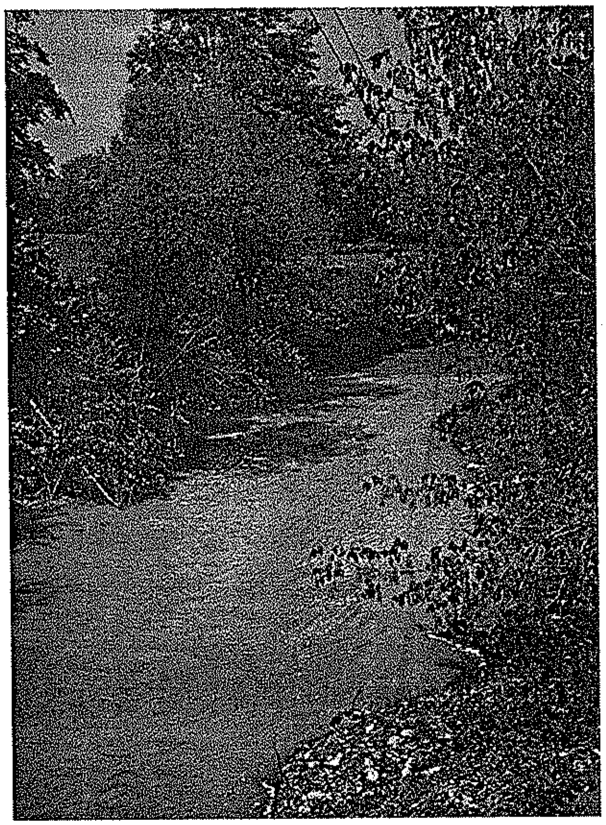
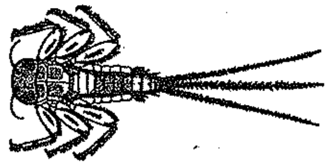
State of Ohio
Environmental Protection Agency

Ecological Assessment
Division of Surface Water

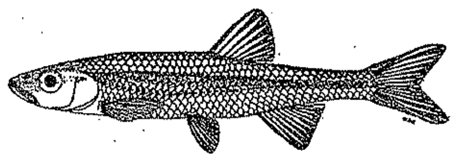
Empirically Derived Guidelines for Determining Water Quality Criteria for Iron Protective of Aquatic Life in Ohio Rivers and Streams

1998

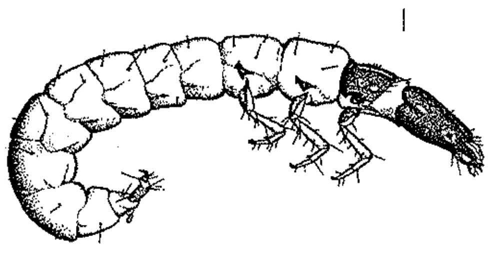




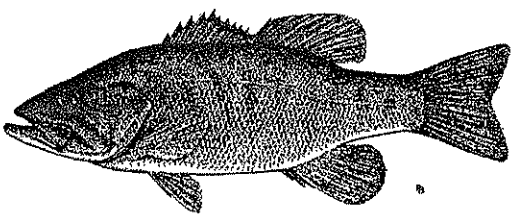
Mayfly *(Stenonema)*



Rosyface Shiner *(Notropis rubellus)*



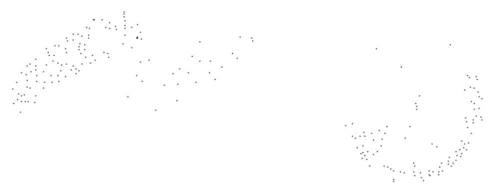
Caddisfly *(Dolophilodes distinctus)*

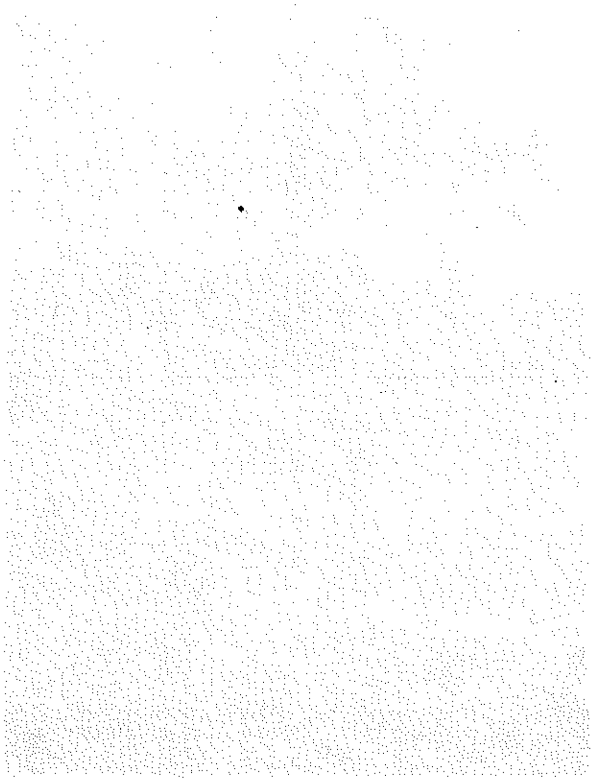


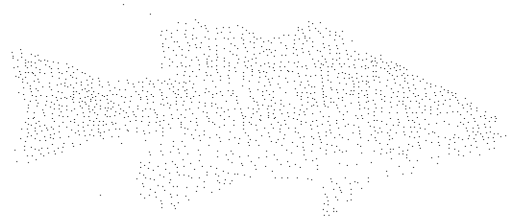
Smallmouth Bass *(Micropterus dolomieui)*

Oct 16, 1998

P.O. Box 1049, 1800 WaterMark Dr., Columbus, Ohio 43266-1049







Empirically Derived Guidelines for Determining Water Quality Criteria For Iron Protective of Aquatic Life in Ohio Rivers and Streams

Ohio EPA Technical Bulletin MAS/1998-9-1

October 16, 1998

State of Ohio Environmental Protection Agency
Division of Surface Water
Monitoring and Assesssment Section
1685 Westbelt Drive
Columbus, Ohio 43228

NOTICE TO USERS

Ohio EPA incorporated biological criteria into the Ohio Water Quality Standards (WQS; Ohio Administrative Code 3745-1) regulations in February 1990 (effective May 1990). These criteria consist of numeric values for the Index of Biotic Integrity (IBI) and Modified Index of Well-Being (MIwb), both of which are based on fish assemblage data, and the Invertebrate Community Index (ICI), which is based on macroinvertebrate assemblage data. Criteria for each index are specified for each of Ohio's five ecoregions (as described by Omernik 1987), and are further organized by organism group, index, site type, and aquatic life use designation. These criteria, along with the existing chemical and whole effluent toxicity evaluation methods and criteria, figure prominently in the monitoring and assessment of Ohio's surface water resources.

The following documents support the use of biological criteria by outlining the rationale for using biological information, the methods by which the biocriteria were derived and calculated, the field methods by which sampling must be conducted, and the process for evaluating results:

Ohio Environmental Protection Agency. 1987a. Biological criteria for the protection of aquatic life: Volume I. The role of biological data in water quality assessment. Div. Water Qual. Monit. & Assess., Surface Water Section, Columbus, Ohio.

Ohio Environmental Protection Agency. 1987b. Biological criteria for the protection of aquatic life: Volume II. Users manual for biological field assessment of Ohio surface waters. Div. Water Qual. Monit. & Assess., Surface Water Section, Columbus, Ohio.

Ohio Environmental Protection Agency. 1989b. Addendum to Biological criteria for the protection of aquatic life: Volume II. Users manual for biological field assessment of Ohio surface waters. Div. Water Qual. Plan. & Assess., Ecological Assessment Section, Columbus, Ohio.

Ohio Environmental Protection Agency. 1989c. Biological criteria for the protection of aquatic life: Volume III. Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities. Div. Water Quality Plan. & Assess., Ecol. Assess. Sect., Columbus, Ohio.

Ohio Environmental Protection Agency. 1990. The use of biological criteria in the Ohio EPA surface water monitoring and assessment program. Div. Water Qual. Plan. & Assess., Ecol. Assess. Sect., Columbus, Ohio.

Rankin, E.T. 1989. The qualitative habitat evaluation index (QHEI): rationale, methods, and application. Div. Water Qual. Plan. & Assess., Ecol. Assess. Sect., Columbus, Ohio.

Since the publication of the preceding guidance documents new publications by Ohio EPA have become available. The following publications should also be consulted as they represent the latest information and analyses used by Ohio EPA to implement the biological criteria.

DeShon, J.D. 1995. Development and application of the invertebrate community index (ICI), pp. 217-243. in W.S. Davis and T. Simon (eds.). Biological Assessment and Criteria: Tools for Risk-based Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Rankin, E. T. 1995. The use of habitat assessments in water resource management programs, pp. 181-208. in W. Davis and T. Simon (eds.). Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Yoder, C.O. and E.T. Rankin. 1995. Biological criteria program development and implementation in Ohio, pp. 109-144. in W. Davis and T. Simon (eds.). Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Yoder, C.O. and E.T. Rankin. 1995. Biological response signatures and the area of degradation value: new tools for interpreting multimetric data, pp. 263-286. in W. Davis and T. Simon (eds.). Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Yoder, C.O. 1995. Policy issues and management applications for biological criteria, pp. 327-344. in W. Davis and T. Simon (eds.). Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Yoder, C.O. and E.T. Rankin. 1995. The role of biological criteria in water quality monitoring, assessment, and regulation. Environmental Regulation in Ohio: How to Cope With the Regulatory Jungle. Inst. of Business Law, Santa Monica, CA. 54 pp.

These documents and this report can be obtained by writing to:

Ohio EPA, Division of Surface Water
Monitoring and Assessment Section
1685 Westbelt Drive
Columbus, Ohio 43228-3809
(614) 728-3401

FOREWORD

Statewide Biological and Water Quality Monitoring & Assessment
Ohio EPA routinely conducts biological and water quality surveys, or "biosurveys", on a systematic basis statewide. A biosurvey is an interdisciplinary monitoring effort coordinated on a waterbody specific or watershed scale. Such efforts may involve a relatively simple setting focusing on one or two small streams, one or two principal stressors, and a handful of sampling sites or a much more complex effort including entire drainage basins, multiple and overlapping stressors, and tens of sites. Each year Ohio EPA conducts biosurveys in 10-15 different study areas with an aggregate total of 250-300 sampling sites. Biological, chemical, and physical monitoring and assessment techniques are employed in biosurveys in order to meet three major objectives: 1) determine the extent to which use designations assigned in the Ohio Water Quality Standards (WQS) are either attained or not attained; 2) determine if use designations assigned to a given water body are appropriate and attainable; and 3) determine if any changes in key ambient biological, chemical, or physical indicators have taken place over time, particularly before and after the implementation of point source pollution controls or best management practices. The data gathered by a biosurvey is processed, evaluated, and synthesized in a biological and water quality report. The findings and conclusions of each biological and water quality study may factor into regulatory actions taken by Ohio EPA and are incorporated into Water Quality Permit Support Documents (WQPSDs), State Water Quality Management Plans, the Ohio Nonpoint Source Assessment, and the Ohio Water Resource Inventory (305[b] report).

Five Year Basin Approach
In 1990 the Ohio EPA initiated an organized, sequential approach to monitoring and assessment termed the Five-Year Basin Approach. One of the principal objectives of this new approach was to better coordinate the collection of ambient monitoring data so that information and reports would be available in time to support water quality management activities such as the reissuance of NPDES permits and periodic revision of the Ohio water quality standards (WQS). The initial step in this process was to section the state into 25 different hydrologic units which represented aggregations of subbasins within the 23 major river basins previously delineated by Ohio EPA for the PEMSO system. The 25 hydrologic areas were each assigned to one of five basin years with respect to the five Ohio EPA districts. Thus within a given year, monitoring takes place within five of the hydrologic areas *and* within each of the five Ohio EPA districts. Five years is required to complete the cycle of monitoring within each of the 25 hydrologic areas. Once the field monitoring is completed, data analysis and reporting takes place. The end product is termed a Technical Support Document (TSD) which contains the summary and integration of the biological, chemical, and physical assessments.

Ohio EPA's approach to surface water monitoring and management via the Five-Year Basin Approach essentially serves as an environmental feedback process taking "cues" from environmental indicators to effect needed changes or adjustments within water quality management. This hierarchy is essentially in place within the TSD process and represents, from a technical assessment and indicators framework standpoint, a watershed approach. The environmental indicators used in this process are categorized as stressor, exposure, and response indicators. *Stressor* indicators generally include activities that impact, but which may or may not degrade the environment. This includes point and nonpoint source loadings, land use changes, and other broad-scale influences that generally result from anthropogenic activities. *Exposure* indicators include chemical-specific, whole effluent toxicity, tissue residues, and biomarkers, each of which suggest or provide evidence of biological exposure to stressor agents. *Response* indicators include the direct measures of the status of use designations. For aquatic life uses the community and population response parameters that are represented by the biological indices that comprise Ohio EPA's biological criteria are the principal response indicators. For human body contact uses (*e.g.,* Primary Contact Recreation) fecal bacteria (*e.g., E. coli,* fecal coliforms) are the principal response indicators. The key to having a successful watershed approach is in using the different types of indicators within the roles that are the most appropriate for each. The inappropriate use of stressor and exposure indicators as substitutes for response indicators is at the root of the national problem of widely divergent 305(b) statistics reported between the States. This issue is discussed in the 1994 Ohio Water Resource Inventory (Ohio EPA 1995).

Monitoring for Status and Trends

An assessment of the impact of multiple sources on the receiving waters of a watershed includes an evaluation of the available chemical/physical (water column, effluent, sediment, flows), biological (fish and macroinvertebrate assemblages), and habitat data which have been collected by Ohio EPA pursuant to the Five-Year Basin Approach. Other data which is evaluated includes, but is not limited to, NPDES permittee self-monitoring data, effluent and mixing zone bioassays conducted by Ohio EPA, the permittee, or U.S. EPA, spills data compiled by Ohio EPA, and fish kill information from the Ohio Division of Wildlife. The integration of this information into a report for each study area is accomplished via the TSD process. Besides reporting on status and trends for the applicable designated uses, the TSD also identifies and describes causal associations of use impairments with the predominant causes and sources of impairment. The completion of this process enables the structured use of the output from the TSD (*i.e.,* the assessment of water bodies) to support virtually any Ohio EPA program where surface water quality is a concern.

Technical Bulletin Series

The systematic monitoring and assessment of Ohio surface waters via the Five-Year Basin Approach since 1990, and overall since 1980, has produced a comprehensive database that can be used to address issues of statewide and program importance. As such, Ohio EPA periodically produces technical bulletins to provide an in-depth analysis of specific issues ranging from the validation of specific water quality criteria to process descriptions for tools such as the biological criteria. These analyses would not have been possible without the systematic baseline monitoring and assessment which are an aggregate outgrowth of the Five-Year Basin Approach.

SUMMARY

This document summarizes the effects of the various forms of iron on aquatic life in streams. While iron is a naturally occurring and common constituent of surface waters, it has been documented to have adverse effects on aquatic life at elevated concentrations and under certain environmental conditions (e.g., low pH). The current criterion in the Ohio Water Quality Standards (WQS) is 1.0 mg/l. However, background and reference sites in Ohio have regularly approached and exceeded the current 1.0 mg/L total iron criterion which calls its relevancy and efficacy into question. This document, through the association and statistical analyses of the relationship of ambient total recoverable iron at regional reference and impacted sites with fish and macroinvertebrate community data, provides:

1) a tiered set of total recoverable iron criteria stratified by aquatic life use and stream size (Table 2);

2) evidence that there is a need to investigate whether there is sufficient data and need to derive a toxicity-based criterion for the ferrous (Fe^{2+}) form of iron which is thought to be the most toxic form; and,

3) identification of the need to monitor the BPA (bathophenanthroline reactive) Fe^{2+} form of iron at reference sites and sites where the ferrous form may be more prevalent (*e.g.*, certain industrial discharges).

Empirically Derived Guidelines for Determining Water Quality Criteria For Iron Protective of Aquatic Life in Ohio Rivers and Streams

INTRODUCTION

Iron is a naturally occurring constituent of the environment and is an essential part of the diets of aquatic organisms. However, at very high levels and under acidic conditions it can cause harm to aquatic ecosystems (Vuori 1995). The relationship between iron concentrations and aquatic life has not been widely studied outside of the effects of acid mine drainage. When aquatic communities have been assessed at more neutral pH levels, elevated concentrations of total iron have not been typically associated with degraded aquatic life at concentrations approaching and exceeding the existing Ohio WQS aquatic life criterion of 1.0 mg/L (this document, Loeffelman *et al.* 1986). Rasmussen and Lindegaard (1988) found a strong relationship between total iron and macroinvertebrate taxa richness and abundance, but not until total iron exceeded 5-10 mg/L.

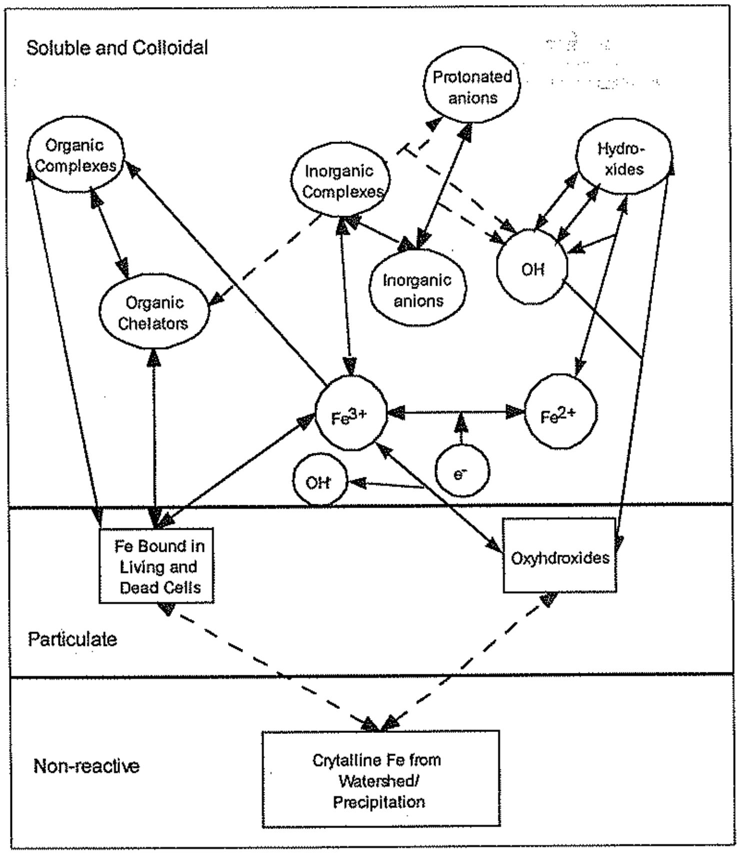


Figure 1. The iron cycle in natural aquatic systems illustrating the complexity of the cycle. Modified from Goldman and Horne (1983).

Part of the difficulty in predicting the effects of iron on aquatic life is the complexity of the iron cycle in the aquatic environment (see Figure 1). The various forms of iron (ferric [Fe^{3+}] and ferrous [Fe^{2+}]) are affected by concentrations of dissolved materials, organic matter, pH, redox potential, the concentration of sulfur compounds, and CO_2. Similar concentrations of total iron in two samples may have different proportions of the ferrous form, considered the most toxic form (Loeffelman *et al.* 1985), depending on the occurrence of the preceding factors. One study found no correlation between total iron and ferrous iron (Fe^{2+}) except at extreme concentrations (Rasmussen and Lindegaard 1988).

Most water quality criteria for aquatic life protection are based primarily on toxicological dose-response studies of aquatic life under laboratory conditions (Perry and Vanderklein 1996).

However, the impetus for the development of water quality criteria was the empirical evidence of adverse ecological effects. Representative aquatic organisms are tested for chronic and acute responses to various compounds in controlled laboratory studies and these studies form the basis for developing water quality criteria. There are valid concerns, however, that the water quality criteria derivation process can result in criteria that are either too stringent (*e.g.,* iron), resulting in poor allocation of pollution control resources, or too lenient because the laboratory conditions do not accurately mimic the fate and transport of the parameters in nature (*e.g.,* total vs dissolved forms of certain metals).

In 1997 Ohio EPA adopted revisions to major portions of the Ohio WQS that included a dissolved form for selected heavy metals in addition to the existing total recoverable form. Such a change originated from evidence that the toxicity of the total recoverable form, which was extrapolated from tests using dissolved forms, may potentially be over estimated because of the complexing of metals to and with inorganic and organic matter in discharges and the receiving waters. Procedures for the implementation of dissolved metals in the wasteload allocation process include the development of dissolved metals translators (DMTs) that define the ratio of total recoverable:dissolved form of a particular heavy metal. Developing a site-specific or regional DMT and the resulting effective dissolved concentration is a prerequisite to calculating a total recoverable wasteload allocation (WLA) and subsequently a water quality based limitation to be included in a NPDES permit. Because this represents a significant departure from past practice, the compatibility of this new approach and aquatic life use attainment was uncertain, particularly given the substantial impairment associated with heavy metals in the recent past (Ohio EPA 1997a [1996 305b report]). This effort resulted in the development of methods and techniques by which modifications to water quality criteria can be validated.

Ohio EPA has also examined the relationship between various chemical and physical (*e.g.*, habitat) parameters of interest in several ways (see Figure 2) to determine whether such parameters may be limiting to aquatic life (D.O.- Ohio EPA 1995; selected heavy metals - Ohio EPA 1997b). Some parameters (*e.g.*, habitat) emerge as basic or common *controlling* factors for aquatic life and the relationships are strong and easily defined with a number of statistical methods (Figure 2, middle). Few, if any ecological relationships are defined significantly by any single parameter (Figure 2, top) under ambient conditions, rather aquatic communities are affected by a wide range of physical and chemical factors. Most parameters exert threshold affects on aquatic life and plots of a single parameter versus the IBI or ICI generally result in a wedge of points with the outer surface approximating these threshold effects (Figure 2, middle). The strength of this relationship can vary with the frequency at which the parameters are controlling. Some parameters are rare controlling variables for aquatic life or they may be controlling in a different form (*e.g.*, dissolved vs. total) than what is typically measured (Figure 3, top). In the majority of Ohio streams, for example, the toxic heavy metals do not naturally occur at concentrations that are detrimental to aquatic life. In addition, where these metals are elevated they typically occur with other parameters that can adversely affect aquatic life.

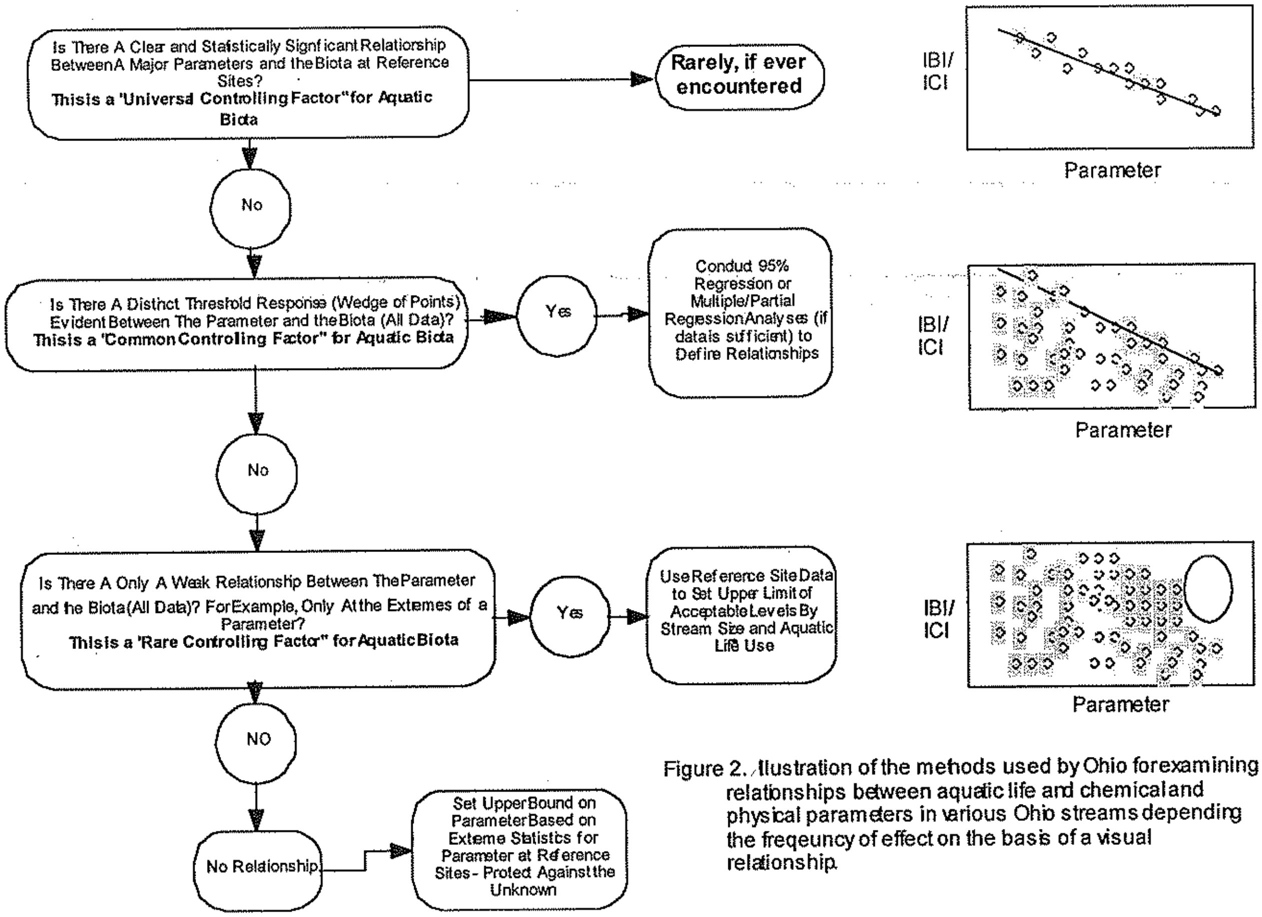


Figure 2. Illustration of the methods used by Ohio for examining relationships between aquatic life and chemical and physical parameters in various Ohio streams depending the freqeuncy of effect on the basis of a visual relationship.

One approach is to identify the threshold concentrations where the risks of aquatic life impairment are considered moderate to high, *i.e.*, we rarely observe attainment of aquatic life above these concentrations. Another approach is to statistically account for important controlling variables (*e.g.*, habitat, ammonia) and perform analyses such as stepwise multiple regression (*e.g.*, this paper) to distinguish the effects caused by a given parameter. The ambient biosurvey data fulfills the role of confirming what concentrations are protective given the uncertainties of fate and transport under natural conditions. For certain parameters or in certain ecoregions the relationship between the parameter and aquatic life may be statistically weak. In such situations the effects of the parameter may not be toxic (*e.g.*, the physical impact of iron precipitate; Gerhardt 1992; Vuori 1995) or the adverse effects may be caused by a form of the parameter that is not typically measured nor is consistently related to the measured form (*e.g.*, total recoverable iron vs. ferrous iron). In such situations, the use of upper percentiles of empirically derived background concentrations at regional reference sites (Figure 2, bottom) are used to set management criteria in conjunction with routine biological monitoring to detect situations where toxic forms may be present on a case-by-case basis.

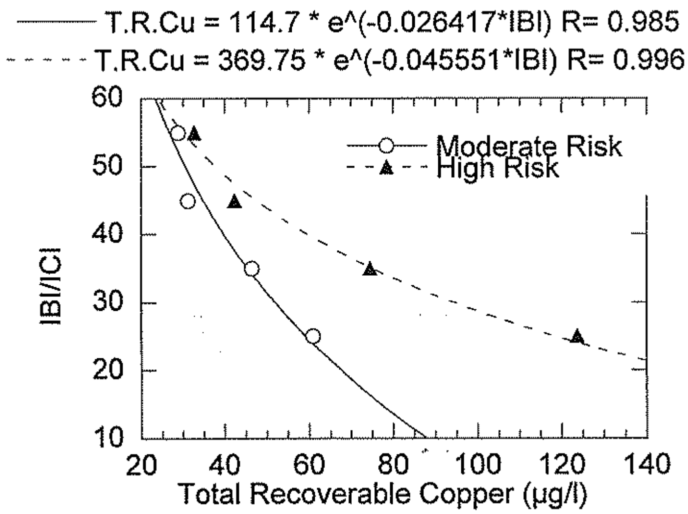


Figure 3. Scatter plot of the statistical maximum (moderate risk level) and 99.5th percentile (high risk level) of total recoverable copper associated with ranges of the IBI or ICI and the exponential curve fit describing the relationship. Good and exceptional IBI or ICI scores are infrequently observed beyond these moderate or high risk levels.

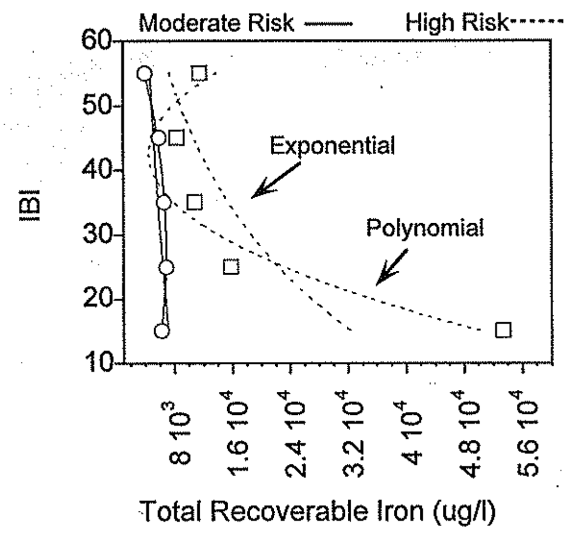


Figure 4. Safety trigger risk levels applied to iron. The polynomial equations provided a better fit to the data than the exponential line for both high (dashed lines) and moderate (solid lines) risk levels.

The approach we have taken for metals with demonstrated laboratory toxicity at relatively low doses (Figure 2, middle: *e.g.*, cadmium, copper, lead and zinc), is to examine scatter plots of the IBI or ICI versus concentration (Ohio 1997b). The outer sloped surface of points approximate the maximum concentrations that have been observed to occur at a given level of aquatic community performance. A line drawn on the outer surface of the data points so that 95% of the points fall to the left or beneath the line is referred to as the "95% line of best fit."

For the IBI and copper, for example (Figure 3), this represents the typically occurring maximum total recoverable copper concentrations at which a corresponding IBI value exists in the statewide database. Lines drawn perpendicular to the X-axis that intersect the IBI biocriterion for the EWH or WWH aquatic life use designation define the maximum total recoverable copper concentration above which there is an increasing risk of non-attainment. The approach described above works well for the heavy metals that exhibit dose-response toxicity at low concentrations (*e.g.*, ug/L), and that are from anthropogenic inputs. Iron represents a case where such an approach may not be entirely suitable (Figures 3 and 4). The present iron water quality criterion of 1.0 mg/l for freshwater aquatic life was derived from three toxicological studies and field observations that found "good fish faunas" at sites with iron concentrations less than 10 mg/l (Ellis 1937). However, the toxicity tests did not address the issue of the available ferrous form (Fe^{2+}) to total recoverable fraction and environmental fate and transport. Median concentrations of total recoverable iron from regional reference sites across all stream size and ecoregion categories (Appendix Figure 3) frequently approach and exceed the current water 1.0 mg/l criterion. This has been commonly observed in the Ohio River, West Virginia streams (Loeffelman *et al.* 1986), and elsewhere (Vuori 1995). Unlike the more toxic metals, a strong relationship between total recoverable iron concentrations and biological index scores does not exist in the statewide database (Figures 5 and 6; A-1 and A-2). Thus iron is considered to be a rare controlling factor.

Background concentrations of iron are influenced by soil type and bedrock parent material (Vuori 1995), factors which operate at the ecoregional scale. For example, exceptional IBI scores are occasionally associated with iron concentrations greater than 10 mg/l in the EOLP ecoregion, whereas exceptional biological index scores in the ECBP are rare at concentrations exceeding 5 mg/l.

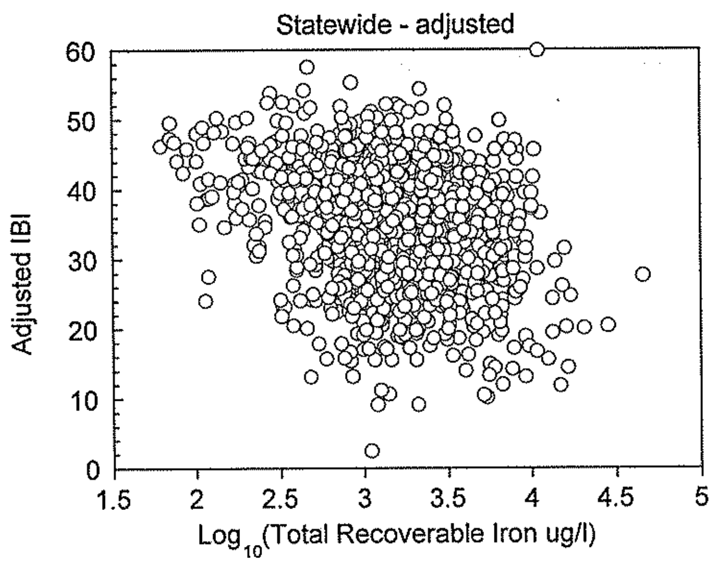


Figure 5. Scatter plot of statewide IBI scores adjusted for habitat and a variety of water quality parameters (see text) by concentrations of total recoverable iron (log_{10}; ug/l).

However, iron also serves as a surrogate indicator for other factors that can control and limit biological performance. Concentrations of total recoverable iron are highly correlated with total suspended solids, given that iron is a major constituent of clayey soils. Total recoverable iron can therefore serve as a surrogate indicator of runoff and sedimentation. The correlation between TSS and total recoverable iron is the most pronounced in the ECBP ecoregion, and reference concentrations of total recoverable iron are highest in the HELP ecoregion where clay content is high (60-80% in fluvial sediment) and agricultural land use and drainage practices facilitate high stream sediment loads (Table 1, see Appendix Figure 3).

Derivation of a New Total Recoverable Iron Criterion

Threshold levels of total recoverable iron that are protective of aquatic life uses (Table 2) were derived from reference concentrations of total recoverable iron after examining the ambient iron data using two other methods. First, mean IBI scores were adjusted for a variety of controlling factors (*e.g.,* QHEI, ammonia, dissolved oxygen, toxic metals) using stepwise regression by ecoregion and stream size (see Figure 2, middle). Adjusted IBI scores within three levels of total recoverable iron (<75th percentile of maximum, >75th to the 90th, and >90th) were then compared by ecoregion and stream size. In five of the 15 stream size/ecoregion combinations higher iron levels showed a significant association with lower IBI scores (see examples in Fig 7).

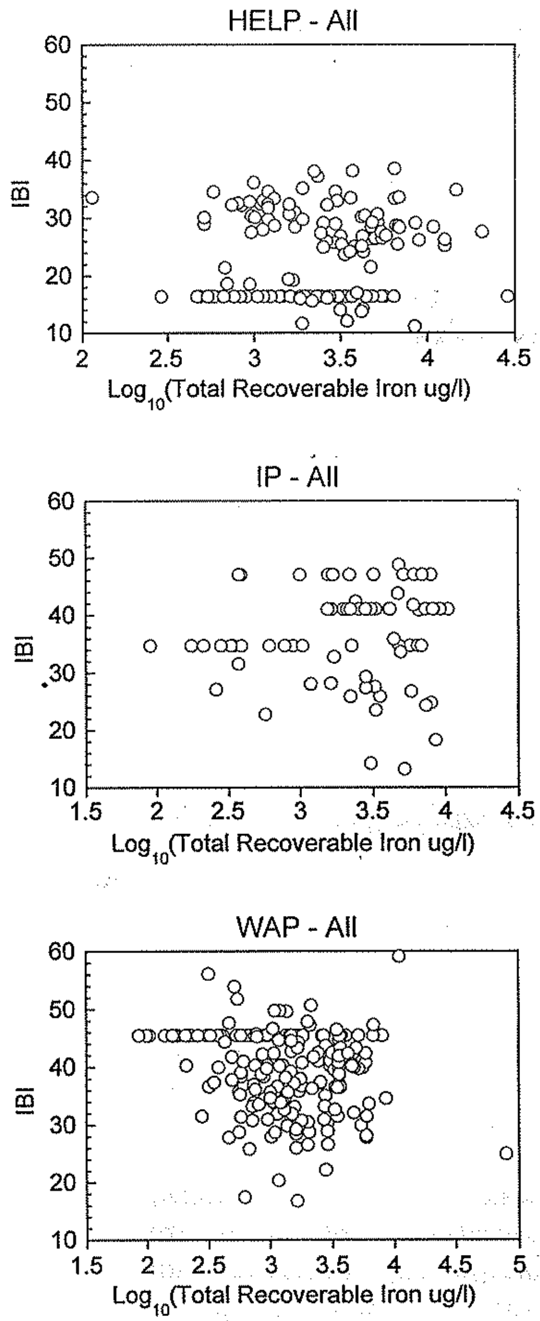


Figure 6. Scatter plots of adjusted IBI scores by total recoverable iron for the HELP, IP and WAP ecoregions. Clusters of equal IBI scores reflect a lack of association with independent variables in the stepwise regression used to adjust the IBI scores within a stream size class (e.g., headwater streams in the HELP ecoregion).

Table 1. Pearson coefficients and probabilities (P) of a greater R for the correlation of Fe and total suspended solids stratified by ecoregion and stream size.

		HELP	IP	EOLP	WAP	ECBP
Headwaters						
	R	0.2643	0.2418	0.2547	0.5546	0.5183
	P>R	0.1581	0.4049	0.0105	0.0001	0.0001
Wadeable						
	R	0.2005	0.0932	0.2773	0.0599	0.4757
	P>R	0.3262	0.6577	0.0009	0.6600	0.0001
Small Rivers						
	R	0.8167	-0.0087	0.3345	0.1636	0.6999
	P>R	0.0001	0.9823	0.0053	0.3130	0.0001
Large Rivers						
	R	0.9691	-0.3621	0.8928	0.1498	0.2562
	P>	0.0001	0.2241	0.0001	0.3692	0.1642

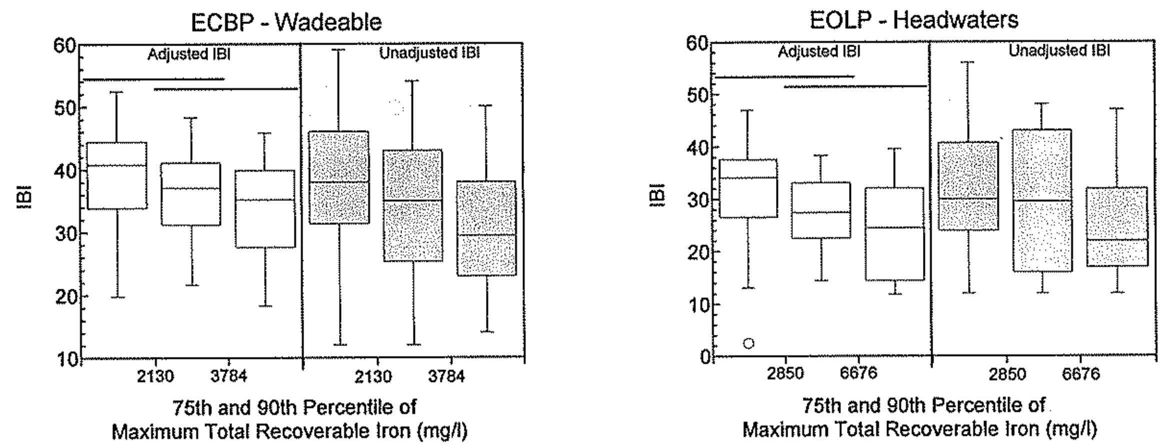


Figure 7. Distributions of adjusted and unadjusted IBI scores for wadeable streams in the ECBP and headwaters streams in the EOLP. Lines spanning box plots indicate similar means

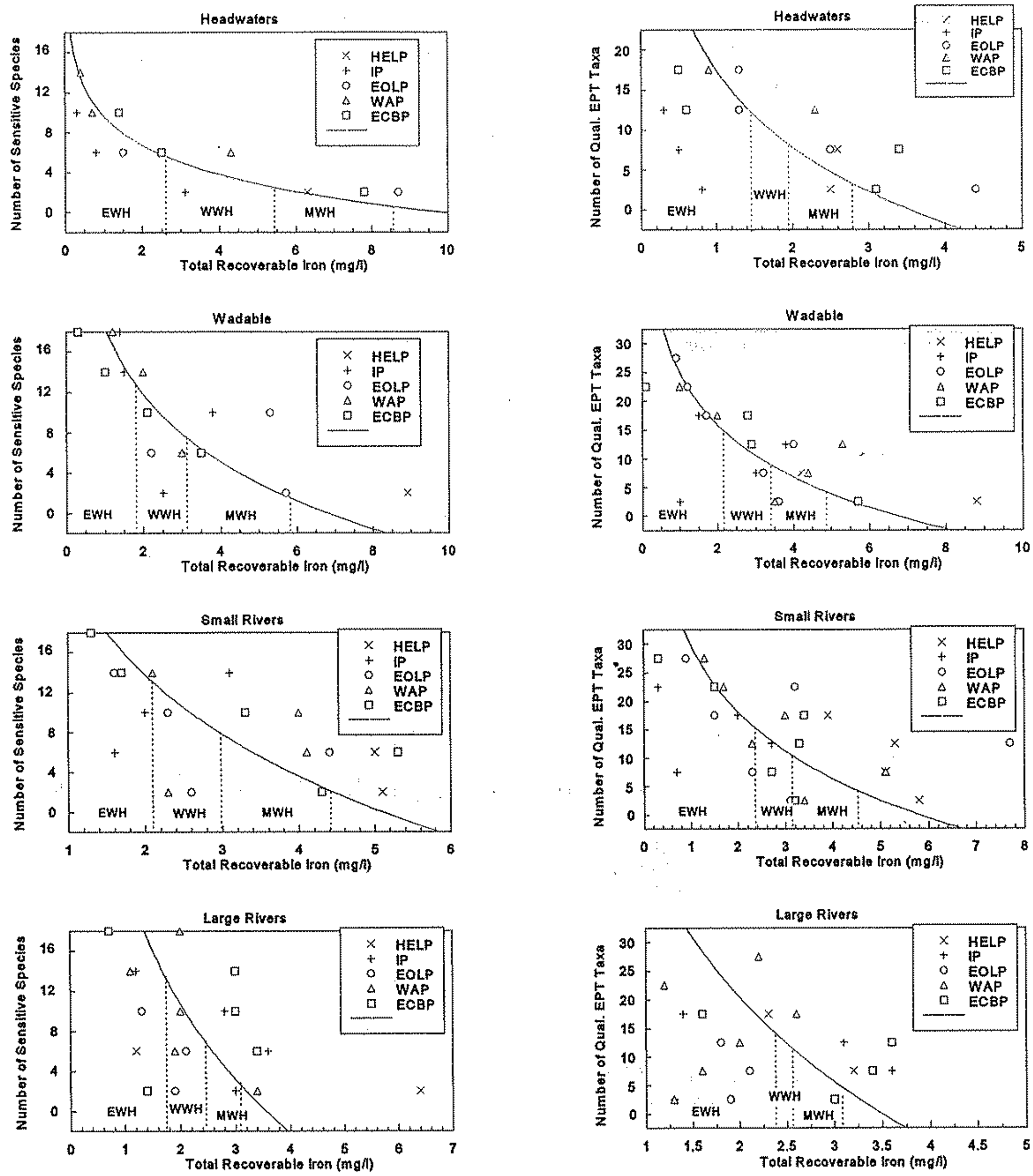


Figure 8. Scatter plots of average iron concentrations within a given range of sensitive fish species (left panel) and Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (right panel) by stream size. The mean number of sensitive fish species or EPT taxa within IBI or ICI ranges define Exceptional Warmwater Habitat (EWH - IBI 50-60; ICI 46-60), Warmwater Habitat (WWH IBI 49-36; ICI 44 - 32) and Modified Warmwater Habitat (MWH IBI <36; ICI <32) boundaries (stippled vertical lines intersecting the x-axis and regression line).

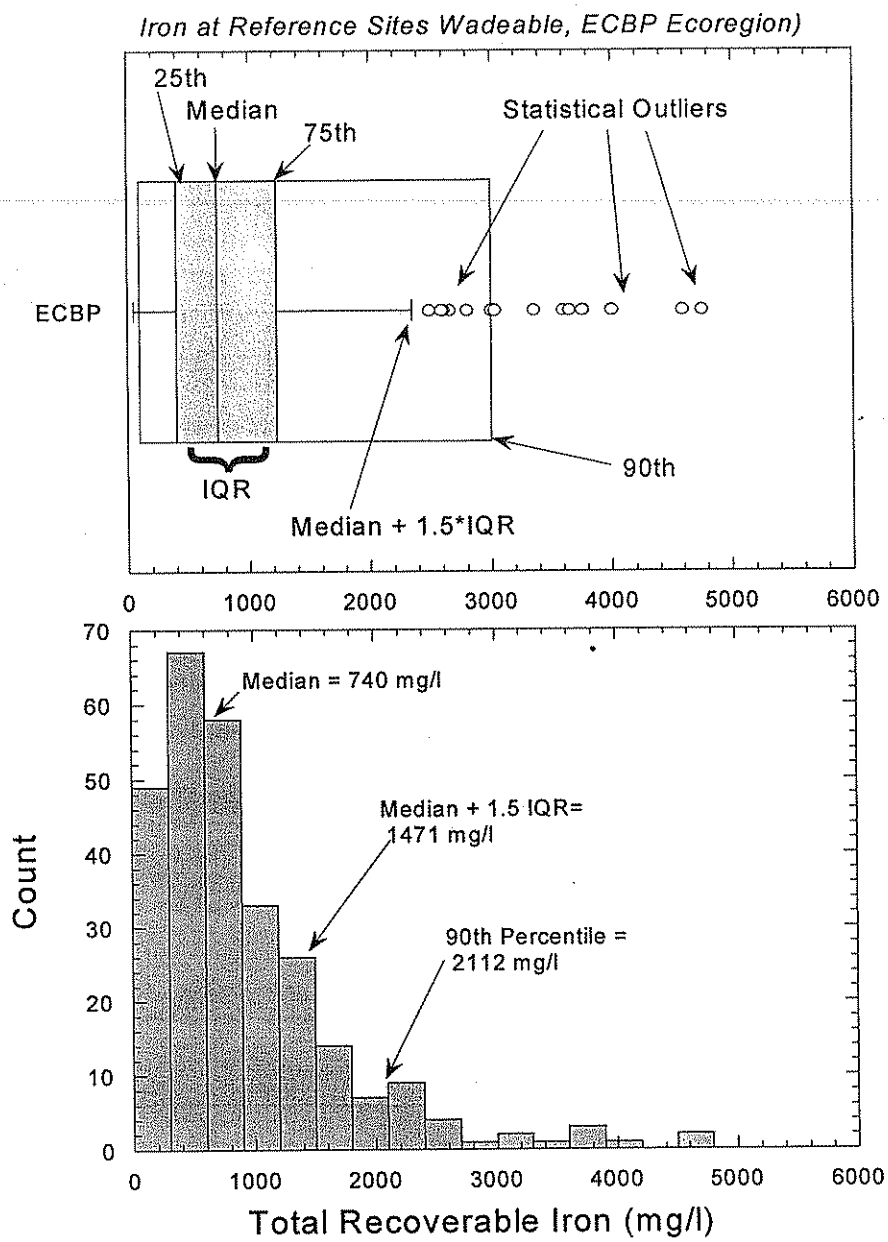


Figure 9. Box and whisker plot (top) illustrating some of the statistics used in selecting water quality criteria from reference data. Boxes on this graph represent various percentiles. The Interquartile range (IQR) is the difference between the 75th and 25th percentiles. "Whiskers" extend 1.5*IQR from the median and values outside of this range are considered outliers. The plot on the bottom illustrates a frequency histogram of this same data. For these plots there were six outliers with iron values greater than 6000 mg/l.

The value of the 75th percentiles used in these analyses provide some insight into levels that are associated with impairment, but do not provide a clear "safe level" for a criteria. As such we examined a second method that included the number of sensitive fish species and number of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa in relation to total recoverable iron analogous to that described for the other toxic heavy metals (Ohio EPA 1997b). For this analysis, the average total recoverable iron concentration, as opposed to the 95th or 99.5th percentile from the statewide database for a given range of the number of sensitive species or EPT taxa, was used to examine iron-aquatic life associations (Figure 8). Iron concentrations were averaged, otherwise the large variation in concentrations would result in 95th or 99.5th percentile lines that would not be appropriate when examined on an ecoregional scale (*e.g.*, headwater streams in the Interior Plateau). Furthermore, because total recoverable iron concentrations tend to be governed by the parent geology, the average concentration better represents ambient conditions as opposed to other more toxic metals that occur as more "episodic" acute events. The graphs of these results in Figure 8 illustrate threshold values where increasing iron is associated with declining fish species and/or macroinvertebrate taxa.

These first two analyses both illustrated associations with aquatic life at relatively high concentrations of total recoverable iron indicating potential "unsafe" concentrations, but do not provide clear "safe levels" for criteria. The values were also higher than the concentrations from regional reference sites (stratified by stream size and ecoregion, Appendix Table 1, Appendix Figure 3) that represent the statistical maximum (median + 1.5* IQR) for reference concentrations. Because of this, we decided to rely on various reference concentrations as protective levels for aquatic life (Table 2). Figure 9 illustrates the various statistics considered and the distribution of iron data with an example from wadeable streams in the ECBP ecoregion.

Table 2. Revised aquatic life criteria for total recoverable iron for Ohio.

Stream Size	Samples	EWH Criteria[a]	WWH Criteria[b]	MWH Criteria[c]
Headwater (≤ 20 sq mi)	399	1.0 mg/l[†]	1.1 mg/l	2.2 mg/l
Wadeable (>20 - 200 sq mi)	852	1.2 mg/l	1.4 mg/l	2.1 mg/l
Small River (>200-1,000 sq mi)	449	1.7 mg/l	2.1 mg/l	3.1 mg/l
Large River > 1,000 sq mi	238	2.0 mg/l	2.4 mg/l	3.9 mg/l

[a] Based on 75th percentile of reference sites unless < 1.0 mg/l.
[b] Based on median + 1.5*IQR of reference sites.
[C] Based on 90th percentile of reference sites.
[†] This value represents the current aquatic life criteria of 1.0 mg/l, the 75th percentile of reference sites was 0.9 mg/l.

The stream size differences in iron concentrations were much greater than among ecoregion differences in background iron concentrations (see Appendix Figure 3), thus we decided to develop criteria statewide by stream size categories and not along ecoregion categories. For warmwater streams we selected the higher of the median + 1.5 times the interquartile range or the existing criteria of 1 mg/l as protective criteria. Although this may appear to be an "extreme" statistic, it is actually below the 90th percentile because of the long "tail" of data at high iron concentrations (see Figure 10). Thus 10-15% of reference sites have values greater than this level and it is higher than the current criteria of 1 mg/l in all cases. For exceptional warmwater streams we selected the greater of the 75th percentile of reference sites or 1.0 mg/l. EWH streams are especially sensitive to disturbance and can be strongly affected by dissolved solids that are associated with higher concentrations of iron. In other than EWH headwater streams, this will result in criteria greater than the current 1.0 mg/l

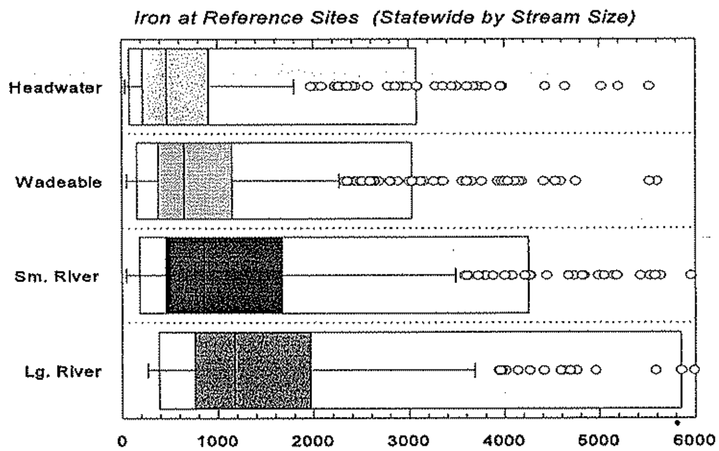


Figure 10. Total recoverable iron (mg/l) at reference sites in Ohio stratified by stream size. See Figure 9 (top) for an explanation of the box and whisker plot.

Aquatic inhabitants of modified streams are generally very tolerant of many environmental stresses. For these streams we selected the 90th percentile of reference values. These streams are typically characterized by high suspended solids and heavy bedloads of fine sediments. Such a value should give more than adequate protection for such waters. The values in Table 2 represent water quality criteria that are designed to act as targets for permits such as NPDES discharge levels. The approach we have taken is conservative; the levels are below where we have observed effects associated with total recoverable iron and 10-15% of reference sites have values greater than the WWH criteria. Because these are conservative targets, we will use a weight of evidence approach when assessing whether total recoverable iron values greater than these criteria are considered as an impairment to an aquatic life use. Such an approach is not likely to result in any adverse environmental impact. Our intensive surveys generally consist of multiple sites, multiple samples (biological, physical, and chemical) and multiple organism groups. Thus we will be able to distinguish among situations where iron may be a significant problem or threat from those situations where high values are statistically abnormal and ecologically insignificant.

Table 3 summarizes the total recoverable iron concentrations where significant associations of aquatic life with iron were detected in the two analyses discussed above. This will be a useful reference for comparison with the criteria derived for total recoverable iron based on reference sites. Future results and the incorporation of data on other forms of iron may result in changes to these criteria in the future.

Ferrous Iron

Recent studies suggest that the ferrous form of iron is the most toxic fraction resulting in recommendations that iron criteria should only appear in the ferrous form (Loeffelman *et al.* 1986). Loeffelman *et al.* (1986) proposed a preliminary criterion of 0.37 mg/L for BPA-reactive ferrous iron[1] which was based on the lowest of five 96-hr LC50 tests (3.7 mg/L for fathead minnow) and using a 0.1 application factor because of the paucity of chronic effects data. This shows the need to develop more ferrous iron toxicity data using a method such as the BPA-reactive analytical method prior to considering this type of criterion. In the Ohio River study the mean BPA Fe^{2+} was 3% of the total recoverable iron (Loeffelman *et al.* 1986). The suggested total recoverable criteria values reported in Table 2 should be protective of most situations in Ohio that do not involve acidic mine drainage and in cases where the proportion of the ferrous form is greater than 3%. Where the ferrous form of iron is a small proportion of the total recoverable iron, the criteria will also protect for situations where total recoverable iron is a surrogate for other factors that can adversely affect aquatic life (suspended solids, sedimentation). The criteria will also reduce the non-toxic, but potentially serious physical effects of iron (precipitates, colloidal forms) that may affect the most sensitive inhabitants of the highest quality streams.

Ohio's system of tiered aquatic life uses is designed to provide an appropriate amount of protection for the types of aquatic organisms that inhabit or could potentially inhabit a given water body. This system recognizes the important influence of regional factors in the inherent potential to support aquatic life as this is related to ecoregion, stream size, and physical habitat quality. This system also allows a more stratified and accurate identification of background levels of commonly measured chemical constituents of each category of water. The result is criteria that are appropriate and are neither over- or under-protective of aquatic life. Although this approach yields a stratified set of numerical criteria, as opposed to a single, statewide criterion, it is represents an objective result that is based on contemporary science and thinking in water quality management. Further collection of other forms of iron could result in a more justified stratification by ecoregion.

The tiered total recoverable iron criteria presented here are the most protective and accurate available based on the data available for analysis. The aquatic life of very few streams are substantially limited by iron alone a conclusion that is based on analyses of the total recoverable form of iron. Areas with very high total recoverable iron concentrations are most frequently associated with acid mine drainage

[1] BPA reactive Fe^{2+} is based on the reagent bathophenanthroline and is considered the "available" Fe^{2+}. HCl reactive Fe^{2+} is also commonly described and is considered total Fe^{2+} (Loeffelman *et al.* 1986).

that has an obvious and unique impact on aquatic life. When sufficient data exists Ohio EPA will investigate whether a ferrous iron criterion in combination with recommended total recoverable criteria is justifiable. In addition, consideration will be given to developing a better ambient database for the ferrous form (*e.g.*, BPA reagent method) especially at regional reference sites and in situations where the ferrous form could be a significant proportion of total recoverable iron (*e.g.*, certain types of industrial discharges). This would allow a future analysis of the direct effect of the ferrous form of iron on aquatic life in Ohio waters.