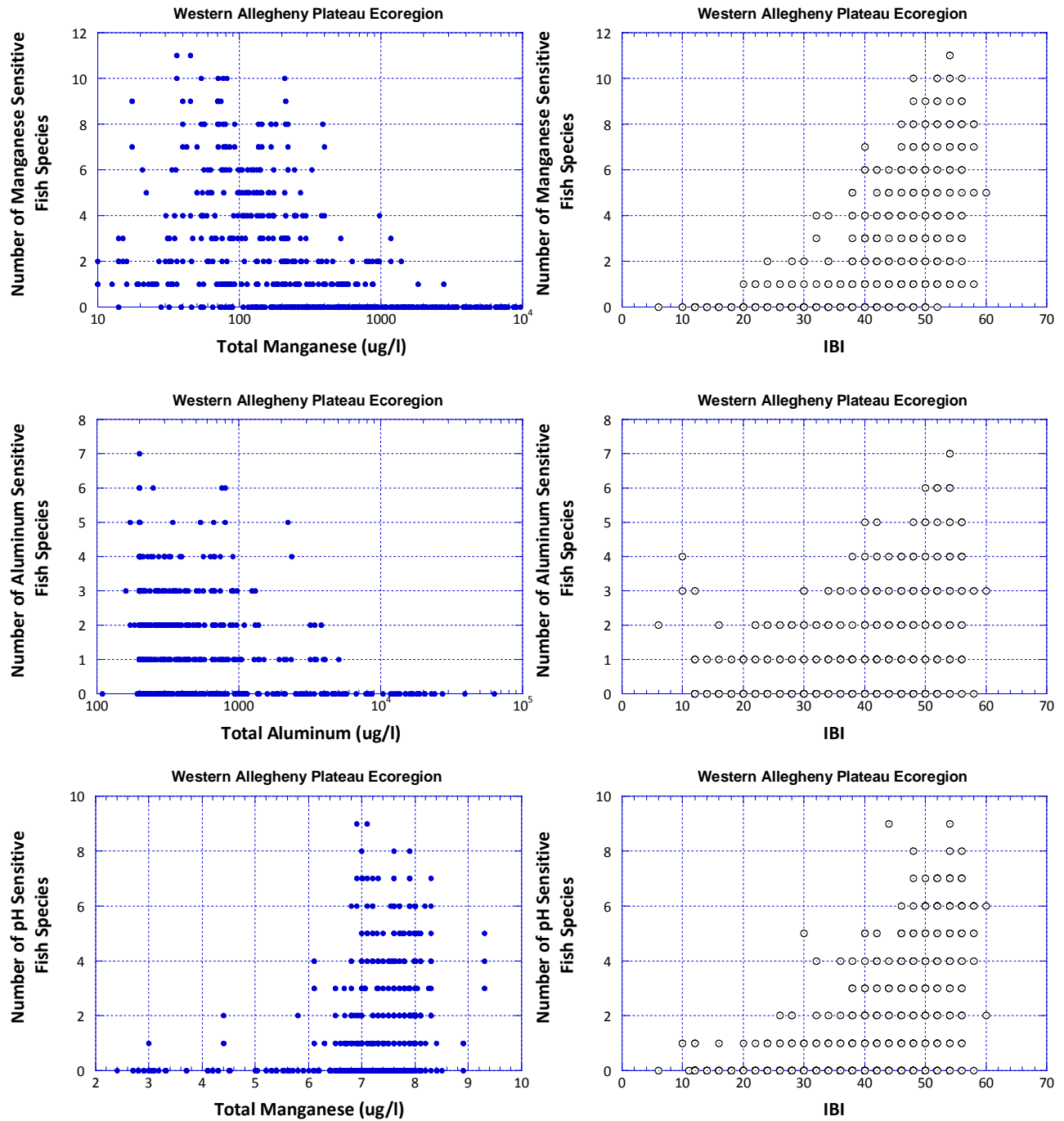


Figure 10. Plots of manganese (top), aluminum (middle) and pH (bottom) sensitive species vs. actual stressor variables (left) or fish IBI (right) for sites in the Western Allegheny Plateau ecoregion of Ohio; wadeable sites only.

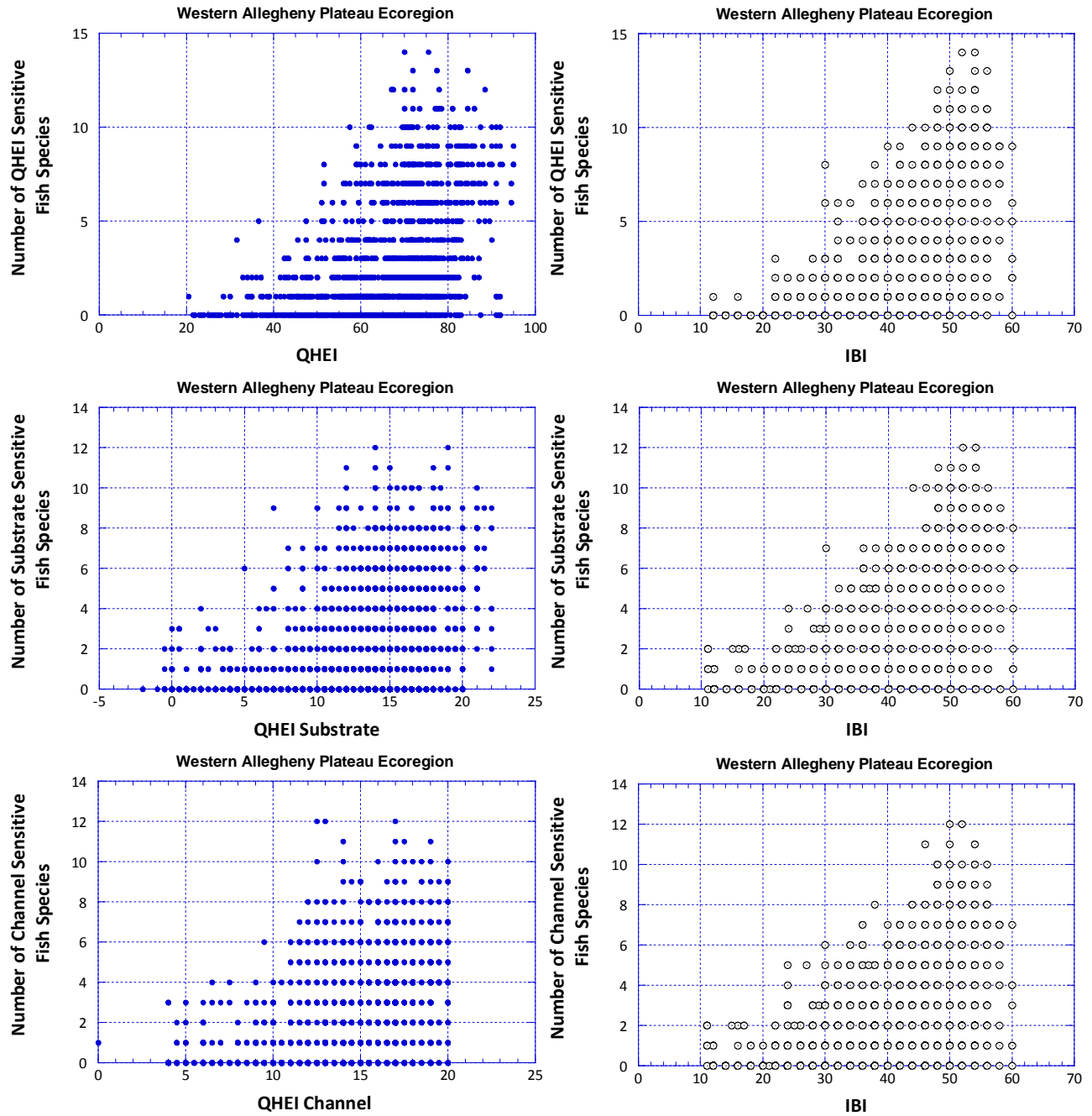


Figure 11. Plots of QHEI (top), substrate (middle) and channel (bottom) sensitive species vs. actual QHEI variables (left) or fish IBI (right) for sites in the Western Allegheny Plateau ecoregion of Ohio; wadeable sites only.

### ***Using Reference Sites Stressor Levels in Stressor Identifications Efforts***

Ohio has used the “reference site” approach (e.g., Stoddard et al. 2006) to determining of aquatic life use attainment/impairment in Ohio streams and rivers. Ohio was an early adopter of the “reference” site approach for assessing the biological quality of streams. Accounting for natural variation in aquatic assemblages at reference sites increases the ability to identify anthropogenic stressors (e.g., alteration of stream flows, habitat) that might affect aquatic condition. For warm water streams, Ohio stratifies aquatic assemblages based on level III ecoregions, which are geographically *dependent*, and stream size, which is geographically *independent*. Stream size is accounted for by having separate fish indices for headwater, wadeable and boatable waters and explicitly calibrating metric expectations by the log of drainage area in many of the metrics used in both the fish and macroinvertebrate indices (IBI, ICI). Ohio also uses water temperature as an important classifying factor embodied in a separate suite of coldwater aquatic life uses (see below), that include lists of characteristic species or taxa found in coldwater streams.

Comparison of chemical or other stressor levels at study sites can be compared to reference site levels or to levels association with ranges of biological performance as measured by the IBI and ICI to estimate the risk that the stressor may be exerting on the aquatic assemblages. Ohio EPA (1999) has developed ranges of stressors (Appendix 1 from Ohio EPA 1999) at biological reference sites (REF; excerpt in Table 4) where increases in the magnitude of stressor values represent increasing levels of risk of impairment. In addition, Ohio associated stressor values with ranges of the fish IBI scores using all available data (ALL; excerpt in Table 5) to create a companion set of “background” values specifically associated with good and excellent biological indices and with a larger dataset than the reference dataset alone. This section was included because the result of the stressor identification efforts using individual species or taxon responses are best done hand-in-hand with an analysis of reference or background stressor concentrations which provide insight into what stressor levels might be reasonably attainable depending on aquatic life use.

**Table 4. Excerpt from Ohio EPA (1999) Appendices illustrating reference site statistics for conductivity in stream of four stream size classes.**

Appendix I. Ohio EPA Water Column Chemistry Statistics for Reference Sites (excluding urban and physically modified sites) by Ecoregion and Stream Size.

IBI Range	Sample Size	Median	75th %tile	90th %tile	95th %tile	Median + 1.5*IQR	Median + 2*IQR
Parameter: Conductivity, Field (umhos/cm)							
Ecoregion: <u>WAP</u>							
Headwaters	93	375.000	573.750	789.000	1251.500	673.1250	772.500
Lg. River	79	610.000	900.000	1050.000	1263.000	1045.0000	1190.000
Sm. River	64	686.000	789.500	900.000	972.400	841.2500	893.000
Wadeable	195	390.000	500.000	800.000	920.000	555.0000	610.000

**Table 5. Excerpt from Ohio EPA (1999) Appendix 2 illustrating site statistics (ALL data) for conductivity in the WAP ecoregion by IBI range in headwater streams.**

Appendix 2. Ohio EPA Water Column Chemistry Statistics for ALL Sites in the Ohio EPA Database by Ecoregion, Stream Size, and IBI Range.

IBI Range	Sample Size	Median	75th %tile	90th %tile	95th %tile	Median + 1.5*IQR	Median + 2*IQR
Ecoregion: <u>WAP</u>							
Headwaters							
12-19	72	990.000	1450.000	1978.000	2634.000	1680.0000	1910.000
20-29	26	1160.000	1440.000	2088.000	2549.000	1580.0000	1720.000
30-39	46	498.500	930.000	1487.500	2144.000	1145.7500	1361.500
40-49	77	475.000	600.000	750.000	1100.000	662.5000	725.000
50-60	39	375.000	580.000	682.000	757.500	682.5000	785.000

### ***Water Quality Standards (WQS)***

Water quality standards (WQS) are an essential cornerstone of water quality management. WQS consist of designated uses and chemical, physical, and biological criteria designed to represent measurable properties of the environment that are consistent with the characteristics and level of protection specified by a designated use. In Ohio these are codified in OAC 3745-1. Use designations consist of two broad categories, aquatic life (ALUSEs) and non-aquatic life uses. WQS to meet aquatic life use criteria frequently are more stringent than WAS to meet non-aquatic life uses, so waters that meet aquatic life criteria generally are suitable for all uses. Therefore, State water quality management programs commonly focus on aquatic life uses.

The Ohio WQS employ a tiered system of refined aquatic life use classifications (TALUs), which is different from the “one-size-fits-all” approach of general uses that are common to many states’ WQS. Tiered uses are based on the reality that reference aquatic assemblages vary locally and regionally, thus their management goals should vary accordingly. The tiered system also offers the opportunity to stratify water quality management goals and end-points, hence reducing the risks of under-protection or over-protection that is inherent to a general use approach. In the Ohio WQS there are five principal aquatic life uses currently designated, which are described as follows.

- 1) Warmwater Habitat (WWH) - this use designation defines the “typical” warmwater assemblage of aquatic organisms for Ohio rivers and streams and biocriteria are stratified by ecoregion and site-type<sup>1</sup>; this use represents the principal restoration target for the majority of water resource management efforts in Ohio. By number, 77.4% of waters are WWH (Ohio EPA 2004).
- 2) Exceptional Warmwater Habitat (EWH) - this use designation is reserved for waters which support “unusual and exceptional” assemblages of aquatic organisms which are characterized by a high diversity of species, particularly those which are highly intolerant and/or rare, threatened, endangered, or special status (i.e., declining species) - biocriteria are set uniformly across ecoregions, but are stratified by site-type; this designation represents a protection goal for water resource management efforts dealing with Ohio’s best water resources. By number, 10.2% of waters are EWH (Ohio EPA 2004).
- 3) Coldwater Habitat (CWH-N and CWH-F) - these uses are intended for waters which support assemblages of native cold water organisms (CWH-N) and/or those which are stocked with salmonids with the intent of providing a put-and-take fishery on a year round basis which is further sanctioned by the Ohio DNR, Division of Wildlife (CWH-F); this use is complimented by the Seasonal Salmonid Habitat (SSH) use which applies to the Lake Erie tributaries which support periodic “runs” of salmonids during the spring, summer, and/or fall. While there are no numeric biocriteria for this use designation, specific fish and macroinvertebrate assemblage attributes are used by Ohio EPA to determine its applicability. By number, 2.4% of waters are CWH (Ohio EPA 2004).
- 4) Modified Warmwater Habitat (MWH) - this use applies to streams and rivers which have

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<sup>1</sup> A site type distinguishes between headwaters (<20 mi<sup>2</sup>), wading (sampled with wading methods), and boat sites (sampled with boat mounted methods) for fish assemblage assessment purposes.

been subjected to extensive, maintained, and essentially permanent hydromodifications such that the biocriteria for the WWH use are not attainable and where restoration to a CWA goal use has been ruled out via a use attainability analysis; the representative aquatic assemblages are predominated by species which are tolerant to low dissolved oxygen, siltation, nutrient enrichment, and poor quality habitat - biocriteria are stratified by ecoregion and site-type; a use attainability analysis conducted in accordance with 40 CFR 131.10[g][1-6] is required to designate this use tier. This use includes stream limited by channelization (MWH-C), impoundments (MWH-I) and by non-acidic mine runoff (MWH-A). By number, 3.8% of waters are MWH (Ohio EPA 2004).

- 5) Limited Resource Water (LRW) - this use applies to small streams (usually <3 mi.<sup>2</sup> drainage area) and other water courses which have been irretrievably altered to the extent that no appreciable assemblage of aquatic life can be supported; such waterways generally include small streams in extensively urbanized areas, those which lie in watersheds with extensive drainage modifications, those which completely lack water on a recurring annual basis (i.e., true ephemeral streams), or other irretrievably altered waterways - these streams are expected to support poor quality biological assemblages at a minimum; a use attainability analysis conducted in accordance with 40 CFR 131.10[g][1-6] is required to designate this use tier. By number, 6.2% of waters are LRW (Ohio EPA 2004); however, many are very small and represent a small proportion of stream miles.

Chemical, physical, and/or biological criteria are assigned to each use designation tier in accordance with the goals and objectives associated with each. As such the system of use designations employed in the Ohio WQS constitutes a “tiered”

approach in that varying and graduated levels of protection are provided by each in accordance with its demonstrated potential to achieve a specific level of biological performance. This is best illustrated by the biological criteria which are stratified across the state by ecoregion, site-type, and designated use (Figure 12). This stratification by use designation and ecoregion has also been applied to selected chemical water quality criteria including parameters such as dissolved oxygen, ammonia-nitrogen, selected heavy metals, and temperature. Stressor identification efforts are best conducted within the

### Ohio Biological Criteria: Adopted May 1990 (OAC 3745-1-07; Table 7-15)

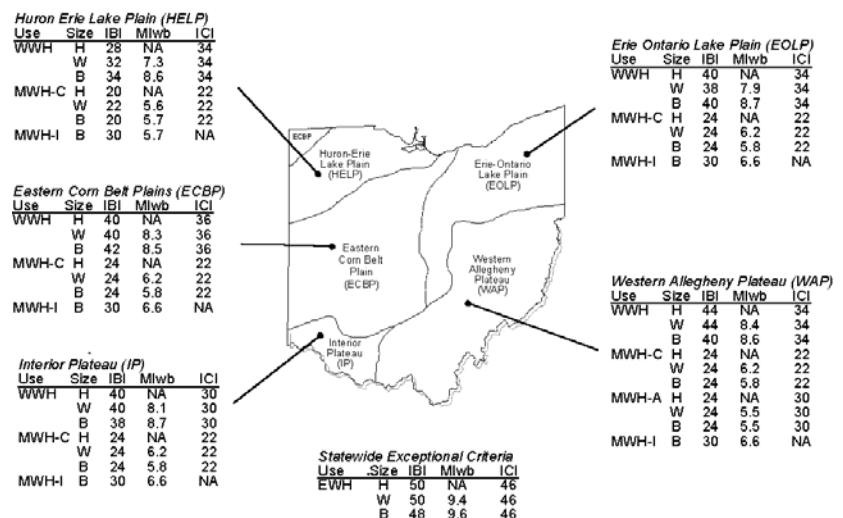


Figure 12. Ohio’s numerical biological criteria (OAC 3745-1-07; Table 7-15) showing the stratification by level III ecoregions and site type (fish assemblage indices) and by tiered aquatic life uses for the fish and macroinvertebrate assemblages. IBI = Index on Biotic Integrity (fish), Mlwb = Modified Index of well-being (fish), ICI = Invertebrate Community Index; Uses are as defined in the text.

tiered aquatic life use framework because the vary aquatic life use potential inherent in these tiers informs the likelihood on these streams having or not having sensitive or intolerant fish and macroinvertebrate species.

### Conclusions

Appendices 1-6 form the core of the products for this contract and this document describes the data and how the data can be used in stressor identification efforts. We originally envision these data and tools to be part of a stressor identification guide for streams in Southeast Ohio. We considered this a beginning effort because there are other statistic tools and techniques that can be applied to this core of data to help develop stressor identification tools, derive better stressor targets or eventually field-based water quality criteria. For example, specific statistical methods have recently been proposed as methods to derive water quality criteria using ambient data like we have in Ohio. In particular the derivation of field-base species sensitivity distributions (SSDs, U.S. EPA 2010; Cormier wt al. 1998) has been used to develop water quality criteria for conductivity. An analysis tool called TITAN (Baker and King 2010) has used species-specific data to derive stressor targets and using a combination of indicator species analyses and change point analyses.

The generation of a field based SSDs uses what is termed an “extirpation concentration” which is the concentration where a genus is effectively absent from a region. US EPA (2010) used this approach to develop a conductivity criteria for the WAP ecoregion. TITAN uses individual species or taxa to identify maximum indicator values along a stressor gradient and then derives, based on all the species in an area, biologically meaningful stressor targets (Baker and King 2010). Both of these methods could be applied to data in the WAP ecoregion of Ohio. The USEPA effort to derive a conductivity criteria for the WAP ecoregion (USEPA 2010) did not included data from Ohio. Ohio may have a naturally higher reference or background level of conductivity due to geology than the WAP ecoregion further south. In addition, their efforts did not considered tiered aquatic life uses which have been existence for 20 years in Ohio, but have not yet been derived for the other States in this ecoregion.

Other analyses which may improve stressor identification efforts include derivation of historical modeled fish and macroinvertebrate assemblages for pre-settlement conditions (Armitage et al. 2009). Armitage et al. (2009) derived historical fish assemblages for the Wabash River based on recent data and historical records and used a modeling approach to infer historical stressor conditions using WSVs they had derived for these species. Ohio has derived newer, continuous IBI and ICI indices that account for potential historical assemblages and that might be more responsive to the range of stressors that includes the range from extremely severe to pre-settlement conditions (Rankin 2010). Recently, advanced in modeling stream flows is providing estimates of daily flows that can be used to derive a suite of flow variables (Poff et al. 2010) that will allow the development of useful flow indicators that can be incorporated into future stressor analyses.

The data files included here provide the data for conducting sound stressor analyses for Southeast Ohio streams immediately, but also provide the basis for improving stressor analyses in the future. It will be important to aggregate and transfer the knowledge derived from current efforts to future work. The



construction of the beginning of a species and taxon-based atlas of stressor response relationships provides a solid basis for these efforts in Southeast Ohio.

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