

# Development of a Fish-Based Index of Biological Integrity for Minnesota's Rivers and Streams



Minnesota Pollution Control Agency

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# 1. Overview

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This report documents the development of a fish community-based Index of Biological Integrity (F-IBI) for Minnesota's streams and rivers. The primary intended use for this tool is the assessment of aquatic life use support by the Minnesota Pollution Control Agency (MPCA). More detailed descriptions of biomonitoring, bioassessments, biological assessment guidance, human disturbance score (HDS), and biological condition gradient (BCG) can be found in other documents.

Passage of Minnesota's Clean Water Legacy Act (CWLA) in 2006 provided a policy framework and resources to accelerate efforts to monitor, assess, and restore impaired waters, and to protect unimpaired waters. With passage of the Clean Water, Land and Legacy Amendment in 2008, additional funding was made available to the MPCA, Minnesota Department of Natural Resources (MnDNR), and partner agencies to continue and expand on efforts outlined in the CWLA.

In 2007, the MPCA initiated a 10-year, rotating watershed approach for comprehensive monitoring and assessment of Minnesota's waters. The MPCA has used indices of biological integrity and chemical measures to assess the integrity of streams since the mid-1990s. However, existing IBIs could not adequately support this statewide monitoring and assessment effort. For example, no biological assessment tools had been developed for the many miles of streams within the Rainy River and Lake Superior Basins, the Lower Mississippi River Basin, and the Red River Basin outside of the Lake Agassiz Plain Ecoregion. Furthermore, existing IBIs had not been developed concurrently, and varied in terms of their analytical approaches, classification frameworks, scoring systems, and taxa attributes. To support comprehensive monitoring and assessment of Minnesota's streams, it was necessary to develop new indicators applicable to the entire state of Minnesota, using a consistent, standardized approach.

Development of the statewide F-IBI utilized a protocol developed by researchers from the United States Environmental Protection Agency (USEPA) and elsewhere. Minnesota's streams and rivers were first partitioned into nine physiographic classes; a unique F-IBI was developed for each stream class. Within each stream class, biological metrics were evaluated using a series of tests. Metrics that passed these tests were ranked and a subset selected for inclusion in each IBI. The final indices included between seven and twelve metrics and demonstrated the ability to distinguish between levels of biological condition.

This document describes the process used in the development of F-IBI for Minnesota's rivers and streams, representing the state's first comprehensive, statewide tool for assessing biological integrity of riverine fish communities. These indices will be used during the first iteration of the 10-year watershed monitoring and assessment cycle, and periodically evaluated to ensure they remain robust and effective tools for assessing aquatic life.

## 2. Introduction

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Waterbody monitoring and condition assessment provide resource managers with information needed to guide restoration and protection efforts. A wide variety of indicators are used in water monitoring and assessment programs, but among the most useful are those that integrate and reflect cumulative impacts to aquatic systems. Degradation of surface waters can be attributed to multiple sources including: chemical pollutants from municipal and industrial point source discharges; agricultural runoff of sediment, nutrients, and pesticides; hydrologic alteration in the form of ditching, drainage, dams, and diversions; and habitat alteration associated with agricultural, urban, and residential development. The timing and magnitude of these impacts may vary through time, and be difficult to detect and measure utilizing traditional chemical evaluations that focus on a single indicator or limited suite of parameters. However, biota reside in these waterbodies utilize the available aquatic habitats, and have life spans ranging from weeks to years. They experience the entire spectrum of environmental conditions, including stressors caused by human activities. Aquatic biota are known to be responsive to a wide variety of anthropogenic impacts and, at the community level, reflect the integrated result of physical, chemical, and biological processes through time (Barbour et al. 1999). In this manner, aquatic communities provide a direct, comprehensive perspective on water quality, and lend themselves well to tools that utilize community-level parameters, such as the Index of Biological Integrity (IBI).

The IBI was originally developed as a tool for assessing the condition of rivers and streams in the Midwestern United States (Karr 1981, Karr et al. 1986). The concept has since been expanded to a wide variety of geographic regions and ecological systems, and has demonstrated its effectiveness in several applications (e.g., condition monitoring, stressor identification). At its core, the IBI provides a framework for translating biological community data into information regarding ecological integrity (“the capability of supporting and maintaining a balanced, integrated, functional organization comparable to that of the natural habitat of the region,” Frey 1977). It utilizes a variety of attributes (“metrics”) of the biological community, each of which responds in a predictable way to anthropogenic disturbance. Metrics are based on ecological traits of species and represent different aspects of ecological structure and function. The metrics are scored numerically to quantify deviation from least-disturbed conditions, and summed together producing a composite IBI score that characterizes biological integrity (Karr et al. 1986).

The composite IBI score is typically compared to a threshold to assess a waterbody’s condition. However, it is also possible to deconstruct the index into its component metrics to determine which aspects of ecological structure and function are particularly robust or diminished. Relationships between specific stressors and the composite IBI or component metrics can be explored, and trait-environment linkages extended to diagnostic (i.e., stressor identification) applications (Culp et al. 2010). Stressor-response relationships are implicit in the IBI concept and may provide information relevant to watershed protection and restoration strategies.

Since the 1990s, the MPCA has utilized the IBI concept in its stream monitoring and assessment program. Narrative language within Minnesota Administrative Rule identifies an IBI calculation as the primary determinant for evaluating impairment of aquatic biota (Minn. R. 7050.0150, subp. 6, Impairment of biological community and aquatic habitat). Details regarding development and calibration of the IBI are included in an associated Statement of Need and Reasonableness, and use of this framework has been upheld in legal proceedings challenging its use.

Between 1993 and 2002, the MPCA developed Fish IBIs for streams in specific ecoregions and major basins of Minnesota, and used them to conduct Aquatic Life Use assessments. Fish IBIs were developed for rivers and streams with the Minnesota River Basin (Bailey et al. 1993), the Lake Agassiz Plain Ecoregion of the Red River Basin (Niemela et al. 1999), the St. Croix River Basin (Niemela and Feist 2000), and the Upper Mississippi River Basin (Niemela and Feist 2002) (Figure 1). However, nearly half of Minnesota's streams and rivers were not covered by these existing IBIs (Table 1).

Index of Biotic Integrity	Stream Miles	Percentage
Lake Agassiz Plain	12057	11.9%
Minnesota River Basin	19264	19.0%
St. Croix River Basin	3775	3.7%
Upper Mississippi River Basin	19942	19.6%
No IBI	46461	45.8%

**Table 1. Estimated sum of Minnesota stream miles previously covered by regional fish IBIs, and percentage of the state's total stream miles covered by each.**

**Figure 1. Map of Minnesota depicting regions previously encompassed by existing (1993-2002) Fish IBIs**

Passage of Minnesota's Clean Water Legacy Act in 2006 and Clean Water, Land and Legacy Amendment in 2008 accelerated efforts to monitor, assess, restore, and protect the state's water resources. With this increased emphasis on water quality, it became evident that monitoring and assessment tools applicable on a statewide scale were needed, and that resources necessary to develop those tools were available. Our objective was to develop a series of IBIs for assessing the condition of fish communities in rivers and streams across the state of Minnesota.

In this document, we describe development and calibration of fish-based IBIs for streams and rivers across the State of Minnesota. Using a methodology developed by researchers at the United States Environmental Protection Agency (Whittier et al. 2007), metrics representing the structure and function of Minnesota's stream fish communities were systematically tested for inclusion in IBIs based on statistical criteria (e.g., responsiveness to disturbance, strong signal, low noise). These IBIs will be used in conjunction with numeric biocriteria to assess biological integrity of Minnesota's rivers and streams, and, in conjunction with water chemistry data and standards, to assess whether waterbodies are meeting designated Aquatic Life Uses as outlined in Minnesota Rules and the federal Clean Water Act.



## 3. Methods

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### 3.1. Study area

The State of Minnesota lies in a water-rich region, at the headwaters of three major continental watersheds (Gulf of Mexico, Laurentian Great Lakes, Hudson Bay) and at the intersection of western prairies, eastern deciduous forests, and northern boreal forests (Figure 2). Much of the state lies in a transition zone between these ecotypes, and its watercourses reflect the diversity of their landscapes. A wide variety of rivers and streams are found within Minnesota's borders, including: short, steep bedrock-controlled cascades; broad, meandering prairie rivers; clear, cold spring-fed creeks; and tannic, low-gradient streams draining large bogs and swamps. The fish fauna is diverse (>140 native species) and dominated by cool- and warm-water taxa, though coldwater assemblages are found in some regions of the state. The distribution of individual fish species has been greatly shaped by glaciations, glacial refugia, and post-glacial barriers to dispersal (Underhill 1989), though several species have been introduced outside of their native range, both intentionally and inadvertently. Dams, pollution, channelization, and diversions have also artificially disrupted movements of migratory species into habitats they historically utilized.

Humans have substantially modified Minnesota's landscape. Most native prairies have been converted to agricultural land, with extensive systems of surface and subsurface drainage. Nearly all of the forested land has been logged at some point in the past 150 years. Urban areas have been steadily expanding in all regions of the state. Associated with this transformation, many of Minnesota's waterbodies have experienced historical and ongoing impacts, including stressors related to agricultural practices, urbanization, mining, logging, channel modification, and industrial discharges. However, substantial portions of the state have retained natural vegetative cover, relatively intact stream habitats, and connectivity within watersheds. The contemporary structure and function of Minnesota's stream ecosystems are shaped by these interacting factors of natural variability and human disturbance; the resulting level of biological integrity can be interpreted by tools such as the IBI.

### 3.2. Program details

Two Biological Monitoring Units within the MPCA's Environmental Analysis and Outcomes Division conduct ecological surveys on rivers and streams across the state. Since the early 1990s, an extensive dataset has been maintained, describing physical, chemical, and biological characteristics of rivers and streams. As of late 2012, more than 5,000 individual fish collection efforts are represented, from more than 4,500 monitoring sites across the state. The vast majority of surveys were conducted by MPCA staff, but the database also includes a limited number of surveys conducted by other agencies and organizations. These data are used to support annual waterbody condition assessments in concordance with state and federal requirements (MPCA 2012, MPCA 2014a, Figure 3).

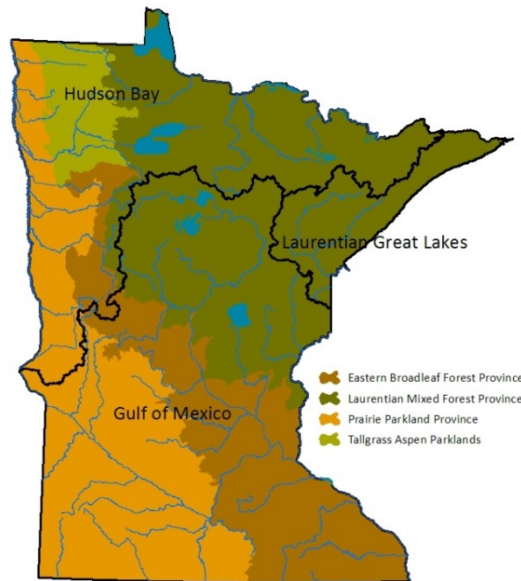


Figure 2. Map of Minnesota depicting major ecotypes (MnDNR Ecological Classification System Provinces), continental watersheds, major rivers and large lakes.

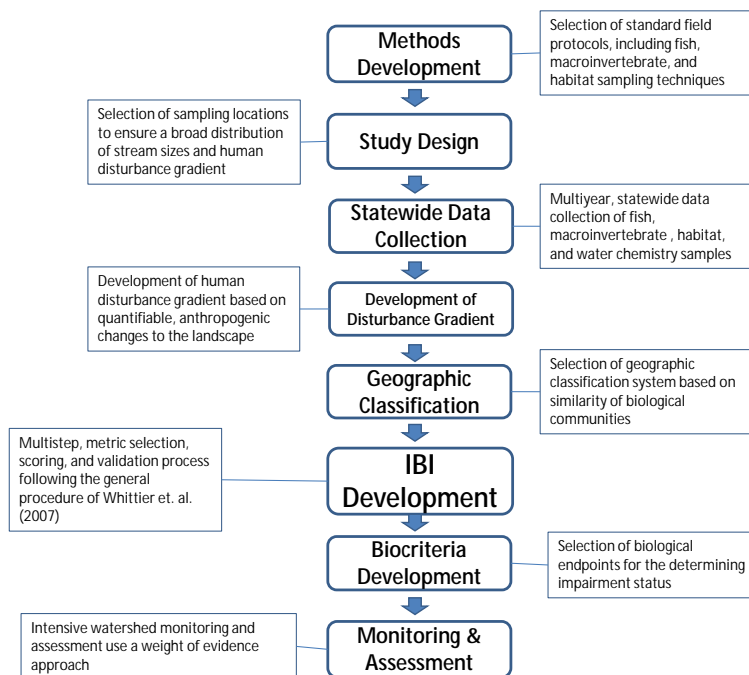


Figure 3. Overview of biological data use by Minnesota Pollution Control Agency.

### 3.3. Field methods

All fish community surveys were conducted using electrofishing techniques during daylight hours under base-flow conditions (generally early June to late September). Crews followed the MPCA's Fish Community Sampling Protocol for Stream Monitoring Sites (Feist 2011). Field methods of partnering agencies (e.g., MnDNR, United States Geological Survey) may have differed, but non-MPCA data was added to the database if methods were deemed similar. Electrofishing distance was typically 35 times mean stream width (at baseflow), with a minimum of 150m for sites less than 4m wide and a maximum of 500m for sites greater than 14m wide.

Fishes were collected using a variety of gear types, depending primarily upon stream width and depth. Backpack electrofishing units with a single anode and single netter were typically used in wadeable streams up to 8m wide. Larger wadeable streams were sampled using a two-anode/two-netter barge-type electrofishing platform ("stream shocker"). Non-wadeable sites were sampled using a boat electrofisher; a single-anode/single-netter jonboat platform ("mini-boom") was used for small or hard to access sites, while a larger two-anode/two-netter boat platform ("boom shocker") was used for large, accessible rivers. Single-pass upstream surveys were used at wadeable sites. Boat electrofishing proceeded in a downstream direction, either a single pass while weaving back and forth into different habitat types (mini-boom) or three separate runs (left bank, right bank, mid-channel) in larger rivers (boom shocker). Regardless of gear type, no physical barriers were deployed to prevent upstream or downstream movement of fishes during the course of the survey.

All fishes greater than 25mm total length were sorted and identified to the species level in the field, with a count, batch weight, and minimum/maximum total length recorded for each species. Small or difficult specimens were often preserved for later identification in a lab setting. Any deformities, eroded fins, lesions, or tumors were noted. Two voucher specimens of each species captured were confirmed and archived by the University of Minnesota's Bell Museum of Natural History. In cases where no small specimens of a species were captured, field identifications were later verified using photographs of distinguishing features (e.g., mouth, fins and caudal peduncle scales for *Moxostoma spp.*).

### 3.4. Human Disturbance Score

A composite Human Disturbance Score (HDS) was developed to represent potential cumulative anthropogenic disturbance experienced by stream environments, assessed at both a reach- and watershed-scale (MPCA 2014b). The disturbance metrics selected for inclusion in the HDS are grounded in the concept of a "Generalized Stressor Gradient" (USEPA 2005), and are evaluated on a site-by-site basis, using readily-available statistics on land use, feedlot and point source density and proximity, reach- and watershed-scale channelization, impervious surfaces, road density, and riparian conditions (Table 2). Eight primary metrics are individually scored on a 0 (highly disturbed) to 10 (minimally disturbed) scale and summed to derive a composite score. Metric scores represent rescaled (0-10) values for each stressor variable, after excluding values greater or less than three times the interquartile range. Up to seven additional "adjustment" metrics are then applied, each of which potentially deducts one point from the composite score. One of the adjustment metrics (watershed road density) may also result in the addition of a point. The final, composite Human Disturbance Score ranges from a minimum of 0 (highly disturbed) to a maximum of 81 (minimally disturbed). Negative composite scores are normalized to 0.

Table 2. Minnesota Pollution Control Agency Human Disturbance Score (HDS) metrics. Metrics are evaluated either at the scale of a site's contributing watershed, or the area immediately adjacent to the sampling location. Several categories of potential anthropogenic disturbance are included (e.g., land use, point sources, riparian condition, channelization). Eight "core metrics" are scored on a 0-10 scale, while six "adjustment" metrics may add or subtract a single point from the composite score.

HDS Metric Description	Scale	Category	Scoring Range
animal unit density	watershed	land use	0-10
percent agricultural land use	watershed	land use	0-10
percent impervious surface	watershed	land use	0-10
feedlot density	watershed	land use	-1
percent agricultural land use within 100m riparian buffer	watershed	land use	-1
road/stream intersection (road crossing) density	watershed	land use	-1 or +1
percent agricultural land use on $\geq 3\%$ slope	watershed	land use	-1
urban land use proximity	local	land use	-1
point source density	watershed	point source	0-10
point source proximity <sup>1</sup>	local	point source	-1
feedlot proximity	local	point source	-1
percent disturbed riparian habitat	watershed	riparian	0-10
riparian condition rating	local	riparian	0-10
percent of stream distance modified by channelization	watershed	channelization	0-10
site channelization rating	local	channelization	0-10

<sup>1</sup> applies only to streams with watershed area <50 square miles

### 3.5. Stream classification

Recognizing that biological communities vary along natural gradients, an effort was undertaken to develop a stream classification framework for Minnesota's riverine fish communities. The goal was to identify natural variables that effectively separated sites into physiographic classes such that the fish community structure was similar among sites within each class, while at the same time distinct from sites in other classes. We considered natural classification variables unaffected by anthropogenic disturbance (e.g., watershed area, stream gradient) to ensure that sites would be classified according to their natural potential rather than by their current state. For example, stream nutrient levels were not considered as a classification variable, because nutrients may be derived from both natural and anthropogenic sources, and ambient levels may reflect anthropogenic disturbance as much or more than natural background. Candidate classification variables included both broad-scale and local variables to encompass the important natural drivers of stream fish community structure.

Stream classification was carried out separately for warm- and coldwater streams. Distinction between the two thermal classes was largely based on whether a site was located on an MnDNR Designated Trout Stream, but consideration was given to whether coldwater fish species (e.g., trout, sculpin) were present or known to have been present in the past. As a result, some sites on Designated Trout Streams were excluded from the Coldwater dataset, and vice-versa. Within each dataset (warmwater, coldwater), a set of least-disturbed sites was identified based on the 75<sup>th</sup> percentile threshold of the HDS distribution. Reach-scale habitat conditions were used to further refine the selection process in a limited number of cases. Classification analyses were carried out using both the least-disturbed dataset

and the full dataset of all sites. While more emphasis was placed on patterns emerging from the least-disturbed dataset, the entire dataset was analyzed in a similar manner to provide supplementary information.

A variety of analytical techniques and statistical tools was used to partition variability in fish community structure into distinct stream classes and evaluate various candidate classification frameworks. For both the “All Sites” and “Least Disturbed” datasets, both presence-absence and relative abundance matrices of fish species observed at each site was analyzed using hierarchical cluster analysis (PC-Ord, Flexible  $\beta$ ,  $\beta=-0.25$ ). Hierarchical cluster analysis is a method for defining groups of objects such that objects within each group are more similar to each other and less similar to objects in other groups; results are often depicted as a dendrogram. Each dataset was clustered into as many as 15 and as few as 2 “species groups.” Following the assignment of sites to species groups, sites were mapped using Geographic Information System software and color-coded by group membership. Sites were color-coded at each level of clustering (from 2 to 15 clusters) and the spatial arrangement of clusters was examined to detect obvious geographic patterns. Summary statistics, distribution plots and box plots were then used to examine the distribution of natural variables (e.g., watershed area, stream gradient, latitude, longitude) for sites comprising each cluster. Ordination (PC-Ord, Non-Metric Multidimensional Scaling) was used to visualize the relative similarity of different clusters, as well as the orientation of environmental gradients and existing regional classification frameworks (e.g., Omernik Ecoregions, MnDNR Ecological Classification System Provinces, HUC4 watersheds) among species clusters. Mean Similarity Analysis (MEANSIM, Van Sickle 1998) was used to evaluate effectiveness of various classification frameworks in partitioning fish community structure variability. This approach determines the classification strength of groupings, evaluated as a combination of both within-class and between-class dissimilarity. Selection and analysis of classification frameworks proceeded in an iterative manner, with candidate variables tested at different levels of partition and in combination with other variables.

While a large number of classes may produce a strong classification, a smaller number of classes might be preferable, given the intended use for the framework. Dozens of classes would likely result in identification of highly localized assemblages and be generally difficult to implement in a bioassessment setting. Fewer classes are preferable, assuming criteria can be identified to separate the dataset into sufficiently distinct and homogenous groups. To compare effectiveness of frameworks containing different numbers of classes, classification strength was calculated at each level of hierarchical clustering based on neutral model classifications (i.e., fish community structure alone) to represent a theoretical optimum to which environmental frameworks with an equivalent number of classes could be compared (Van Sickle and Hughes 2000). For example, classification strength for a 5-class environmental framework would be divided by the classification strength of a 5-cluster grouping of the fish community dataset, and expressed as a percentage of the “optimum.” In this manner, marginal increases in classification strength achieved simply by adding classes could be objectively evaluated with respect to the increased complexity also introduced to the classification system.

Ultimately, a classification framework was developed that divides lotic sites into nine “fish classes,” differentiated by region, drainage area, gradient, and thermal regime (Figure 4, Appendix A). An IBI was developed for each individual fish class, while keeping open the possibility of combining classes if obvious similarities emerged during the metric evaluation process.

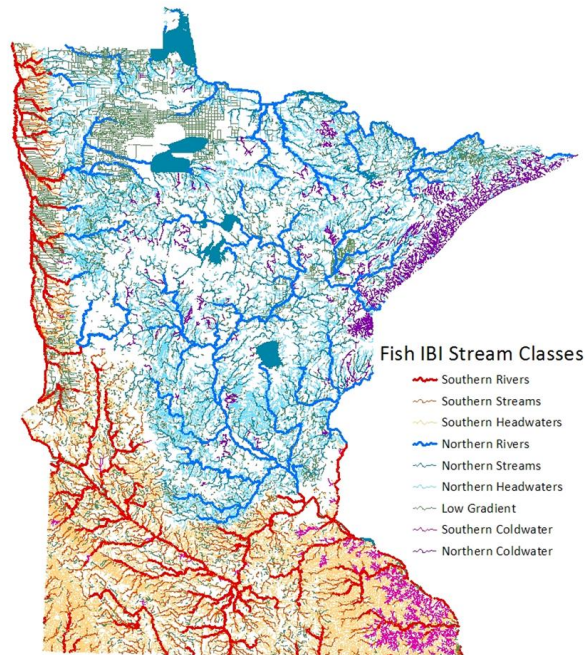


Figure 4. Generalized map of Fish IBI stream classes for the State of Minnesota. For display purposes, reach-specific fish class assignments were derived from the NHD+ spatial dataset. Map is for display purposes only; classification of individual sampling locations should utilize site-specific attributes as outlined in Appendix A.

### 3.6. IBI development dataset

Warmwater streams were prioritized for IBI development because they make up the vast majority (>90%) of Minnesota's stream miles and a sufficient dataset had been established by 2009. Coldwater streams make up less than 10% of Minnesota's stream miles, and preliminary evaluation of existing data in 2009 indicated that additional, targeted sampling was required to assemble a suitable IBI development dataset. The definition of "warmwater stream" used in this analysis encompassed all non-coldwater streams, including some that might be properly classified as "coolwater." Warmwater IBI development began in 2009 and was completed in early 2010; coldwater IBI development began following supplemental field sampling carried out in the summer of 2010, and was completed in early 2011.

The warmwater IBI development dataset consisted of 1,563 sites and 1,918 samples collected between 1990 and 2008 (Figure 5a). Fish sampling was conducted in the course of multiple projects, and included both randomly-located and targeted surveys. In cases where multiple samples were collected from the same site, the fish taxa abundance data were averaged. Sites with within-year repeat visits (n=146) were identified for use in evaluating metric precision.

The coldwater IBI development dataset consisted of 367 sites sampled between 1996 and 2010 (Figure 4b); in cases where multiple samples were collected from the same site, the most recent sample was used. Sites with within-year repeat visits (n=94) were identified for use in evaluating metric precision. Fish sampling was conducted in the course of multiple projects, and included both randomly-located and targeted surveys.

Two-thirds of the sites within each stream class were selected to serve as an IBI development dataset; the remaining one-third was reserved as a validation dataset. Within each dataset (development and validation), sites were sorted into disturbance categories defined by quartile boundaries of the Human Disturbance Score (HDS) for each class. “Least-disturbed” sites were defined as those with an HDS above the 75<sup>th</sup> percentile for a particular class; “most-disturbed” sites were defined as those with an HDS below the 25<sup>th</sup> percentile for a particular class.

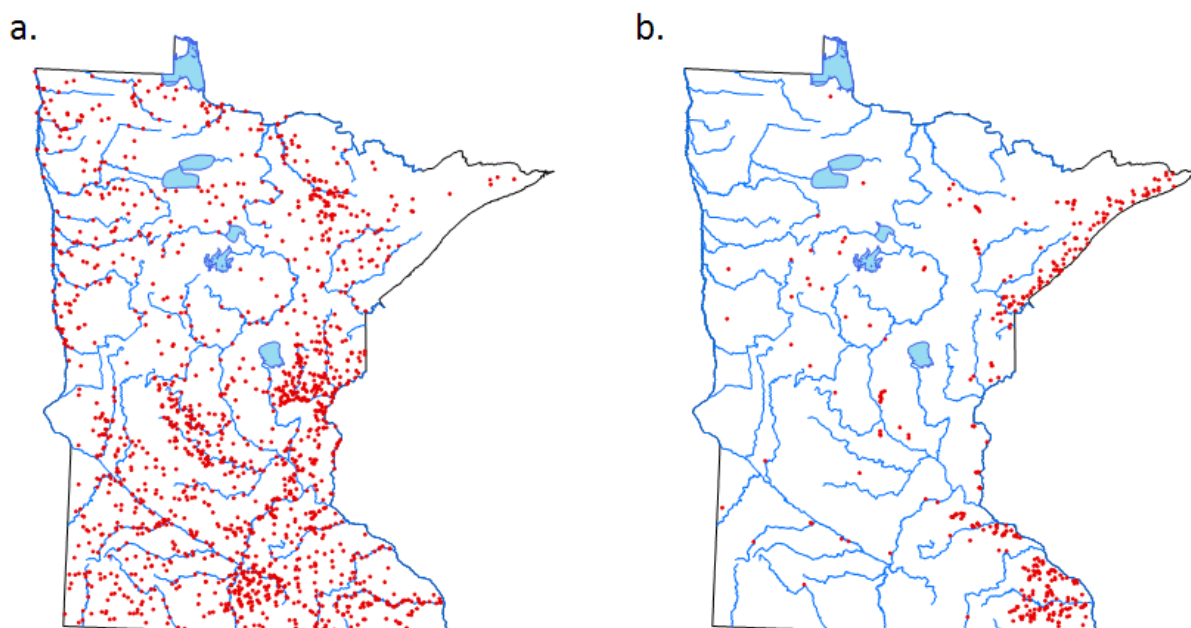


Figure 5. Maps of (a) warmwater and (b) coldwater stream monitoring sites used to develop F-IBI for the State of Minnesota. Large lakes and major rivers are also depicted.

### 3.7. Species characteristics

The IBI development process relies upon commonalities between fish species and combines them into groups related to their taxonomy, morphometry, behavior, habitat requirements, and life history traits. This type of trait-based approach groups species that experience their environment in a similar fashion, and emphasizes the functional structure of fish communities (Karr and Chu 1999). A variety of published and non-published sources were used to assign trophic, reproductive, habitat, tolerance, and life history traits to fish species known to inhabit Minnesota’s rivers and streams (Balon 1975, Pflieger 1975, Becker 1983, Lyons 1992, Barbour et al. 1999, Etnier and Starnes 1999, Goldstein and Meador 2004, Frimpong and Angermeier 2009). We also used a weighted-averaging process (Meador and Carlisle 2007) to calculate species-specific tolerance values for both individual stressors (e.g., nutrients, turbidity, dissolved oxygen, habitat characteristics) and HDS. These data were used to refine existing tolerance attributes that were derived from the literature.



## 3.8. Metric evaluation

For warmwater streams, 240 candidate metrics were calculated from fish community data, utilizing the species characteristics database described above. The coldwater IBI development effort included an additional twelve metrics for a total of 252 (Appendix B). Metrics were summarized in three ways (taxa richness, relative taxa abundance, and relative taxa richness), and were assigned to one of seven metric classes (taxa richness, composition, tolerance, life history, habitat, reproductive, trophic), intended to represent different components of biotic integrity. Abundance of two schooling species (*Notropis atherinoides*, *Dorosoma cepedianum*) was excluded from relative taxa abundance metrics due to the tendency of these species to naturally occur in large numbers such that proportions of other taxa may be heavily skewed, depending on whether a school is encountered while sampling. While other species are known to occur in schools (e.g., *Cyprinella spiloptera*, *Luxilus cornutus*), catches of *N. atherinoides* and *D. cepedianum* were often two to three orders of magnitude larger than other taxa in the same assemblage, a unique situation which justified their exclusion from relative taxa abundance metrics.

To develop each stream-class IBI we evaluated metrics using a series of tests, following the general procedure of Whittier et al.(2007). Metrics were tested, eliminated or selected, and scored separately within each of the nine stream classes using the same methodology throughout. The IBI development dataset was used for each test unless otherwise noted.

### 3.8.1 Range test and metric transformation

Metrics with poor range are unlikely to differentiate disturbed and non-disturbed sites because the response gradient is highly compressed. We eliminated richness metrics if the range was less than three species and eliminated any metric if more than 75% of the values were identical.

In cases where the distribution of metric values within a class was highly skewed, transformation was used to normalize the data (or reduce skew). Several transformations were considered, including: log<sub>10</sub>, natural log, square root, and arcsine square root. Metrics were not automatically rejected if a normal distribution could not be achieved. In general, we attempted to reduce absolute skew values to less than 1 through transformation. The metric scoring process (described below) also reduced skewness in most cases.

### 3.8.2. Natural gradient metric correction

The classification of sites into nine different stream classes minimized the influence of natural gradients on metric response. However, we also evaluated each metric against natural gradients within each class to further ensure that metric response was not obscured or amplified. To minimize the potentially covarying effect of human disturbance, natural gradient relationships were evaluated using the subset of least-disturbed sites within each class. We used simple linear regression to evaluate the relationship between metric values, watershed area, and stream gradient, examined plots of the data points, and calculated correlation coefficients for the relationship. For metrics where a significant ( $p \leq 0.05$ ) relationship existed and the correlation coefficient ( $R^2$ ) was greater than 0.3 we derived a natural-gradient corrected metric by calculating the residual based on the regression equation. The residual then replaced the original metric value in the IBI development process (Figure 6). Calibration and validation datasets were combined to test whether natural gradient correction was necessary.



### 3.8.3. Responsiveness Test

To test metric responsiveness to human disturbance, we used the non-parametric Mann-Whitney U test to evaluate the difference between metric values at least- and most-disturbed sites. The magnitude of the Mann-Whitney p-value was used to gauge responsiveness, essentially the ability of a metric to distinguish least-disturbed sites from most-disturbed sites. Spearman rank correlation between metric values and HDS was also used to evaluate metric responsiveness, primarily by ranking metrics with similar p-values according to their Spearman  $r_s$  value. Finally, box plots of metric values within each disturbance quartile were also used to visually assess metric responsiveness. Non-responsive metrics (i.e., those with non-significant  $U$ -statistics at the  $p=0.05$  level) were eliminated from the candidate metric pool. The validation dataset was used to confirm the responsiveness of metrics with significant Mann-Whitney p-values; if a metric's validation dataset produced a non-significant difference, it was eliminated. In a few cases, metrics at or near the responsiveness threshold were allowed to pass the test if a strong conceptual rationale existed for inclusion. IBI development and validation datasets were evaluated separately, and metrics were considered responsive if they passed this test for both datasets within a class.

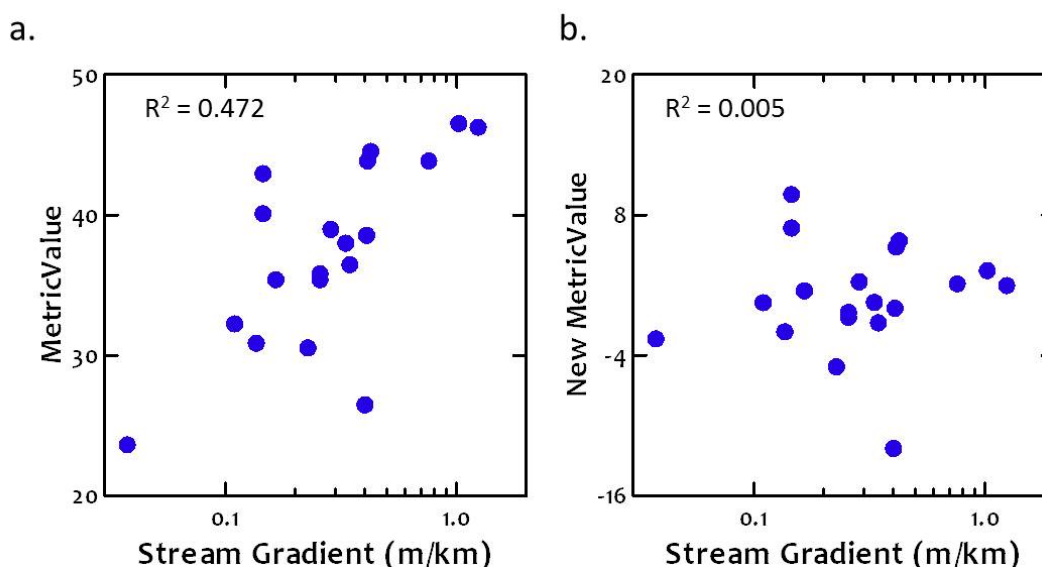


Figure 6. Example of metric value relationships with a natural gradient before and after correction. Metric value is Sensitive Taxa Percentage in the Northern Rivers F-IBI class. Raw metric values (a) demonstrate a positive relationship with stream gradient. Replacing metric values with the residual values from a simple linear regression (b) eliminates the natural gradient relationship.

### 3.8.4. Signal-to-Noise Test (S:N)

Precision of metric values can be evaluated by comparing variance among sites ("signal") to variance within sites ("noise") (Kaufmann et al. 1999). This statistic was calculated using the subset of sites that were sampled twice within the index period of the same year. This type of "repeat" sampling is a normal component of the MPCA's monitoring design; approximately 10% of monitoring sites are sampled twice each year, with an attempt made to distribute repeat sampling events evenly across the spatial extent and stream characteristics encompassed by a particular year's monitoring effort.

Low “signal-to-noise” ratios indicate low-precision metrics that are unable to distinguish well among sites (Kaufman et al. 1999, Whittier et al. 2007). While few well-established guidelines exist for evaluating S:N ratio, some researchers have suggested that signal-to-noise ratios greater than 3 characterize sufficiently precise data (Kaufmann et al. 1999). However, we used a conservative approach in evaluating metric precision, calculating S:N on a statewide basis rather than individually within each class. As a result, we utilized a slightly lower S:N threshold, where metrics with a ratio value less than 2 were eliminated from the candidate metric pool. In a few cases, metrics with S:N values slightly below 2 were allowed to “pass” this test if a strong conceptual basis existed for inclusion.

### 3.8.5. Metric redundancy

A correlation matrix of metric values was created to examine metric redundancy and avoid selecting IBI metrics that contained redundant information. We evaluated redundancy using the subset of least-disturbed sites within each class, to avoid rejecting metrics simply because their response to disturbance was similar. We also evaluated metric redundancy using all sites, regardless of disturbance level, but more emphasis was given to correlations in the least-disturbed dataset. In general, we considered metrics to be redundant when their Spearman correlation coefficients were greater than 0.7. However, “conceptual redundancy” was also considered in cases where the Spearman coefficient approached the threshold; metrics were sometimes included despite Spearman correlations greater than 0.7 if we considered them to represent distinct components of biological integrity, and sometimes rejected despite Spearman correlations less than 0.7 if we considered them to be conceptually redundant.

Within each class, metrics that passed the Range, Signal-to-Noise, and Responsiveness tests were ranked by their Responsiveness p-value (most responsive to least responsive). Metrics were selected for inclusion in the IBI in order of descending responsiveness, provided they were not redundant with more-responsive metrics. To obtain representation across the seven metric classes, a maximum of two non-redundant metrics from any single metric class was chosen until each class was represented by at least one metric. In some cases, it was not possible to select a metric from each metric class, due either to a lack of metrics passing earlier tests, or redundancy with highly-responsive metrics.

### 3.8.6. Range of metric scores

In cases where box plots and scatter plots indicated that a majority of sites within a class would receive the same metric score regardless of disturbance level, the metric was rejected. When metrics were eliminated by this test, we returned to the metric selection process described in the previous step and replaced it with the next most responsive metric.

### 3.8.7. Metric scoring

Each selected metric was scored on a continuous scale from 0 to 10 (with some exceptions, see below). Maximum and minimum values for each metric were defined as the 5<sup>th</sup> and 95<sup>th</sup>, or 10<sup>th</sup> and 90<sup>th</sup> percentile observed across all sites within each class. Southern Rivers, Southern Streams, Southern Headwaters, Southern Coldwater, and Northern Coldwater were scored using the 5<sup>th</sup>/95<sup>th</sup> threshold values. Northern Rivers, Northern Streams, and Northern Headwaters were scored using the 10<sup>th</sup>/90<sup>th</sup> threshold values. The two slightly different approaches resulted from an observation that few sites in the Northern Rivers, Northern Streams, and Northern Headwaters classes were achieving composite IBI scores near the theoretical minimum and maximum values. This may have occurred due to the generally high quality of sites in northern Minnesota, such that any given site was unlikely to achieve the maximum score for multiple metrics.

For positive metrics (those that decrease with disturbance), values less than the defined minimum were given a score of 0; those with values greater than the defined maximum were given a score of 10. Metric values between the minimum and maximum values were scored based on linear interpolation. Negative metrics (those that increase with disturbance) were scored in the same manner, with the minimum defined as the 95<sup>th</sup> or 90<sup>th</sup> percentile value and the maximum defined as the 5<sup>th</sup> or 10<sup>th</sup> percentile value. Metrics that passed all tests but still exhibited a skewed distribution of metric scores were scored discretely. These metrics typically received scores of 0, 5, or 10 depending on breakpoints in metric score distributions. Metric scores were summed within each class, and the resulting value re-scaled to a 0-100 range (multiplied by 10, divided by the number of metrics within each index).

Very low catch rates, either in terms of number of individuals or number of taxa, are generally indicators of severe degradation in permanent, warm- and coolwater Minnesota streams (Niemela and Feist 2000). In these special cases the presence of a few individuals may artificially inflate the IBI score and possibly mask a serious impairment. This is particularly concerning for proportional metrics (individual percentage and taxa percentage), where very low counts of “non-tolerant” individuals may result in extremely high metric scores for negative metrics. To address this issue, we implemented “Low End Scoring” criteria, under which individual percentage metrics in non-coldwater IBIs received a score of 0 when fewer than 25 individuals were captured, and taxa richness and taxa percentage received a score of 0 when fewer than 6 taxa were captured. Low End Scoring taxa richness and taxa percentage metric adjustments were applied to the Southern Rivers, Southern Streams, Northern Rivers and Northern Streams IBIs. Because fish assemblages of small, perennial headwaters may be relatively depauperate under natural conditions, the Low End Scoring threshold for taxa richness and taxa percentage metrics in Northern Headwaters, Southern Headwaters, and Low Gradient IBIs was reduced to fewer than 4 taxa. Low End Scoring criteria were not applied to Southern Coldwater and Northern Coldwater IBIs because these systems may exhibit extremely low taxa richness or number of individuals under natural, undisturbed conditions.

Each IBI was evaluated for overall responsiveness and correlation with natural gradients, using ANOVA and Pearson correlation. Sensitivity analysis was used to determine whether removal of individual metrics would dramatically improve overall responsiveness of the index or reduce correlation with natural gradients. If major improvement in these parameters was observed following temporary exclusion of one or more metrics, they were considered for permanent removal from the index.

## 4. Results

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Within each class, a set of robust metrics was selected for inclusion in a final, class-specific IBI. The number of metrics in any given IBI ranged from seven to twelve (Table 3), but most included at least nine metrics. Each IBI included a combination of metrics that increase with disturbance (negative metrics) and metrics that decrease with disturbance (positive metrics), though some IBIs were more heavily weighted towards one or the other. Trophic and Tolerance metrics were utilized most frequently, Life History and Habitat metrics least frequently. The IBIs generally included greater proportions of Individual Percentage and Taxa Percentage metrics and fewer Taxa Richness metrics. Taxa Richness metrics were relatively uncommon; four of the nine IBIs featured only a single richness metric and the Northern Rivers IBI lacked richness metrics completely. A “total taxa count” metric (i.e., overall taxa richness) was not included in any class-specific IBI. In contrast, taxa percentage and individual percentage metrics demonstrated widespread effectiveness in distinguishing least- from most-disturbed sites. Four different IBIs each included four taxa percentage metrics, and each IBI

included at least two. Individual percentage metrics were even more commonly-used, with each IBI including at least three and one IBI (Northern Rivers) included seven. A Catch-Per-Unit-Effort (CPUE) metric (number of individuals per meter, excluding Tolerant species) was included in both the Northern Headwaters and Low Gradient IBIs.

In most cases, a few effective metrics were excluded from the final IBI due to the earlier selection of more robust metrics from the same category, or quantitative/conceptual redundancy with more robust candidates. In addition, the “FishDELTpct” metric (see Appendix C for metric descriptions) was included in each IBI, and the “DomTwoPct” was included in the Southern Rivers, Southern Streams, Northern Rivers, and Northern Streams IBIs. These metrics failed to pass one or more tests, but were included due to their conceptual importance as indicators of severe environmental stress (Sanders et al.1999). The FishDELTpct metric was scored discretely based on the assumption that this metric is most responsive at the highly disturbed end of the spectrum. Two other metrics (Northern Rivers: ExoticPct, Southern Coldwater: HerbvPct) were scored discretely due to a highly skewed distribution of metric scores that could not be adequately corrected through transformation. Among all metrics included in the final class-specific IBIs, four required log transformation, and nine were adjusted for natural gradients (4 for watershed area, 5 for stream gradient).

Within each class, F-IBI scores differed between least- and most-disturbed sites (Table 4, Figure 7). Correlations between F-IBI scores and HDS were generally strong, while correlations between IBI scores and natural gradients (watershed area and stream gradient) were generally weak to moderately-strong (Table 5).

**Table 3. Summary of metric count, trait category, metric type, and response type for each Fish IBI.**

FishClass	Trait Category							Type				Response	
	number of metrics	Composition	Habitat	Life History	Reproductive	Tolerance	Trophic	CPUE	Individual Percent	Taxa Richness	Taxa Percent	Positive	Negative
Southern Rivers	12	2	1	1	2	2	4	6	2	4	4	8	
Southern Streams	9	2		1	1	3	2	4	1	4	2	7	
Southern Headwaters	7	1		1	1	2	2	3	1	3	1	6	
Northern Rivers	11	3	1		3	2	2	7		4	4	7	
Northern Streams	12	3			3	3	3	6	2	4	6	6	
Northern Headwaters	11	4	1	1	1	2	2	1	3	4	3	8	3
Low Gradient	10	3	2	1	1	2	1	1	3	3	3	6	4
South Coldwater	8	1	2	1		2	2	5	1	2	3	5	
North Coldwater	9	2	1	1	1	3	1	5	1	3	3	6	
Grand Total		21	8	7	13	21	19	2	42	15	30	37	43

Table 4. Analysis of variance results testing for difference in F-IBI scores between least- and most-disturbed sites within each Fish IBI class.

FishClass	F-Ratio	Error df	R <sup>2</sup>	p-Value
Southern Rivers	60.3	89	0.404	<0.001
Southern Streams	43.1	142	0.233	<0.001
Southern Headwaters	13.8	124	0.100	<0.001
Northern Rivers	75.2	53	0.587	<0.001
Northern Streams	92.1	118	0.438	<0.001
Northern Headwaters	180.8	148	0.550	<0.001
Low Gradient	106.7	84	0.560	<0.001
Southern Coldwater	43.6	92	0.321	<0.001
Northern Coldwater	76.9	136	0.361	<0.001

Table 5. Pearson correlation coefficients for F-IBI versus Human Disturbance Score (HDS), watershed area, and stream gradient within each Fish IBI class.

FishClass	HDS	watershed area	stream gradient
Southern Rivers	0.521	0.407	-0.075
Southern Streams	0.385	0.197	0.249
Southern Headwaters	0.238	0.140	0.142
Northern Rivers	0.731	0.346	0.089
Northern Streams	0.599	0.133	0.275
Northern Headwaters	0.649	0.252	-0.069
Low Gradient	0.665	0.150	0.074
Southern Coldwater	0.184	-0.164	0.344
Northern Coldwater	0.557	-0.038	0.561

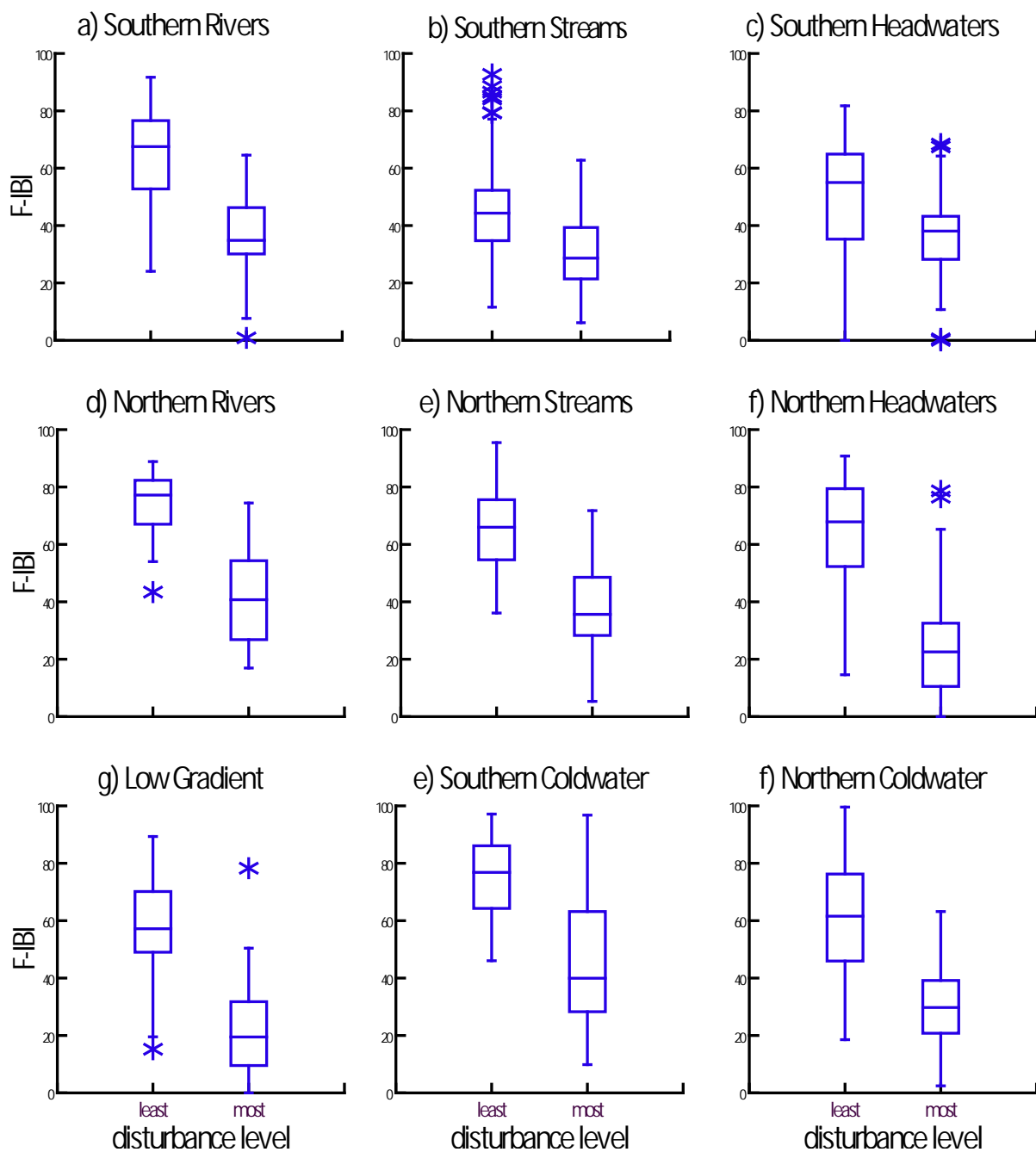


Figure 7. Boxplots of F-IBI scores among least- and most-disturbed sites within each fish class. Top edge, middle line, and bottom edge of boxes represent 75<sup>th</sup>, 50<sup>th</sup>, and 25<sup>th</sup> percentile values, respectively. Tails represent 1.5 times the interquartile range. Asterisks represent values between 1.5 and 3 times the interquartile range. All class-specific differences in F-IBI scores are significant ( $p < 0.001$ ).

## 4.1. Southern Rivers

**Table 6.** Metrics selected for the Southern Rivers F-IBI, listed in order of responsiveness. The p-values are from a one-way Mann-Whitney U test to distinguish between least- and most-disturbed sites. The signal-to-noise ratio (S:N) is the ratio of variance among sites to that within sites. Floor and ceiling values are 5<sup>th</sup> and 95<sup>th</sup> percentile metric values used to define minimum and maximum metric scores.

Metric Name	Metric Type	Metric Description	Category	Response	p-value	S:N	floor	ceiling
Insectivore-Tol_Pct	IndPct	Percent insectivorous individuals (excludes tolerant species)	trophic	positive	<0.001	4.01	12.01	82.00
SimpleLithophil <sup>1</sup>	Richness	Simple lithophilic taxa	reproductive	positive	<0.001	7.84	-6.71	2.59
GeneralistFeeder_Pct	IndPct	Percent generalist feeder individuals	trophic	negative	<0.001	4.42	5.64	64.72
VeryTolerant_TxPct	TXPct	Percent very tolerant taxa	tolerance	negative	<0.001	2.11	5.04	33.33
SerialSpawner_TxPct	TXPct	Percent serial spawner taxa	reproductive	negative	<0.001	2.20	14.40	38.04
Tolerant_Pct	IndPct	Percent tolerant individuals	tolerance	negative	<0.001	9.23	5.38	82.30
ShortLived_Pct	IndPct	Percent short-lived individuals	life history	negative	0.001	3.43	0.83	60.10
Sensitive_TxPct <sup>1</sup>	TXPct	Percent sensitive taxa	tolerance	positive	0.002	6.58	-23.59	15.82
Detritivore_TxPct	TXPct	Percent detritivorous taxa	trophic	negative	0.002	2.66	15.38	41.62
Piscivore	Richness	Piscivorous taxa	trophic	positive	0.011	5.22	1.00	7.90
DominanceTwoTaxa_Pct <sup>2</sup>	IndPct	Combined relative abundance of the two most abundant taxa	composition	negative			30.39	75.00
FishDELT_Pct <sup>3</sup>	IndPct	Percent of individuals with Deformities, Eroded fins, Lesions, Tumors	composition	negative				

<sup>1</sup> metric scoring adjusted for stream gradient

<sup>2</sup> metric included based on conceptual importance

<sup>3</sup> metric included based on conceptual importance, scored discretely

A total of 60 metrics failed either the Range or Signal-to-Noise Test in the Southern Rivers class. Twenty-four metrics showed significant relationships with either watershed area or stream gradient and were replaced by natural gradient-corrected metrics. The Responsiveness Test eliminated an additional 91 non-responsive metrics, leaving a total of 86 metrics that met all testing criteria. Twelve metrics spanning five metric categories were selected for inclusion in the final Southern Rivers F-IBI (Table 5). Two F-IBI metrics were included based on their conceptual importance, and two required adjustment for stream gradient. We observed a strong correlation between F-IBI and HDS, a moderate correlation between F-IBI and watershed area, and a weak correlation between F-IBI and stream gradient (Table 4). “Low End Scoring” criteria apply to this IBI, under which individual percentage metrics receive a score of 0 when fewer than 25 individuals are captured, and taxa richness and taxa percentage receive a score of 0 when fewer than 6 taxa are captured.

## 4.2. Southern Streams

Table 7. Metrics selected for the Southern Streams F-IBI, listed in order of responsiveness. The p-values are from a one-way Mann-Whitney U test to distinguish between least- and most-disturbed sites. The signal-to-noise ratio (S:N) is the ratio of variance among sites to that within sites. Floor and ceiling values are 5<sup>th</sup> and 95<sup>th</sup> percentile metric values used to define minimum and maximum metric scores.

Metric Name	Metric Type	Metric Description	Category	Response	p-value	S:N	floor	ceiling
BenthicInsectivore-Tol_TxPct	TXPct	Percent benthic insectivore taxa (excludes tolerant species)	trophic	positive	<0.001	3.64	0.00	40.00
Sensitive_TxPct	TXPct	Percent sensitive taxa	tolerance	positive	<0.001	6.58	0.00	45.11
Detritivore_TxPct	TXPct	Percent detritivorous taxa	trophic	negative	<0.001	2.66	14.13	46.38
ShortLived	Richness	Short-lived taxa	life history	negative	<0.001	3.06	1.00	7.00
Tolerant_TxPct	TXPct	Percent tolerant taxa	tolerance	negative	<0.001	5.55	27.99	84.81
MatureAge<2_Pct	IndPct	Percent early-maturing individuals	reproductive	negative	<0.001	2.74	29.68	97.68
Tolerant_Pct	IndPct	Percent tolerant individuals	tolerance	negative	0.060	9.23	27.93	75.00
DominanceTwoTaxa_Pct <sup>1</sup>	IndPct	Combined relative abundance of the two most abundant taxa	composition	negative			34.00	75.00
FishDELT_Pct <sup>2</sup>	IndPct	Percent of individuals with Deformities, Eroded fins, Lesions, Tumors	composition	negative				

<sup>1</sup> metric included based on conceptual importance

<sup>2</sup> metric included based on conceptual importance, scored discretely

A total of 76 metrics failed either the Range or Signal-to-Noise Test in the Southern Streams class. No metrics in this class required adjustment for natural gradients. The Responsiveness Test eliminated an additional 79 non-responsive metrics, leaving a total of 82 metrics that met all testing criteria. Nine metrics spanning five metric categories were selected for inclusion in the final Southern Streams IBI (Table 6). Two of these metrics were included based on their conceptual importance. The TolPct metric was included despite showing only moderately strong differences between least- and most-disturbed sites (Responsiveness p-value 0.06). The conceptual importance of the proportion of tolerant individuals, coupled with the high Signal-To-Noise ratio observed for this metric, justified its inclusion. We observed a moderate correlation between F-IBI and HDS, and weak correlations between F-IBI, watershed area, and stream gradient (Table 4). “Low End Scoring” criteria apply to this IBI, under which individual percentage metrics receive a score of 0 when fewer than 25 individuals are captured, and taxa richness and taxa percentage receive a score of 0 when fewer than 6 taxa are captured.



## 4.3. Southern Headwaters

Table 8. Metrics selected for the Southern Headwaters F-IBI, listed in order of responsiveness. The p-values are from a one-way Mann-Whitney U test to distinguish between least- and most-disturbed sites. The signal-to-noise ratio (S:N) is the ratio of variance among sites to that within sites. Floor and ceiling values are 5<sup>th</sup> and 95<sup>th</sup> percentile metric values used to define minimum and maximum metric scores.

Metric Name	Metric Type	Metric Description	Category	Response	p-value	S:N	floor	ceiling
Sensitive	Richness	Sensitive taxa	tolerance	positive	0.001	9.97	0.00	4.00
Detritivore_TxPct	TXPct	Percent detritivorous taxa	trophic	negative	0.002	2.66	0.00	50.00
GeneralistFeeder_TxPct	TXPct	Percent generalist feeder taxa	trophic	negative	0.010	3.79	31.92	76.53
SerialSpawner_Pct	IndPct	Percent serial spawner individuals	reproductive	negative	0.029	4.38	0.00	76.92
VeryTolerant_TxPct	TXPct	Percent very tolerant taxa	tolerance	negative	0.045	2.11	0.00	58.71
ShortLived_Pct	IndPct	Percent short-lived individuals	life history	negative	0.061	3.43	0.14	98.73
FishDELT_Pct <sup>1</sup>	IndPct	Percent of individuals with Deformities, Eroded fins, Lesions, Tumors	composition	negative				

<sup>1</sup> metric included based on conceptual importance, scored discretely

A total of 63 metrics failed either the Range or Signal-to-Noise Test in the Southern Headwaters class. No metrics in the Southern Headwaters class required adjustment for natural gradients. The Responsiveness Test eliminated an additional 126 non-responsive metrics, leaving a total of 48 metrics that met all testing criteria. Seven metrics spanning four metric categories were selected for inclusion in the final Southern Headwaters IBI (Table 7). One of these metrics was included based on its conceptual importance. Southern Headwaters F-IBI scores differed significantly ( $\alpha=0.05$ ) between least- and most-disturbed sites (Table 3, Figure 2). We observed weak correlations between F-IBI and HDS, watershed area, and stream gradient (Table 4). “Low End Scoring” criteria apply to this IBI, under which individual percentage metrics receive a score of 0 when fewer than 25 individuals are captured, and taxa richness and taxa percentage receive a score of 0 when fewer than 4 taxa are captured.

## 4.4. Northern Rivers

Table 9. Metrics selected for the Northern Rivers F-IBI, listed in order of responsiveness. The p-values are from a one-way Mann-Whitney U test to distinguish between least- and most-disturbed sites. The signal-to-noise ratio (S:N) is the ratio of variance among sites to that within sites. Floor and ceiling values are 5<sup>th</sup> and 95<sup>th</sup> percentile metric values used to define minimum and maximum metric scores.

Metric Name	Metric Type	Metric Description	Category	Response	p-value	S:N	floor	ceiling
Sensitive_TxPct <sup>1</sup>	TXPct	Percent sensitive taxa	tolerance	positive	<0.001	6.58	-16.39	7.04
Sensitive_Pct <sup>1</sup>	IndPct	Percent sensitive individuals	tolerance	positive	<0.001	3.94	-33.70	17.75
Detritivore_Pct	IndPct	Percent detritivorous individuals	trophic	negative	<0.001	5.40	0.39	46.93
VeryTolerant_TxPct	TXPct	Percent very tolerant taxa	tolerance	negative	<0.001	2.11	0.00	20.00
Exotic_Pct <sup>2</sup>	IndPct	Percent exotic individuals	composition	negative	0.001	0.71		
SerialSpawner_TxPct	TXPct	Percent serial spawner taxa	reproductive	negative	0.001	2.20	8.70	29.22
Insectivore-Tol_Pct	IndPct	Percent insectivorous individuals (excludes tolerant species)	trophic	positive	0.006	4.01	28.94	74.99
NonLithophilicNester_Pct	IndPct	Percent non-lithophilic nest-building individuals	reproductive	negative	0.012	2.13	8.74	46.14
SimpleLithophil_TxPct	TXPct	Percent simple lithophilic taxa	reproductive	positive	0.015	1.67	26.28	48.32
DominanceTwoTaxa_Pct <sup>3</sup>	IndPct	Combined relative abundance of the two most abundant taxa	composition	negative	0.077	1.83	34.86	75.00
FishDELT_Pct <sup>4</sup>	IndPct	Percent of individuals with Deformities, Eroded fins, Lesions, Tumors	composition	negative				

<sup>1</sup> metric scoring adjusted for stream gradient

<sup>2</sup> metric scored discretely

<sup>3</sup> metric included based on conceptual importance

<sup>4</sup> metric included based on conceptual importance, scored discretely

A total of 54 metrics failed either the Range or Signal-to-Noise Test in the Northern Rivers class. Eighteen metrics showed significant relationships with either watershed area or stream gradient and were replaced by natural gradient-corrected metrics. The Responsiveness test eliminated an additional 134 non-responsive metrics, leaving a total of 52 metrics that met all testing criteria. Eleven metrics spanning five metric categories were selected for inclusion in the final Northern Rivers IBI (Table 8). Two metrics required adjustment for stream gradient, and two metrics were included based on their conceptual importance. Northern Rivers F-IBI scores differed significantly ( $\alpha=0.05$ ) between least- and most-disturbed sites (Table 3, Figure 2). We observed a strong correlation between F-IBI and HDS, a moderate correlation between F-IBI and watershed area, and a weak correlation between F-IBI and stream gradient (Table 4). "Low End Scoring" criteria apply to this IBI, under which individual percentage metrics receive a score of 0 when fewer than 25 individuals are captured, and taxa richness and taxa percentage receive a score of 0 when fewer than 6 taxa are captured.

## 4.5. Northern Streams

Table 10. Metrics selected for the Northern Streams F-IBI, listed in order of responsiveness. The p-values are from a one-way Mann-Whitney U test to distinguish between least- and most-disturbed sites. The signal-to-noise ratio (S:N) is the ratio of variance among sites to that within sites. Floor and ceiling values are 5<sup>th</sup> and 95<sup>th</sup> percentile metric values used to define minimum and maximum metric scores.

Metric Name	Metric Type	Metric Description	Category	Response	p-value	S:N	floor	ceiling
Sensitive_TxPct	TXPct	Percent sensitive taxa	tolerance	positive	<0.001	6.58	5.69	44.00
Intolerant_Pct	IndPct	Percent intolerant individuals	tolerance	positive	<0.001	3.51	0.00	41.98
Insectivore-Tol_TxPct	TXPct	Percent insectivorous taxa (excludes tolerant species)	trophic	positive	<0.001	3.36	26.12	50.50
MatureAge>3-Tol_Pct	IndPct	Percent late-maturing individuals (excludes tolerant species)	reproductive	positive	<0.001	6.25	0.00	34.09
GeneralistFeeder	Richness	Generalist taxa	trophic	negative	<0.001	3.50	2.20	7.00
SerialSpawner_TxPct	TXPct	Percent serial spawner taxa	reproductive	negative	<0.001	2.20	6.25	33.33
Detritivore_Pct	IndPct	Percent detritivorous individuals	trophic	negative	<0.001	5.40	1.01	38.98
VeryTolerant	Richness	Very tolerant taxa	tolerance	negative	<0.001	4.77	1.00	5.00
DarterSculpinSucker_TxPct	TXPct	Percent darter, sculpin, and sucker taxa	composition	positive	0.003	3.22	6.42	27.78
SimpleLithophil_Pct	IndPct	Percent simple lithophilic individuals	reproductive	positive	0.011	3.57	3.11	67.34
DominanceTwoTaxa_Pct <sup>1</sup>	IndPct	Combined relative abundance of the two most abundant taxa	composition	negative			37.64	75.00
FishDELT_Pct <sup>2</sup>	IndPct	Percent of individuals with Deformities, Eroded fins, Lesions, Tumors	composition	negative				

<sup>1</sup> metric included based on conceptual importance

<sup>2</sup> metric included based on conceptual importance, scored discretely

A total of 70 metrics failed either the Range or Signal-to-Noise Test in the Northern Streams class. No metrics in this class required adjustment for natural gradients. The Responsiveness Test eliminated an additional 76 non-responsive metrics, leaving a total of 91 metrics that met all testing criteria. Twelve metrics spanning five metric categories were selected for inclusion in the final Northern Streams IBI (Table 9). Two of these metrics were included based on their conceptual importance. Northern Streams F-IBI scores differed significantly ( $\alpha=0.05$ ) between least- and most-disturbed sites (Table 3, Figure 2). We observed a strong correlation between F-IBI and HDS, a weak correlation between F-IBI and watershed area, and a moderate correlation between F-IBI and stream gradient (Table 4). “Low End Scoring” criteria apply to this IBI, under which individual percentage metrics receive a score of 0 when fewer than 25 individuals are captured, and taxa richness and taxa percentage receive a score of 0 when fewer than 6 taxa are captured.

## 4.6. Northern Headwaters

Table 11. Metrics selected for the Northern Headwaters F-IBI, listed in order of responsiveness. The p-values are from a one-way Mann-Whitney U test to distinguish between least- and most-disturbed sites. The signal-to-noise ratio (S:N) is the ratio of variance among sites to that within sites. Floor and ceiling values are 5<sup>th</sup> and 95<sup>th</sup> percentile metric values used to define minimum and maximum metric scores.

Metric Name	Metric Type	Metric Description	Category	Response	p-value	S:N	floor	ceiling
Sensitive	Richness	Sensitive taxa	tolerance	positive	<0.001	9.97	0.00	4.00
Minnow-Tol_Pct	IndPct	Percent cyprinid individuals (excludes tolerant species)	composition	positive	<0.001	2.50	0.00	51.48
Insectivore-Tol_TxPct	TXPct	Percent insectivorous taxa (excludes tolerant species)	trophic	positive	<0.001	3.36	0.00	42.87
NumPerMeter-Tol	CPUE	Number of fish per meter (excludes tolerant species)	composition	positive	<0.001	2.00	0.01	1.82
InsectivorousCyprinid_Pct	IndPct	Percent insectivorous cyprinid individuals	trophic	positive	<0.001	2.27	0.00	20.85
HeadwaterSpecialist-Tol	Richness	Headwater taxa (excludes tolerant taxa)	habitat	positive	<0.001	6.88	0.00	3.00
DarterSculpin	Richness	Darter and sculpin taxa	composition	positive	<0.001	3.57	0.00	2.00
SimpleLithophil	Richness	Simple lithophilic taxa	reproductive	positive	<0.001	7.84	0.00	4.28
Tolerant_TxPct	TXPct	Percent tolerant taxa	tolerance	negative	<0.001	5.55	33.33	80.00
Pioneer_TxPct	TXPct	Percent pioneer taxa	life history	negative	0.002	2.97	10.00	33.33
FishDELT_Pct <sup>1</sup>	IndPct	Percent of individuals with Deformities, Eroded fins, Lesions, Tumors	composition	negative				

<sup>1</sup> metric included based on conceptual importance, scored discretely

A total of 73 metrics failed either the Range or Signal-to-Noise Test in the Northern Headwaters class. No metrics in the Northern Headwaters class required adjustment for natural gradients. The Responsiveness Test eliminated an additional 75 metrics, leaving a total of 89 metrics that met all testing criteria. Eleven metrics spanning seven metric categories were selected for inclusion in the final Northern Headwaters IBI (Table 10). One metric was included based on its conceptual importance. Northern Headwaters F-IBI scores differed significantly ( $\alpha=0.05$ ) between least- and most-disturbed sites (Table 3, Figure 2). We observed a strong correlation between F-IBI and HDS, a moderate correlation between F-IBI and watershed area, and a weak correlation between F-IBI and stream gradient (Table 4). “Low End Scoring” criteria apply to this IBI, under which individual percentage metrics receive a score of 0 when fewer than 25 individuals are captured, and taxa richness and taxa percentage receive a score of 0 when fewer than 4 taxa are captured.

## 4.7. Low Gradient

Table 12. Metrics selected for the Low Gradient F-IBI, listed in order of responsiveness. The p-values are from a one-way Mann-Whitney U test to distinguish between least- and most-disturbed sites. The signal-to-noise ratio (S:N) is the ratio of variance among sites to that within sites. Floor and ceiling values are 5<sup>th</sup> and 95<sup>th</sup> percentile metric values used to define minimum and maximum metric scores.

Metric Name	Metric Type	Metric Description	Category	Response	p-value	S:N	floor	ceiling
Minnow-Tol_Pct	IndPct	Percent cyprinid individuals (excludes tolerant species)	composition	positive	<0.001	2.50	0.00	52.29
Wetland-Tol	Richness	Wetland taxa (excludes tolerant species)	habitat	positive	<0.001	2.03	0.00	4.10
Sensitive	Richness	Sensitive taxa	tolerance	positive	<0.001	9.97	0.00	4.00
NumPerMeter-Tol	CPUE	Number of fish per meter (excludes tolerant species)	composition	positive	<0.001	2.00	0.00	1.89
HeadwaterSpecialist-Tol_Pct	IndPct	Percent headwater individuals (excludes tolerant species)	habitat	positive	<0.001	4.96	0.00	34.77
SimpleLithophil	Richness	Simple lithophilic taxa	reproductive	positive	<0.001	7.84	0.00	4.00
Omnivore_TxPct	TXPct	Percent omnivorous taxa	trophic	negative	<0.001	3.27	0.00	40.00
Tolerant_TxPct	TXPct	Percent tolerant taxa	tolerance	negative	<0.001	5.55	33.33	85.80
Pioneer_TxPct	TXPct	Percent pioneer taxa	life history	negative	0.005	2.97	0.00	35.71
FishDELT_Pct <sup>1</sup>	IndPct	Percent of individuals with Deformities, Eroded fins, Lesions, Tumors	composition	negative				

<sup>1</sup> metric included based on conceptual importance, scored discretely

A total of 81 metrics failed either the Range or Signal-to-Noise test in the Low Gradient class. No metrics required adjustment for natural gradients. The Responsiveness Test eliminated an additional 85 metrics, leaving a total of 71 metrics that met all testing criteria. Ten metrics spanning six metric categories were selected for inclusion in the final Low Gradient IBI (Table 11). One metric was included based on its conceptual importance. Low Gradient F-IBI scores differed significantly ( $\alpha=0.05$ ) between least- and most-disturbed sites (Table 3, Figure 2). We observed a strong correlation between F-IBI and HDS, and weak correlations between F-IBI, watershed area, and stream gradient (Table 4). “Low End Scoring” criteria apply to this IBI, under which individual percentage metrics receive a score of 0 when fewer than 25 individuals are captured, and taxa richness and taxa percentage receive a score of 0 when fewer than 4 taxa are captured.

## 4.8. Southern Coldwater

Table 13. Metrics selected for the Southern Coldwater F-IBI, listed in order of responsiveness. The p-values are from a one-way Mann-Whitney U test to distinguish between least- and most-disturbed sites. The signal-to-noise ratio (S:N) is the ratio of variance among sites to that within sites. Floor and ceiling values are 5<sup>th</sup> and 95<sup>th</sup> percentile metric values used to define minimum and maximum metric scores.

Metric Name	Metric Type	Metric Description	Category	Response	p-value	S:N	floor	ceiling
ColdwaterNative_Pct <sup>1</sup>	IndPct	Percent native, coldwater individuals	habitat	positive	0.001	4.38	0.00	1.96
SensitiveColdwater_Pct <sup>2</sup>	IndPct	Percent sensitive individuals (specific to coldwater streams)	tolerance	positive	0.006	5.24	-76.14	17.59
DetritivoreMinor_TxPct <sup>2</sup>	TXPct	Percent detritivore (at least 5% of diet) taxa	trophic	negative	0.010	2.40	-14.35	28.09
TolerantColdwater <sup>2</sup>	Richness	Tolerant taxa (specific to coldwater streams)	tolerance	negative	0.011	3.80	-1.04	4.24
Pioneer_Pct	IndPct	Percent pioneer individuals	life history	negative	0.016	4.76	0.00	55.02
Herbivore_Pct <sup>3</sup>	IndPct	Percent herbivorous individuals	trophic	negative	0.018	1.91		
ColdwaterNative_TxPct <sup>2</sup>	TXPct	Percent native, coldwater taxa	habitat	positive	0.040	12.66	-32.45	28.48
FishDELT_Pct <sup>4</sup>	IndPct	Percent of individuals with Deformities, Eroded fins, Lesions, Tumors	composition	negative				

<sup>1</sup> metric value transformed ( $\log_{10} + 1$ )

<sup>2</sup> metric scoring adjusted for watershed area

<sup>3</sup> metric scored discretely

<sup>4</sup> metric included based on conceptual importance, scored discretely

Nine metrics failed the Range Test in the Southern Coldwater class. Of the remaining metrics, 118 showed a significant relationship with watershed area and required natural gradient correction before responsiveness testing. The Responsiveness Test eliminated an additional 173 non-responsive metrics, leaving a total of 70 metrics that met all testing criteria. Eight metrics spanning four metric categories were selected for inclusion in the final Southern Coldwater IBI (Table 12). Four metrics required adjustment for watershed area, and one metric was included based on its conceptual importance. Southern Coldwater F-IBI scores differed significantly ( $\alpha=0.05$ ) between least- and most-disturbed sites (Table 3, Figure 2). We observed weak correlations between F-IBI, HDS, and watershed area, and a moderate correlation between F-IBI and stream gradient (Table 4).

## 4.9. Northern Coldwater

Table 14. Metrics selected for the Northern Coldwater F-IBI, listed in order of responsiveness. The p-values are from a one-way Mann-Whitney U test to distinguish between least- and most-disturbed sites. The signal-to-noise ratio (S:N) is the ratio of variance among sites to that within sites. Floor and ceiling values are 5<sup>th</sup> and 95<sup>th</sup> percentile metric values used to define minimum and maximum metric scores.

Metric Name	Metric Type	Metric Description	Category	Response	p-value	S:N	floor	ceiling
Coldwater	Richness	Coldwater taxa	habitat	positive	<0.001	2.91	0.00	2.00
IntolerantColdwater_Pct	IndPct	Percent intolerant individuals (specific to coldwater streams)	tolerance	positive	<0.001	17.52	0.00	83.65
SensitiveColdwater_TxPct <sup>1</sup>	TXPct	Percent sensitive taxa (specific to coldwater streams)	tolerance	positive	<0.001	11.60	-27.66	25.90
TolerantColdwater_Pct <sup>2</sup>	IndPct	Percent tolerant individuals (specific to coldwater streams)	tolerance	negative	<0.001	11.45	0.00	1.49
NonLithophilicNester_Pct <sup>2</sup>	IndPct	Percent non-lithophilic nest-building individuals	reproductive	negative	<0.001	6.14	0.00	1.68
Omnivore_TxPct	TXPct	Percent omnivorous taxa	trophic	negative	<0.001	2.87	0.00	20.00
Pioneer_TxPct	TXPct	Percent pioneer taxa	life history	negative	<0.001	6.41	0.00	33.33
Perciformes_Pct <sup>2</sup>	IndPct	Percent of individuals belonging to Order Perciformes	composition	negative	0.002	3.78	0.00	1.52
FishDELT_Pct <sup>3</sup>	IndPct	Percent of individuals with Deformities, Eroded fins, Lesions, Tumors	composition	negative				

<sup>1</sup> metric scoring adjusted for stream gradient

<sup>2</sup> metric value transformed ( $\log_{10} + 1$ )

<sup>3</sup> metric included based on conceptual importance, scored discretely

Nine metrics failed the Range Test in the Northern Coldwater class. Of the remaining metrics, 62 showed a significant relationship with a natural gradient and required correction before responsiveness testing. The Responsiveness Test eliminated an additional 156 metrics, leaving a total of 93 metrics that met all testing criteria. Nine metrics spanning six metric categories were selected for inclusion in the final Northern Coldwater IBI (Table 13). One metric required adjustment for stream gradient, and one metric was included based on its conceptual importance. Northern Coldwater F-IBI scores differed significantly ( $\alpha=0.05$ ) between least- and most-disturbed sites (Table 3, Figure 2). We observed strong correlations between F-IBI, HDS, and stream gradient, and a weak correlation between F-IBI and watershed area (Table 4).

## 5. Discussion

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The class-specific indices described here represent the first comprehensive, statewide tool for assessing the biological integrity of riverine fish communities in the State of Minnesota. Our statewide approach encompassed the geographic extent and variety of lotic environments found across the state, including large rivers, moderate-sized streams, headwaters, low-gradient and coldwater streams. Some rare and/or transitional habitats (such as estuaries, impoundments, wetland flowages, and “Great Rivers”) fell beyond the scope of this project but future work may focus on development and application of fish community-based indicators for these systems.

Stream classification frameworks used to standardize IBIs have typically incorporated some aspect of regionalization (e.g., ecoregions, basins) along physical stream characteristics (e.g., water temperature, watershed area), under the assumption that the biological communities within each resulting class are relatively homogenous. This process of identifying discrete breakpoints across what are inherently continuous environmental gradients can be challenging, and requires that a balance be struck between precision and practical application of the tool. For example, a highly refined classification framework that identifies 100 different stream classes (and consequently, 100 different IBIs) would likely improve within-class precision of the IBI tool. However, the application of such a tool would be overly complex and burdensome for water managers and stakeholders alike. On the other hand, an overly simplified classification framework might be easily implemented, but provide an unacceptably low level of precision.

Our intent in this effort was to develop a framework that would work for most rivers and streams throughout the state but also offer precision at a management-relevant scale. The importance of recognizing issues of scale cannot be overemphasized when developing an indicator that will be used to detect often subtle changes in biological condition. IBIs used to detect broad patterns of change in biological condition across very large regions of the country, as was the objective of Whittier et al. (2007), might fail to detect more subtle changes in resource quality within a relatively undisturbed watershed in Northern Minnesota. Likewise, an IBI developed for low gradient headwater streams in Minnesota would not be an appropriate tool applied across broad regions of North America. In both cases the classification framework and metric selection process have been optimized to detect change at the most relevant scale to specific objectives and resource conditions.

Previous work related to biological indicators for Minnesota’s rivers and streams was primarily organized at the major basin scale (e.g., St. Croix Basin, Upper Mississippi Basin), though at least one ecoregion-specific IBI was developed (Niemela et al. 1999). Each of these IBIs typically identified a single set of metrics applicable to all streams, with unique scoring criteria identified for different stream types (typically differentiated by watershed area). Our approach differed in that we first identified a set of distinct stream types, and then evaluated metrics individually within each class. While we acknowledged the possibility of combining classes if the IBI development process revealed significant convergence between classes, we wanted to explore the possibility that certain metrics might be excellent indicators for one stream type, but not for others. This approach emphasized within-class metric precision, and likely improved the performance of each IBI, but added complexity to the resulting classification framework.

We evaluated several potential regional frameworks for use in IBI development. Existing regionalizations based on landscape features (e.g. ecoregions, ecological provinces) and large watersheds (e.g., HUC4) showed some utility in partitioning variability in stream fish community structure, but neither was ideal.



While ecoregions have a long history associated with biomonitoring applications in the United States, their use may have more to do with convenience than effectiveness (Hawkins et al. 2000). Minnesota's location at a transition zone between several distinct ecoregions, coupled with the commonplace occurrence of river networks crossing (and sometimes re-crossing) ecoregion boundaries may partially explain why existing regionalizations demonstrated weaker classification strength. Ecoregions also fail to account for certain landscape features relevant to fish community structure across the state. For example, within the St. Croix Basin, several species of fish are native to rivers and streams below Taylors Falls but are absent upstream; as a result, distinct differences exist between the fish assemblages above and below this barrier (Fago and Hatch 1993). Alternatively, frameworks based on major basins failed to adequately account for certain abrupt transitions between ecotypes, such as between the forested, higher-gradient headwaters of Red River tributaries and the low-gradient (former) prairie region surrounding the lower reaches of these same rivers.

We ultimately decided on a customized regional framework that (for the most part) utilizes watershed lines corresponding to post-glacial barriers to fish movement. For example, the importance of St. Anthony Falls as a fish migration barrier is well documented in the literature and is reflected in a much smaller number of native fish species above the falls ( $n=64$ ) compared to below ( $n=123$ ) (Eddy et al. 1963). We established a regional classification line at St. Anthony Falls which separates streams of the Upper Mississippi Basin from those of the Lower Mississippi, Minnesota, and Lower St. Croix basins. In a similar fashion, we established a regional line at Taylors Falls in the St. Croix Basin, also the location of a historic barrier to fish migration.

The importance of watershed area in structuring stream fish communities has been well-documented (Hugueny et al. 2010). Our framework partitions streams into three general size classes ("headwaters," "streams," and "rivers") based on watershed area – this approach is intuitive, given widespread understanding that the fish communities of large rivers differ greatly from those of small streams. However, the specific watershed area thresholds used to segregate each class required careful scrutiny of the distribution of different fish assemblages across a wide gradient of watershed area. We were able to identify watershed area thresholds that effectively partition natural variability in fish community structure, with the caveat that sites near a particular watershed area threshold value should be evaluated on a case-by-case basis to determine the most appropriate class. In a small number of cases, classification by watershed area was either not feasible or insufficient to completely account for its influence on metric values, requiring derivation of watershed area "corrected" metrics (e.g., *Percentage of sensitive coldwater individuals* metric in the Southern Coldwater IBI).

In a similar manner, stream gradient proved to be a useful variable in segregating the fish communities of headwater streams. In small watersheds, a unique "low gradient" fish assemblage was typically observed when stream gradient was less than 0.5 m/km. However, the method used to calculate stream gradient was somewhat imprecise. Essentially, the change in elevation between the two topographic lines that bracket a particular site was divided by the length of stream channel between them. Imprecision in the gradient value may result from errors in the location of topographic lines or landscape features that are not accurately depicted. In a similar manner to classification by watershed area, sites with gradient values at or near this threshold should be evaluated on a case-by-case basis to determine the most appropriate class. Secondary characteristics of sites with stream gradient  $>0.5$  m/km may be evaluated for application of the Low Gradient IBI, including features such as substrate composition, flow velocity, and the nature of in-channel and riparian vegetation. In a small number of cases, classification by stream gradient area was either not feasible or insufficient to completely account for its influence on metric values, requiring derivation of stream gradient "corrected" metrics (e.g., *Taxa richness of simple lithophilic spawners* metric in the Southern Rivers IBI). Ongoing work by the MPCA is exploring the use of

high-resolution digital topographic data (i.e., LIDAR) to estimate stream gradient, which may offer increased accuracy and precision for the purposes of IBI classification and scoring.

Our chosen method of correcting for natural gradient relationships was consistent with the method used by Whittier et al. (2007). This method regressed metric values from least-disturbed sites against natural gradients; where strong relationships existed, we replaced the original metric values with natural-gradient corrected metric values equal to the offset (plus or minus) from the regression. This method appeared to be effective in reducing the potential for covariance between disturbance and natural gradients to confound or obscure “true” metric relationships with disturbance. Corrected metric values demonstrated minimal correlation with natural gradients, and inspection of scatterplots indicated that the range of corrected metric values was not biased towards either end of the natural gradient. Alternative approaches to define and correct for natural gradient relationships might be explored in future IBI development efforts, including quantile regression and/or expression of corrected metric values as a percentile of the regression rather than a raw offset value.

Our initial decision to identify distinct stream classes and proceed through metric selection within each class likely aided us in developing effective IBIs for certain types of streams. In particular, low-gradient, wetland-influenced streams have presented bioassessment challenges in Minnesota and other states. These streams are common in Minnesota and are often dominated by fish species tolerant of natural conditions that, in higher-gradient systems, could be considered signals of degradation (e.g., low dissolved oxygen, dominance of fine-grained substrates, limited habitat complexity). Both taxa richness and number of individuals tends to be lower in these systems than in higher-gradient streams, and using the metrics and scoring criteria of most traditional IBIs, even the fish assemblages of Minnesota’s minimally-impacted low-gradient streams would probably score poorly. We were aware of these circumstances going into the stream classification and metric selection processes, and anticipated challenges in constructing an effective IBI for low gradient and/or wetland-influenced streams. However, a distinct “low gradient” stream type was identified by the classification analysis, and a relatively large number (n=71) of metrics passed all metric selection tests; nine highly responsive metrics were identified for inclusion in the Low Gradient IBI. We observed excellent separation in IBI scores between least- and most-disturbed sites, and the correlation between Low Gradient IBI and HDS was among the highest across all fish classes. While the Low Gradient IBI included some non-conventional metrics (e.g., *Taxa richness of wetland species, excluding tolerants*), it also included several that were used in other IBIs and could be considered “traditional” metrics (e.g., *Taxa richness of Sensitive species*, *Percentage of tolerant taxa*). It is possible that a broader metric selection approach, conducted independently of stream class, would have identified metrics applicable across a wide variety of streams. However, we feel that our class-specific approach was largely successful in encapsulating natural variability in stream fish communities, and likely will improve the accuracy of bioassessment in systems such as low gradient headwaters.

The classification of streams into either “warmwater” or “coldwater” systems may have obscured a substantial amount of natural thermal variability, but was necessitated by an absence of better alternatives. The thermal regime of rivers and streams is complex, spatially and temporally dynamic, and can be dramatically altered by anthropogenic impacts. While ambient thermal conditions can be used to classify the current thermal regime, it may be difficult to distinguish impacted coldwater systems from non-impacted cool- and warmwater systems. For example, disturbances such as water appropriation and the clearing of riparian vegetation may artificially warm streams. The Designated Trout Stream framework established and maintained by MnDNR is typically based on historical records of stream conditions and several years of thermal monitoring. While the MnDNR classifications may not precisely describe the thermal conditions of all streams and rivers, in general this framework effectively separates

coldwater streams from cool- and warmwater systems. We acknowledge that, in some cases, this classification may not adequately represent the natural thermal potential of a particular stream; these special cases may be identified and dealt with on an individual basis, and the most appropriate IBI determined following interagency review of available historic and contemporary data. Future work should explore whether a more accurate thermal classification system can be developed, but any such system will largely depend on the ability to isolate the natural thermal potential of streams from changes due to anthropogenic influence.

The approach outlined by Whittier et al. (2007) provided an objective methodological template for metric evaluation. Using a series of standardized metric tests, we developed sensitive, robust, community-based indices that provide reliable information about biological integrity. This method differed from a traditional, often-utilized approach, which is to essentially employ the original Karr (1981) IBI as a template and substitute individual metrics when deemed appropriate. While Minnesota lies in relatively close proximity to the geographic region where the original IBI was developed, we realized that some “unconventional” metrics might show potential as biological indicators, due to unique aspects of the state’s ichthyofauna, river networks, and lotic habitats.

Our approach maintained the conceptual foundation of the IBI – a trait-based, multi-metric index demonstrably sensitive to anthropogenic disturbance – but assumed little regarding the *a priori* utility of specific metrics and considered a wider variety of candidates. However, while the metric selection tests were designed with objective criteria for removing candidates from the pool, those with test values slightly over the threshold for a particular test were sometimes allowed to “pass” if a sound conceptual basis for doing so could be identified. While few of these “borderline” metrics made it into the final indices, this interplay between a conceptual and quantitative approach strengthened our understanding of how fish communities respond to anthropogenic disturbance and ensured the resulting indices were well-balanced and representative of biological integrity.

In general, this approach worked well – most of the fish classes spanned a suitable range of disturbance, and we were able to identify a number of robust, responsive, non-redundant metrics within each class. Highly significant differences in IBI score were observed between least- and most-disturbed sites, and correlations with HDS were generally strong. While certain metrics included in the final IBIs might be considered relatively novel (e.g., *Percentage of serial spawning individuals*) and other “traditional” metrics were not included (e.g., *Total taxa richness*), a legitimate conceptual rationale could be identified for each metric that was included. In many cases, “traditional” metrics were excluded not because they failed a particular test, but instead because they were demonstrably less responsive than and/or redundant with other, more responsive metrics. It is also conceivable that the prevalence of non-traditional metrics reflects a shift in the dominant ecological stressors in streams and rivers since the initial development of the IBI concept in the late 1970s. For example, widespread improvements have been made in the areas of wastewater treatment and reductions of toxic effluents. At the same time, stressors related to hydrologic alteration, geomorphic destabilization, and habitat modification have possibly increased in relative importance. While any IBI should be responsive to a wide variety of stressors, the most important stressors affecting aquatic systems may change over time, and these changes will be manifested in terms of biological community response.

We used a composite human disturbance gradient (HDS) to select least- and most-disturbed sites and evaluate metric responsiveness. Our disturbance gradient included only variables that were unaffected by natural factors, excluding others where ambient conditions reflect both natural and anthropogenic contributions (e.g., nutrient levels, sediment characteristics). By focusing on variables unaffected by natural variability, this approach offers a greater degree of confidence that the observed metric and index responses are truly attributable to human influence rather than natural variability.

Other anthropogenic disturbances proved too difficult to quantify for inclusion in the HDS. For example, hydrologic alteration through water appropriation or diversion often varies from year to year, and manifests both chronic and acute effects on stream biota. This type of anthropogenic disturbance is also difficult to quantify, since even minimally-impacted hydrologic regimes are inherently dynamic. Furthermore, the data necessary to accurately estimate degree of hydrologic alteration (e.g., reference hydrographs, estimates of streamflow reduction or increase) are notably lacking for most of Minnesota's riverine habitats. As a result, the degree to which a stream's hydrologic regime has been altered was impossible to explicitly incorporate into the HDS. Disruptions to stream network connectivity (e.g., dams, perched culverts) are also difficult to quantify in an accurate manner at the scale required for this analysis, and were likewise not included. However, some HDS metrics, such as those related to road density and impervious surface, may provide surrogate representation for these types of stressors. While our HDS approach likely does not quantify all relevant anthropogenic disturbances, we feel confident that it provides a reasonable estimate of human-induced stress across the state.

The Southern Headwaters class lacked a large number of responsive metrics, despite featuring a relatively broad range of disturbance (HDS interquartile range = 20.7). Only 48 metrics passed all tests in the Southern Headwaters class, with more than half of the candidates (126 of 240) failing the Responsiveness Test. Of the metrics that passed all tests and were identified as candidates for inclusion in the final Southern Headwaters IBI, few demonstrated the highly significant Responsiveness p-values ( $<0.01$ ) that were common among metrics in other classes. While the composite Southern Headwaters IBI scores were significantly different between least- and most-disturbed sites, the F-Ratio for this comparison was the lowest across all fish classes (Table 3) and the correlation between IBI score and HDS was relatively weak. The Southern Headwaters IBI was also heavily weighted towards "negative" metrics, with six of seven metrics increasing with human disturbance.

Streams in the Southern Headwaters class are relatively small (watershed area  $<30$  square miles), and may be disproportionately impacted by poorly-quantified anthropogenic disturbances (e.g., hydrologic alteration, loss of network connectivity). At the same time, the effects of human disturbance on these streams may be partially mitigated by natural features providing resilience (e.g., localized groundwater inputs, small-scale habitat features). Some of these factors may be more relevant to the biological communities of small streams than to larger systems. For example, the fish communities of small headwater streams may be dependent on uninterrupted stream network connectivity due to the need for downstream refugia during periods of natural stress (e.g., periodic drying, winter freezing). A disturbance gradient that better accounts for some of these factors could possibly reveal obscured stress-response relationships for Southern Headwaters.

By any reasonable measure, few examples of minimally-impacted Southern Headwater stream communities exist in Minnesota. While the interquartile range of HDS was relatively broad, the median HDS score for Southern Headwaters (37) was the lowest of any F-IBI class. The generally degraded condition of streams in this class may partially explain why few positive metrics were selected, as well as relatively weak correlation between HDS and F-IBI score. Future approaches to metric selection in this class might consider either including high-quality sites from other classes (e.g., Northern Headwaters, Southern Streams), or "hindcasting" hypothetical minimally-impacted communities (Kilgour and Stanfield 2006) for comparison.

The Southern Coldwater class also demonstrated a relatively weak correspondence between the disturbance gradient and IBI. Due to consistent patterns of land use within this class, upper- and lower-quartile HDS values were separated by a relatively narrow range of scores (HDS interquartile range = 11.9). While reach-scale variables are included in the HDS, it was necessary to utilize habitat scores and site photographs as secondary criteria for sorting sites into the least- and most-disturbed categories. As

a result, some sites with “good” HDS scores were excluded from the least-disturbed dataset, and vice-versa. While this method diverged slightly from the quartile method used for all other fish classes, and resulted in a lower correlation between IBI score and HDS, it likely provided a better overall assessment of anthropogenic disturbance for the Southern Coldwater class. Localized groundwater features (e.g., springs, seeps) may also contribute resilience at some sites, and could possibly confound the influence of subtle differences in human disturbance within this class.

The influence of fish management also may have contributed to the relatively weak correspondence between HDS and IBI in the Southern Coldwater class. Between 2002 and 2009, MnDNR was responsible for the stocking of nearly 6 million trout in these streams (MnDNR, unpublished data). Trout are generally considered to be a positive indicator in aquatic systems, as they are sensitive to anthropogenic disturbances such as warming, siltation, and habitat degradation, though some researchers have indicated that stocking of non-native trout may lower biological integrity (Mundahl and Simon 1999). While significant natural reproduction of trout does occur in many Southern Coldwater streams, and a large proportion of these streams are not regularly stocked, it is difficult to account for the influence of stocking and other management practices (e.g., addition of in-stream habitat structures, harvest regulations) in the IBI development process. For example, it is possible that the large numbers of trout in some “disturbed” streams may be the result of stocking, while lower numbers of trout in non-stocked “least-disturbed” streams may be due to a management emphasis on natural reproduction in higher-quality habitats. In either case, it was impossible to distinguish “stocked” from “wild” trout in our datasets, which has been an important component of other coldwater IBIs (Lyons et al. 1996). Although the Southern Coldwater IBI was demonstrably effective in separating least- from most-disturbed sites, corresponded well with an independent assessment of biological condition (Gerritsen et al. 2012), and has proved to be an effective tool for waterbody assessment in Minnesota, index scores may be confounded in the cases of heavily-stocked and/or managed trout streams. Management practices may also influence fish community structure and function in other stream classes, though the exact nature of the resulting effects (if any) on IBI is largely unknown. Future efforts to identify and account for these effects should be pursued.

The MPCA has committed extensive time and effort towards the development of biological indicators and a framework for their use in its surface water monitoring and assessment process (Anderson et al. 2012). The stream classification system and fish-based Indices of Biological Integrity described in this document have been utilized (in concert with other indicators) since 2010 to annually assess the condition of aquatic life in Minnesota’s rivers and streams. Continuing work may attempt to expand the IBI concept to waterbodies not covered here, including lakes, reservoirs, and large rivers. Diagnostic applications of the IBI and its component metrics will also be explored. Large-scale changes in environmental condition across Minnesota, or advances in the science of biological indicators may require periodic evaluation of these indices to ensure their relevancy as assessment tools.

## 6. Literature Cited

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## 7. Appendices

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### Appendix A. Classification criteria for Minnesota river and stream Fish IBI

- 1a. Northern.....5
- 1b. Southern.....2

#### Southern

- 2a. coldwater.....Southern Coldwater
- 2b. warmwater.....3
  - 3a. Drainage area >300 sq mi.....Southern Rivers
  - 3b. Drainage area <300 sq mi.....4
    - 4a. Drainage area >30 sq mi..... Southern Streams
    - 4b. Drainage area <30 sq mi.....5
      - 5a. Gradient >0.50 m/km.....Southern Headwaters
      - 5b. Gradient <0.50 m/km.....Low-Gradient

#### Northern

- 5a. coldwater.....Northern Coldwater
- 5b. warmwater.....6
  - 6a. Basin = Red.....7
  - 6b. Basin = other.....8
    - 7a. Drainage area >350 sq mi.....Northern Rivers
    - 7b. Drainage area <350 sq mi.....9
      - 8a. Drainage area >500 sq mi.....Northern Rivers
      - 8b. Drainage area <500 sq mi.....9
        - 9a. Drainage area >50.....Northern Streams
        - 9b. Drainage area <50.....10
          - 10a. Gradient >0.50 m/km.....Northern Headwaters
          - 10b. Gradient <0.50 m/km.....Low-Gradient



**Appendix B. Metrics evaluated for F-IBI. (+) - metric satisfied all testing criteria. (IBI metric) - metric was included in F-IBI. (NT) - metric was not tested. See Appendix C for metric descriptions, Appendix D for trait assignments**

Metric Name	Southern Rivers	Southern Streams	Southern Headwaters	Northern Rivers	Northern Streams	Northern Headwaters	Low Gradient	Southern Coldwater	Northern Coldwater
BenthicFeeder			+			+			
BenthicFeeder_Pct									
BenthicFeeder_TxPct									
BenthicInsectivore	+					+			
BenthicInsectivore_Pct						+			
BenthicInsectivore_TxPct		+			+	+			
BenthicInsectivore-Tol	+	+			+	+	+		
BenthicInsectivore-Tol_Pct	+				+	+	+		
BenthicInsectivore-Tol_TxPct		IBI metric			+	+			
BenthicMinnowDarter	+					+			
BenthicMinnowDarter_Pct						+			
BenthicMinnowDarter_TxPct		+				+			
Carnivore							+		
Carnivore_Pct	+								+
Carnivore_TxPct									+
Centrarchid									
Centrarchid_Pct									
Centrarchid_TxPct		+							
Centrarchid-Tol									
Centrarchid-Tol_Pct	+								
Centrarchid-Tol_TxPct									
Coldwater								+	IBI metric
Coldwater_Pct								+	+
Coldwater_TxPct								+	+
ColdwaterCoolwater					+	+	+		
ColdwaterCoolwater_Pct								+	+
ColdwaterCoolwater_TxPct					+	+		+	+
ColdwaterCoolwaterNative					+	+	+		
ColdwaterCoolwaterNative_Pct		+							+
ColdwaterCoolwaterNative_TxPct		+			+	+			

## Appendix B (continued)

Metric Name	Southern Rivers	Southern Streams	Southern Headwaters	Northern Rivers	Northern Streams	Northern Headwaters	Low Gradient	Southern Coldwater	Northern Coldwater
ColdwaterNative								+	+
ColdwaterNative_Pct								IBI metric	+
ColdwaterNative_TxPct								IBI metric	+
ComplexLithophil						+	+		
ComplexLithophil_Pct					+		+		+
ComplexLithophil_TxPct									+
Coolwater						+	+		
Coolwater_Pct		+							
Coolwater_TxPct		+							
CoolwaterNative						+	+		
CoolwaterNative_Pct		+							
CoolwaterNative_TxPct		+							
CountofTaxa			+			+	+		
Darter		+				+			+
Darter_Pct									+
Darter_TxPct									+
DarterSculpin		+			+		IBI metric		
DarterSculpin_Pct									
DarterSculpin_TxPct									
DarterSculpinNoturus		+			+	+			
DarterSculpinNoturus_Pct									
DarterSculpinNoturus_TxPct									
DarterSculpinSucker		+			+	+	+		+
DarterSculpinSucker_Pct									+
DarterSculpinSucker_TxPct		+			IBI metric	+			
Detritivore		+	+	+	+			+	+
Detritivore_Pct	+			IBI metric	IBI metric				+
Detritivore_TxPct	IBI metric	IBI metric	IBI metric	+	+	+	+		+
DetritivoreMinor	+	+	+	+	+			+	
DetritivoreMinor_TxPct	+	+	+	+	+			IBI metric	+
DetritivoreMinorI_Pct	+			+	+				
DetritivorePlanktivore		+	+	+	+			+	
DetritivorePlanktivore_Pct	+			+	+				

## Appendix B (continued)

Metric Name	Southern Rivers	Southern Streams	Southern Headwaters	Northern Rivers	Northern Streams	Northern Headwaters	Low Gradient	Southern Coldwater	Northern Coldwater
DetritivorePlanktivore_TxPct		+	+	+	+				
DominanceOneTaxa_Pct									
DominanceThreeTaxa_Pct			+						
DominanceTwoTaxa_Pct	IBI metric	IBI metric	+	IBI metric	IBI metric				
Exotic									
Exotic_Pct				IBI metric				+	
Exotic_TxPct				+					
FilterFeeder									
FilterFeeder_Pct									
FilterFeeder_TxPct				+					
FishDELT_Pct	IBI metric	IBI metric	IBI metric	IBI metric	IBI metric	IBI metric	IBI metric	IBI metric	IBI metric
GeneralistFeeder	+		+	+	IBI metric				
GeneralistFeeder_Pct	IBI metric				+			+	
GeneralistFeeder_TxPct	+		IBI metric	+	+				
GeneralistFeederFrim					+	+		+	
GeneralistFeederFrim_Pct		+			+	+			+
GeneralistFeederFrim_TxPct								+	
HeadwaterSpecialist						+	+		
HeadwaterSpecialist_Pct									
HeadwaterSpecialist_TxPct									
HeadwaterSpecialist-Tol					+	IBI metric	+		
HeadwaterSpecialist-Tol_Pct					+	+	IBI metric		
HeadwaterSpecialist-Tol_TxPct					+	+	+		
Herbivore	+					+		+	+
Herbivore_Pct	+	+				+	+	IBI metric	
Herbivore_TxPct	+	+						+	+
HerbivorousNWQ						+	+	+	
HerbivorousNWQ_Pct									
HerbivorousNWQ_TxPct									
Insectivore	+					+	+		
Insectivore_Pct					+				+
Insectivore_TxPct	+								
Insectivore-Tol	+	+			+	+	+		

## Appendix B (continued)

Metric Name	Southern Rivers	Southern Streams	Southern Headwaters	Northern Rivers	Northern Streams	Northern Headwaters	Low Gradient	Southern Coldwater	Northern Coldwater
Insectivore-Tol_Pct	IBI metric			IBI metric			+	+	+
Insectivore-Tol_TxPct	+	+			IBI metric	IBI metric	+		
InsectivorousCyprinid						+	+		
InsectivorousCyprinid_Pct			+			IBI metric	+		
InsectivorousCyprinid_TxPct					+	+	+		
Intolerant	+	+		+	+	+	+	+	+
Intolerant_Pct	+	+			IBI metric	+	+	+	+
Intolerant_TxPct	+	+		+	+	+	+	+	+
IntolerantColdwater	NT	NT	NT	NT	NT	NT	NT	+	+
IntolerantColdwater_Pct	NT	NT	NT	NT	NT	NT	NT	+	IBI metric
IntolerantColdwater_TxPct	NT	NT	NT	NT	NT	NT	NT	+	+
InvertivoreNWO	+					+	+		
InvertivoreNWO_Pct									
InvertivoreNWO_TxPct									
LargeRiver	+								
LargeRiver_Pct	+								
LargeRiver_TxPct									
Lithophil	+					+	+		
Lithophil_Pct		+				+	+		+
Lithophil_TxPct		+		+		+	+		+
LongLived	+								
LongLived_Pct		+							+
LongLived_TxPct									+
MatureAge<1									
MatureAge<1_Pct	+	+				+		+	
MatureAge<1_TxPct									+
MatureAge<2			+		+	+	+		
MatureAge<2_Pct	+	IBI metric			+			+	
MatureAge<2_TxPct	+							+	+
MatureAge<2Vtol	+	+	+	+	+			+	+
MatureAge<2Vtol_Pct	+	+	+	+	+	+		+	+
MatureAge<2Vtol_TxPct			+	+				+	+
MatureAge>3	+				+	+	+		+

## Appendix B (continued)

Metric Name	Southern Rivers	Southern Streams	Southern Headwaters	Northern Rivers	Northern Streams	Northern Headwaters	Low Gradient	Southern Coldwater	Northern Coldwater
MatureAge>3_Pct	+	+			+			+	+
MatureAge>3_TxPct	+	+			+		+	+	+
MatureAge>3-Tol	+				+	+	+	+	+
MatureAge>3-Tol_Pct		+			IBI metric		+	+	
MatureAge>3-Tol_TxPct	+	+			+		+	+	
MatureAge>4	+								
MatureAge>4_Pct	+								
MatureAge>4_TxPct									
MeagerSpawner						+	+		
MeagerSpawner_Pct									+
MeagerSpawner_TxPct									
Migratory						+	+		
Migratory_Pct		+				+	+		+
Migratory_TxPct									+
Minnow		+	+		+	+	+		
Minnow_Pct	+	+	+			+			
Minnow_TxPct	+	+	+	+	+				
Minnow-Tol					+	+	+		
Minnow-Tol_Pct					+	IBI metric		IBI metric	
Minnow-Tol_TxPct					+	+	+		
Native			+			+	+		
Native_Pct				+				+	
Native_TxPct				+					
NonBenthicGeneralist	+	+	+	+	+				
NonBenthicGeneralist_Pct	+	+	+		+			+	
NonBenthicGeneralist_TxPct	+	+	+	+	+			+	
NonBenthicGeneralistTol	+	+	+	+	+				
NonBenthicGeneralistTol_Pct	+	+	+	+	+		+	+	
NonBenthicGeneralistTol_TxPct	+	+	+	+	+	+	+		
NonLithophilicNester			+		+				
NonLithophilicNester_Pct		+		IBI metric		+	+	+	IBI metric
NonLithophilicNester_TxPct	+			+	+	+	+		+
NumPerMeter									

## Appendix B (continued)

Metric Name	Southern Rivers	Southern Streams	Southern Headwaters	Northern Rivers	Northern Streams	Northern Headwaters	Low Gradient	Southern Coldwater	Northern Coldwater
NumPerMeter-Tol						IBI metric	IBI metric	+	
NumPerMin			+						
NumPerMin-Tol						+	+		
Ominivore	+	+	+	+	+			+	+
Ominivore_Pct	+			+	+		+		+
Ominivore_TxPct				+	+	+	IBI metric		IBI metric
OmnivorousCyprinid							+	+	
OmnivorousCyprinid_Pct		+		+	+	+	+	+	
OmnivorousCyprinid_TxPct		+		+	+	+	+	+	
Parasitic									
Parasitic_Pct									
Parasitic_TxPct									
Perciformes									+
Perciformes_Pct	+								IBI metric
Perciformes_TxPct		+							
Perciformes-Tol									+
Perciformes-Tol_Pct	+								+
Perciformes-Tol_TxPct				+					
Pioneer	+		+	+	+				+
Pioneer_Pct	+	+		+	+			IBI metric	+
Pioneer_TxPct	+			+	+	IBI metric	IBI metric		IBI metric
Piscivore	IBI metric								
Piscivore_Pct								+	+
Piscivore_TxPct									+
Planktivore	+		+						
Planktivore_Pct		+							
Planktivore_TxPct	+	+							
ProlificSpawner									
ProlificSpawner_Pct									+
ProlificSpawner_TxPct									
Rhinichthys									
Rhinichthys_Pct			+			+			
Rhinichthys_TxPct									

## Appendix B (continued)

Metric Name	Southern Rivers	Southern Streams	Southern Headwaters	Northern Rivers	Northern Streams	Northern Headwaters	Low Gradient	Southern Coldwater	Northern Coldwater
RiffleSpecialist						+			
RiffleSpecialist_Pct		+					+		
RiffleSpecialist_TxPct	+					+	+		
RoundBodiedSucker									
RoundBodiedSucker_Pct		+							
RoundBodiedSucker_TxPct									
Salmonid									+
Salmonid_Pct								+	+
Salmonid_TxPct								+	+
Sensitive		+	IBI metric	+	+	IBI metric	IBI metric		
Sensitive_Pct	+	+	+	IBI metric	+	+	+	+	+
Sensitive_TxPct	IBI metric	IBI metric	+	IBI metric	IBI metric	+	+	+	+
SensitiveColdwater	NT	NT	NT	NT	NT	NT	NT	+	+
SensitiveColdwater_Pct	NT	NT	NT	NT	NT	NT	NT	IBI metric	+
SensitiveColdwater_TxPct	NT	NT	NT	NT	NT	NT	NT	+	IBI metric
SerialSpawner			+	+	+				
SerialSpawner_Pct	+	+	IBI metric		+				
SerialSpawner_TxPct	IBI metric	+	+	IBI metric	IBI metric				
ShortLived		IBI metric	+		+			+	+
ShortLived_Pct	IBI metric	+	IBI metric		+			+	
ShortLived_TxPct	+	+			+				+
SimpleLithophil	IBI metric					IBI metric	IBI metric		
SimpleLithophil_Pct	+	+			IBI metric	+	+		
SimpleLithophil_TxPct				IBI metric					
SimpleLithophilFrim	+				+	+	+		
SimpleLithophilFrim_Pct	+	+				+	+		
SimpleLithophilFrim_TxPct									
StickebackMudminnow									
StickebackMudminnow_Pct		+							+
StickebackMudminnow_TxPct									+
SubterminalMouth	+	+	+		+				
SubterminalMouth_Pct	+	+	+						
SubterminalMouth_TxPct	+	+	+						



## Appendix B (continued)

Metric Name	Southern Rivers	Southern Streams	Southern Headwaters	Northern Rivers	Northern Streams	Northern Headwaters	Low Gradient	Southern Coldwater	Northern Coldwater
Sucker						+			+
Sucker_Pct		+							+
Sucker_TxPct									+
SuckerCatfish									
SuckerCatfish_Pct									
SuckerCatfish_TxPct									
Tolerant	+	+	+	+	+			+	+
Tolerant_Pct	IBI metric	IBI metric		+	+	+	+	+	+
Tolerant_TxPct	+	IBI metric	+	+	+	IBI metric	IBI metric	+	+
TolerantColdwater	NT	NT	NT	NT	NT	NT	NT	IBI metric	+
TolerantColdwater_Pct	NT	NT	NT	NT	NT	NT	NT	+	IBI metric
TolerantColdwater_TxPct	NT	NT	NT	NT	NT	NT	NT	+	+
VeryTolerant	+	+	+	+	IBI metric	+			+
VeryTolerant_Pct	+	+		+	+	+		+	+
VeryTolerant_TxPct	IBI metric	+	IBI metric	IBI metric	+	+	+		+
VeryTolerantColdwater	NT	NT	NT	NT	NT	NT	NT		+
VeryTolerantColdwater_Pct	NT	NT	NT	NT	NT	NT	NT	+	+
VeryTolerantColdwater_TxPct	NT	NT	NT	NT	NT	NT	NT	+	+
Wetland								+	+
Wetland_Pct		+				+		+	+
Wetland_TxPct								+	+
Wetland-Tol	+				+	+	IBI metric		
Wetland-Tol_Pct	+				+	+			
Wetland-Tol_TxPct	+				+				

## Appendix C. List of metrics evaluated for inclusion in F-IBI, metric category assignments, and metric descriptions

Metric Name	Metric Category	Metric Description
BenthicFeeder	Trophic	Taxa richness of benthic feeders
BenthicFeeder_Pct	Trophic	Relative abundance (%) of benthic feeding individuals
BenthicFeeder_TxPct	Trophic	Relative abundance (%) of benthic feeding species
BenthicInsectivore	Trophic	Taxa richness of benthic insectivores
BenthicInsectivore_Pct	Trophic	Relative abundance (%) of benthic insectivore individuals
BenthicInsectivore_TxPct	Trophic	Relative abundance (%) of benthic insectivore species
BenthicInsectivore-Tol	Trophic	Taxa richness of benthic insectivores (excludes tolerant species)
BenthicInsectivore-Tol_Pct	Trophic	Relative abundance (%) of benthic insectivore individuals (excludes tolerant species)
BenthicInsectivore-Tol_TxPct	Trophic	Relative abundance (%) of benthic insectivore species (excludes tolerant species)
BenthicMinnowDarter	Trophic	Taxa richness of benthic insectivore minnows and darters
BenthicMinnowDarter_Pct	Trophic	Relative abundance (%) of benthic insectivore minnow and darter individuals
BenthicMinnowDarter_TxPct	Trophic	Relative abundance (%) of benthic insectivore minnow and darter species
Carnivore	Trophic	Taxa richness of carnivores
Carnivore_Pct	Trophic	Relative abundance (%) of carnivorous individuals
Carnivore_TxPct	Trophic	Relative abundance (%) of carnivorous species
Centrarchid	Richness	Taxa richness of Centrarchids
Centrarchid_Pct	Composition	Relative abundance (%) of Centrarchid individuals
Centrarchid_TxPct	Composition	Relative abundance (%) of Centrarchid species
Centrarchid-Tol	Richness	Taxa richness of Centrarchids (excludes tolerant species)
Centrarchid-Tol_Pct	Composition	Relative abundance (%) of Centrarchid individuals (excludes tolerant species)
Centrarchid-Tol_TxPct	Composition	Relative abundance (%) of Centrarchid species (excludes tolerant species)
Coldwater	Habitat	Taxa richness of coldwater species
Coldwater_Pct	Habitat	Relative abundance (%) of coldwater individuals
Coldwater_TxPct	Habitat	Relative abundance (%) of coldwater taxa
ColdwaterCoolwater	Habitat	Taxa richness of coldwater and coolwater species
ColdwaterCoolwater_Pct	Habitat	Relative abundance (%) of coldwater and coolwater individuals
ColdwaterCoolwater_TxPct	Habitat	Relative abundance (%) of coldwater and coolwater species
ColdwaterCoolwaterNative	Habitat	Taxa richness of native coldwater and coolwater species
ColdwaterCoolwaterNative_Pct	Habitat	Relative abundance (%) of native coldwater and coolwater individuals
ColdwaterCoolwaterNative_TxPct	Habitat	Relative abundance (%) of native coldwater and coolwater species
ColdwaterNative	Habitat	Taxa richness of native coldwater species
ColdwaterNative_Pct	Habitat	Relative abundance (%) of native coldwater individuals
ColdwaterNative_TxPct	Habitat	Relative abundance (%) of native coldwater species

## Appendix C (continued)

Metric Name	Metric Category	Metric Description
ComplexLithophil	Reproductive	Taxa richness of complex lithophilic spawners
ComplexLithophil_Pct	Reproductive	Relative abundance (%) of complex lithophilic individuals
ComplexLithophil_TxPct	Reproductive	Relative abundance (%) of complex lithophilic species
Coolwater	Habitat	Taxa richness of coolwater species
Coolwater_Pct	Habitat	Relative abundance (%) of coolwater individuals
Coolwater_TxPct	Habitat	Relative abundance (%) of coolwater species
CoolwaterNative	Habitat	Taxa richness of native coolwater species
CoolwaterNative_Pct	Habitat	Relative abundance (%) of native coolwater individuals
CoolwaterNative_TxPct	Habitat	Relative abundance (%) of native coolwater species
CountofTaxa	Richness	Total taxa richness
Darter	Richness	Taxa richness of darters
Darter_Pct	Composition	Relative abundance (%) of darter individuals
Darter_TxPct	Composition	Relative abundance (%) of darter species
DarterSculpin	Richness	Taxa richness of darters and sculpins
DarterSculpin_Pct	Composition	Relative abundance (%) of darter and sculpin individuals
DarterSculpin_TxPct	Composition	Relative abundance (%) of darter and sculpin species
DarterSculpinNoturus	Richness	Taxa richness of darters, sculpins, and Noturus species
DarterSculpinNoturus_Pct	Composition	Relative abundance (%) of darter, sculpin, and Noturus individuals
DarterSculpinNoturus_TxPct	Composition	Relative abundance (%) of darter, sculpin, and Noturus species
DarterSculpinSucker	Richness	Taxa richness of darters, sculpins, and round-bodied suckers
DarterSculpinSucker_Pct	Composition	Relative abundance (%) of darter, sculpin, and round-bodied sucker individuals
DarterSculpinSucker_TxPct	Composition	Relative abundance (%) of darter, sculpin, and round-bodied sucker species
Detritivore	Trophic	Taxa richness of detritivores
Detritivore_Pct	Trophic	Relative abundance (%) of detritivorous individuals
Detritivore_TxPct	Trophic	Relative abundance (%) of detritivorous species
DetritivoreMinor	Trophic	Taxa richness of species where detritus constitutes at least 5% of their diet
DetritivoreMinor_Pct	Trophic	Relative abundance (%) of individuals where detritus constitutes at least 5% of their diet
DetritivoreMinor_TxPct	Trophic	Relative abundance (%) of species where detritus constitutes at least 5% of their diet
DetritivorePlanktivore	Trophic	Taxa richness of detritivores and planktivores
DetritivorePlanktivore_Pct	Trophic	Relative abundance (%) of detritivorous and planktivorous individuals
DetritivorePlanktivore_TxPct	Trophic	Relative abundance (%) of detritivorous and planktivorous species
DominanceOneTaxa_Pct	Composition	Relative abundance (%) of individuals of the most abundant species
DominanceThreeTaxa_Pct	Composition	Relative abundance (%) of individuals of the three most abundant species
DominanceTwoTaxa_Pct	Composition	Relative abundance (%) of individuals of the two most abundant species
Exotic	Richness	Taxa richness of exotic species
Exotic_Pct	Composition	Relative abundance (%) of exotic individuals
Exotic_TxPct	Composition	Relative abundance (%) of exotic species

## Appendix C (continued)

Metric Name	Metric Category	Metric Description
FilterFeeder	Trophic	Taxa richness of filter feeders
FilterFeeder_Pct	Trophic	Relative abundance (%) of filter feeding individuals
FilterFeeder_TxPct	Trophic	Relative abundance (%) of filter feeding species
FishDELT_Pct	Composition	Relative abundance (%) of individuals with DELT anomalies (deformities, eroded fins, lesions, or tumors)
GeneralistFeeder	Trophic	Taxa richness of trophic generalists
GeneralistFeeder_Pct	Trophic	Relative abundance (%) of trophic generalist individuals
GeneralistFeeder_TxPct	Trophic	Relative abundance (%) of trophic generalist species
GeneralistFeederFrim	Trophic	Taxa richness of trophic generalists
GeneralistFeederFrim_Pct	Trophic	Relative abundance (%) of trophic generalist individuals
GeneralistFeederFrim_TxPct	Trophic	Relative abundance (%) of trophic generalist species
HeadwaterSpecialist	Habitat	Taxa richness of headwater specialists
HeadwaterSpecialist_Pct	Habitat	Relative abundance (%) of headwater specialist individuals
HeadwaterSpecialist_TxPct	Habitat	Relative abundance (%) of headwater specialist species
HeadwaterSpecialist-Tol	Habitat	Taxa richness of headwater specialists (excludes tolerant species)
HeadwaterSpecialist-Tol_Pct	Habitat	Relative abundance (%) of headwater specialist individuals (excludes tolerant species)
HeadwaterSpecialist-Tol_TxPct	Habitat	Relative abundance (%) of headwater specialist species (excludes tolerant species)
Herbivore	Trophic	Taxa richness of herbivores
Herbivore_Pct	Trophic	Relative abundance (%) of herbivorous individuals
Herbivore_TxPct	Trophic	Relative abundance (%) of herbivorous species
HerbivorousNWQ	Trophic	Taxa richness of herbivores
HerbivorousNWQ_Pct	Trophic	Relative abundance (%) of herbivorous individuals
HerbivorousNWQ_TxPct	Trophic	Relative abundance (%) of herbivorous species
Insectivore	Trophic	Taxa richness of insectivores
Insectivore_Pct	Trophic	Relative abundance (%) of insectivorous individuals
Insectivore_TxPct	Trophic	Relative abundance (%) of insectivorous species
Insectivore-Tol	Trophic	Taxa richness of insectivores (excludes tolerant species)
Insectivore-Tol_Pct	Trophic	Relative abundance (%) of insectivorous individuals (excludes tolerant species)
Insectivore-Tol_TxPct	Trophic	Relative abundance (%) of insectivorous species (excludes tolerant species)
InsectivorousCyprinid	Trophic	Taxa richness of insectivorous Cyprinids
InsectivorousCyprinid_Pct	Trophic	Relative abundance (%) of insectivorous Cyprinid individuals
InsectivorousCyprinid_TxPct	Trophic	Relative abundance (%) of insectivorous Cyprinid species
Intolerant	Tolerance	Taxa richness of intolerant species
Intolerant_Pct	Tolerance	Relative abundance (%) of intolerant individuals
Intolerant_TxPct	Tolerance	Relative abundance (%) of intolerant species
IntolerantColdwater	Tolerance	Taxa richness of species considered Intolerant in coldwater streams
IntolerantColdwater_Pct	Tolerance	Relative abundance (%) of individuals considered Intolerant in coldwater streams
IntolerantColdwater_TxPct	Tolerance	Relative abundance (%) of species considered Intolerant in coldwater streams

## Appendix C (continued)

Metric Name	Metric Category	Metric Description
InvertivoreNWQ	Trophic	Taxa richness of invertivores
InvertivoreNWQ_Pct	Trophic	Relative abundance (%) of invertivorous individuals
InvertivoreNWQ_TxPct	Trophic	Relative abundance (%) of invertivorous species
LargeRiver	Habitat	Taxa richness of species that predominately utilize large river habitats
LargeRiver_Pct	Habitat	Relative abundance (%) of individuals that predominately utilize large river habitats
LargeRiver_TxPct	Habitat	Relative abundance (%) of species that predominately utilize large river habitats
Lithophil	Reproductive	Taxa richness of lithophilic spawners
Lithophil_Pct	Reproductive	Relative abundance (%) of lithophilic individuals
Lithophil_TxPct	Reproductive	Relative abundance (%) of lithophilic species
LongLived	Life History	Taxa richness of long-lived species
LongLived_Pct	Life History	Relative abundance (%) of long-lived individuals
LongLived_TxPct	Life History	Relative abundance (%) of long-lived species
MatureAge<1	Reproductive	Taxa richness of species with a female mature age <=1
MatureAge<1_Pct	Reproductive	Relative abundance (%) of individuals with a female mature age <=1
MatureAge<1_TxPct	Reproductive	Relative abundance (%) of species with a female mature age <=1
MatureAge<2	Reproductive	Taxa richness of species with a female mature age <=2
MatureAge<2_Pct	Reproductive	Relative abundance (%) of individuals with a female mature age <=2
MatureAge<2_TxPct	Reproductive	Relative abundance (%) of species with a female mature age <=2
MatureAge<2Vtol	Reproductive	Taxa richness of species with a female mature age <=2 that are also considered Very Tolerant
MatureAge<2Vtol_Pct	Reproductive	Relative abundance (%) of individuals with a female mature age <=2 that are also considered Tolerant
MatureAge<2Vtol_TxPct	Reproductive	Relative abundance (%) of species with a female mature age <=2 that are also considered Very Tolerant
MatureAge>3	Reproductive	Taxa richness of species with a female mature age >=3
MatureAge>3_Pct	Reproductive	Relative abundance (%) of individuals with a female mature age >=3
MatureAge>3_TxPct	Reproductive	Relative abundance (%) of species with a female mature age >=3
MatureAge>3-Tol	Reproductive	Taxa richness of species with a female mature age >=3 (excludes tolerant species)
MatureAge>3-Tol_Pct	Reproductive	Relative abundance (%) of individuals with a female mature age >=3 (excludes tolerant species)
MatureAge>3-Tol_TxPct	Reproductive	Relative abundance (%) of species with a female mature age >=3 (excludes tolerant species)
MatureAge>4	Reproductive	Taxa richness of species with a female mature age >=4
MatureAge>4_Pct	Reproductive	Relative abundance (%) of individuals with a female mature age >=4
MatureAge>4_TxPct	Reproductive	Relative abundance (%) of species with a female mature age >=4
MeagerSpawner	Reproductive	Taxa richness of meager spawners
MeagerSpawner_Pct	Reproductive	Relative abundance (%) of meager spawning individuals
MeagerSpawner_TxPct	Reproductive	Relative abundance (%) of meager spawning species
Migratory	Life History	Taxa richness of migratory species
Migratory_Pct	Life History	Relative abundance (%) of migratory individuals
Migratory_TxPct	Life History	Relative abundance (%) of migratory species

## Appendix C (continued)

Metric Name	Metric Category	Metric Description
Minnow	Richness	Taxa richness of Cyprinids
Minnow_Pct	Composition	Relative abundance (%) of Cyprinid individuals
Minnow_TxPct	Composition	Relative abundance (%) of Cyprinid species
Minnow-Tol	Richness	Taxa richness of Cyprinids (excludes tolerant species)
Minnow-Tol_Pct	Composition	Relative abundance (%) of Cyprinid individuals (excludes tolerant species)
Minnow-Tol_TxPct	Composition	Relative abundance (%) of Cyprinid species (excludes tolerant species)
Native	Richness	Taxa richness of native species
Native_Pct	Composition	Relative abundance (%) of native individuals
Native_TxPct	Composition	Relative abundance (%) of native species
NonBenthicGeneralist	Trophic	Taxa richness of species that feed within the surface water column (not benthic exclusively) and are generalist feeders
NonBenthicGeneralist_Pct	Trophic	Relative abundance (%) of individuals that feed within the surface water column (not benthic exclusively) and are generalist feeders
NonBenthicGeneralist_TxPct	Trophic	Relative abundance (%) of species that feed within the surface water column (not benthic exclusively) and are generalist feeders
NonBenthicGeneralistTol	Trophic	Taxa richness of species that feed within the surface water column (not benthic exclusively), are considered tolerant, and are generalist feeders
NonBenthicGeneralistTol_Pct	Trophic	Relative abundance (%) of individuals that feed within the surface water column (not benthic exclusively), are considered tolerant, and are generalist feeders
NonBenthicGeneralistTol_TxPct	Trophic	Relative abundance (%) of species that feed within the surface water column (not benthic exclusively), are considered tolerant, and are generalist feeders
NonLithophilicNester	Reproductive	Taxa richness of non-lithophilic nest-guarders
NonLithophilicNester_Pct	Reproductive	Relative abundance (%) of non-lithophilic, nest-guarding individuals
NonLithophilicNester_TxPct	Reproductive	Relative abundance (%) of non-lithophilic, nest-guarding species
NumPerMeter	Composition	Number of individuals per meter of stream sampled
NumPerMeter-Tol	Composition	Number of individuals per meter of stream sampled (excludes individuals of tolerant species)
NumPerMin	Composition	Number of individuals per minute of sampling time
NumPerMin-Tol	Composition	Number of individuals per minute of sampling time (excludes individuals of tolerant species)

## Appendix C (continued)

Metric Name	Metric Category	Metric Description
Ominivore	Trophic	Taxa richness of omnivores
Ominivore_Pct	Trophic	Relative abundance (%) of omnivorous individuals
Ominivore_TxPct	Trophic	Relative abundance (%) of omnivorous species
OmnivorousCyprinid	Trophic	Taxa richness of omnivorous Cyprinids
OmnivorousCyprinid_Pct	Trophic	Relative abundance (%) of omnivorous Cyprinid individuals
OmnivorousCyprinid_TxPct	Trophic	Relative abundance (%) of omnivorous Cyprinid species
Parasitic	Trophic	Taxa richness of parasitic species
Parasitic_Pct	Trophic	Relative abundance (%) of parasitic individuals
Parasitic_TxPct	Trophic	Relative abundance (%) of parasitic species
Perciformes	Richness	Taxa richness of Perciformids
Perciformes_Pct	Composition	Relative abundance (%) of Perciformid individuals
Perciformes_TxPct	Composition	Relative abundance (%) of Perciformid species
Perciformes-Tol	Habitat	Taxa richness of Perciformids (excludes tolerant species)
Perciformes-Tol_Pct	Composition	Relative abundance (%) of Perciformid individuals (excludes tolerant species)
Perciformes-Tol_TxPct	Composition	Relative abundance (%) of Perciformid species (excludes tolerant species)
Pioneer	Life History	Taxa richness of pioneer species
Pioneer_Pct	Life History	Relative abundance (%) of pioneer individuals
Pioneer_TxPct	Life History	Relative abundance (%) of pioneer species
Piscivore	Trophic	Taxa richness of piscivores
Piscivore_Pct	Trophic	Relative abundance (%) of piscivorous individuals
Piscivore_TxPct	Trophic	Relative abundance (%) of piscivorous species
Planktivore	Trophic	Taxa richness of planktivores
Planktivore_Pct	Trophic	Relative abundance (%) of planktivorous individuals
Planktivore_TxPct	Trophic	Relative abundance (%) of planktivorous species
ProlificSpawner	Reproductive	Taxa richness of prolific spawners
ProlificSpawner_Pct	Reproductive	Relative abundance (%) of prolific spawning individuals
ProlificSpawner_TxPct	Reproductive	Relative abundance (%) of prolific spawning species
Rhinichthys	Richness	Taxa richness of Rhinichthyds
Rhinichthys_Pct	Composition	Relative abundance (%) of Rhinichthyd individuals
Rhinichthys_TxPct	Composition	Relative abundance (%) of Rhinichthyd species
RiffleSpecialist	Habitat	Taxa richness of species that predominately utilize riffle habitats
RiffleSpecialist_Pct	Habitat	Relative abundance (%) of individuals that predominately utilize riffle habitats
RiffleSpecialist_TxPct	Habitat	Relative abundance (%) of species that predominately utilize riffle habitats
RoundBodiedSucker	Richness	Taxa richness of round-bodied suckers (excludes Catostomus commersonii)
RoundBodiedSucker_Pct	Composition	Relative abundance (%) of round-bodied sucker individuals (excludes Catostomus commersonii)
RoundBodiedSucker_TxPct	Composition	Relative abundance (%) of round-bodied sucker species (excludes Catostomus commersonii)

## Appendix C (continued)

Metric Name	Metric Category	Metric Description
Salmonid	Richness	Taxa richness of Salmonids
Salmonid_Pct	Composition	Relative abundance (%) of Salmonid individuals
Salmonid_TxPct	Composition	Relative abundance (%) of Salmonid species
Sensitive	Tolerance	Taxa richness of sensitive species
Sensitive_Pct	Tolerance	Relative abundance (%) of sensitive individuals
Sensitive_TxPct	Tolerance	Relative abundance (%) of sensitive species
SensitiveColdwater	Tolerance	Taxa richness of species considered Sensitive in coldwater streams
SensitiveColdwater_Pct	Tolerance	Relative abundance (%) of individuals considered Sensitive in coldwater streams
SensitiveColdwater_TxPct	Tolerance	Relative abundance (%) of species considered Sensitive in coldwater streams
SerialSpawner	Reproductive	Taxa richness of serial spawners
SerialSpawner_Pct	Reproductive	Relative abundance (%) of serial spawning individuals
SerialSpawner_TxPct	Reproductive	Relative abundance (%) of serial spawning species
ShortLived	Life History	Taxa richness of short-lived species
ShortLived_Pct	Life History	Relative abundance (%) of short-lived individuals
ShortLived_TxPct	Life History	Relative abundance (%) of short-lived species
SimpleLithophil	Reproductive	Taxa richness of simple lithophils
SimpleLithophil_Pct	Reproductive	Relative abundance (%) of simple lithophilic individuals
SimpleLithophil_TxPct	Reproductive	Relative abundance (%) of simple lithophilic species
SimpleLithophilFrim	Reproductive	Taxa richness of simple lithophils
SimpleLithophilFrim_Pct	Reproductive	Relative abundance (%) of simple lithophilic individuals
SimpleLithophilFrim_TxPct	Reproductive	Relative abundance (%) of simple lithophilic species
StickebackMudminnow	Richness	Taxa richness of Umbra limi and Culaea inconstans
StickebackMudminnow_Pct	Composition	Relative abundance (%) of Umbra limi and Culaea inconstans individuals
StickebackMudminnow_TxPct	Composition	Relative abundance (%) of Umbra limi and Culaea inconstans species
SubterminalMouth	Trophic	Taxa richness of subterminal-mouthed Cyprinids (excludes exotic species)
SubterminalMouth_Pct	Trophic	Relative abundance (%) of subterminal-mouthed Cyprinid individuals (excludes exotic species)
SubterminalMouth_TxPct	Trophic	Relative abundance (%) of subterminal-mouthed Cyprinid species (excludes exotic species)
Sucker	Richness	Taxa richness of Catostomids
Sucker_Pct	Composition	Relative abundance (%) of Catostomid individuals
Sucker_TxPct	Composition	Relative abundance (%) of Catostomid species
SuckerCatfish	Richness	Taxa richness of Catostomids and Ictalurids
SuckerCatfish_Pct	Composition	Relative abundance (%) of Catostomid and Ictalurid individuals
SuckerCatfish_TxPct	Composition	Relative abundance (%) of Catostomid and Ictalurid species
Tolerant	Tolerance	Taxa richness of tolerant species
Tolerant_Pct	Tolerance	Relative abundance (%) of tolerant individuals
Tolerant_TxPct	Tolerance	Relative abundance (%) of tolerant species
TolerantColdwater	Tolerance	Taxa richness of species considered Tolerant in coldwater streams
TolerantColdwater_Pct	Tolerance	Relative abundance (%) of individuals considered Tolerant in coldwater streams



## Appendix C (continued)

Metric Name	Metric Category	Metric Description
TolerantColdwater_TxPct	Tolerance	Relative abundance (%) of species considered Tolerant in coldwater streams
VeryTolerant	Tolerance	Taxa richness of very tolerant species
VeryTolerant_Pct	Tolerance	Relative abundance (%) of very tolerant individuals
VeryTolerant_TxPct	Tolerance	Relative abundance (%) of very tolerant species
VeryTolerantColdwater	Tolerance	Taxa richness of species considered Very Tolerant in coldwater streams
VeryTolerantColdwater_Pct	Tolerance	Relative abundance (%) of individuals considered Very Tolerant in coldwater streams
VeryTolerantColdwater_TxPct	Tolerance	Relative abundance (%) of species considered Very Tolerant in coldwater streams
Wetland	Habitat	Taxa richness of species that utilize wetland habitats
Wetland_Pct	Habitat	Relative abundance (%) of individuals that utilize wetland habitats
Wetland_TxPct	Habitat	Relative abundance (%) of species that utilize wetland habitats
Wetland-Tol	Habitat	Taxa richness of species that utilize wetland habitats (excludes tolerant species)
Wetland-Tol_Pct	Habitat	Relative abundance (%) of individuals that utilize wetland habitats (excludes tolerant taxa)
Wetland-Tol_TxPct	Habitat	Relative abundance (%) of species that utilize wetland habitats (excludes tolerant taxa)

## Appendix D. List of Minnesota fish species and attributes used to calculate F-IBI metrics

Composition		Reproductive		Trophic	
DSS	DarterSculpinSucker	MA<2	MatureAge<2	BI-T	BenthicInsectivore-Tol
DS	DarterSculpin	MA>3-T	MatureAge>3-Tol	DE	Detritivore
EX	Exotic	NE	NonLithophilicNester	DEM	DetritivoreMinor
MIN-T	Minnow-Tol	SER	SerialSpawner	GE	GeneralistFeeder
PERC	Perciformes	SILI	SimpleLithophil	HE	Herbivore
Habitat		Tolerance		IN-T	Insectivore-Tol
CW	Coldwater	I	Intolerant	IN_CYP	InsectivorousCyprinid
CW_N	ColdwaterNative	I_CW	IntolerantColdwater	OM	Ominivore
HW-T	HeadwaterSpecialist-Tol	S	Sensitive	PI	Piscivore
WE-T	Wetland-Tol	S_CW	SensitiveColdwater		
Life History		T	Tolerant		
PI	Pioneer	T_CW	TolerantColdwater		
SL	ShortLived	VT	VeryTolerant		

CommonName	ScientificName	Composition	Habitat	Life History	Reproductive	Tolerance	Trophic
Lampreys	Petromyzontidae					S	
lamprey ammocoete	Petromyzontidae larvae					I,S	
chestnut lamprey	Ichthyomyzon castaneus				MA>3-T	I,S	PI
northern brook lamprey	Ichthyomyzon fossor				MA>3-T	I,ICW,S,SCW	
southern brook lamprey	Ichthyomyzon gagei				MA>3-T	I,ICW,S,SCW	DE
silver lamprey	Ichthyomyzon unicuspis				MA>3-T	S	PI
American brook lamprey	Lampetra appendix		HW-T		MA>3-T	I,ICW,S,SCW	
sea lamprey	Petromyzon marinus	EX			MA>3-T		DE,PI
Sturgeons	Acipenseridae				SILI		BI-T,IN-T
lake sturgeon	Acipenser fulvescens				MA>3-T,SILI	I,S	BI-T,IN-T
shovelnose sturgeon	Scaphirhynchus platyrhynchus				MA>3-T,SILI		BI-T,IN-T
Paddlefishes	Polyodontidae						
paddlefish	Polyodon spathula				MA>3-T,SILI	I,S	
Gars	Lepisosteidae						PI
longnose gar	Lepisosteus osseus				MA>3-T,SER		PI
shortnose gar	Lepisosteus platostomus				MA>3-T		PI
Bowfins	Amiidae						
bowfin	Amia calva				MA>3-T		PI
Mooneyes	Hiodontidae						IN-T
goldeye	Hiodon alosoides				MA>3-T		IN-T
mooneye	Hiodon tergisus					S	IN-T

## Appendix D (continued)

Eels	Anguillidae						
American eel	Anguilla rostrata					PI	
Herrings	Clupeidae						
skipjack herring	Alosa chrysochloris					PI	
alewife	Alosa pseudoharengus	EX		MA>3-T			
gizzard shad	Dorosoma cepedianum			MA<2,SER		DEM	
Minnows	Cyprinidae						
central stoneroller	Campostoma anomalum				T,TCW	HE	
largescale stoneroller	Campostoma oligolepis	MIN-T		MA<2		DEM,HE	
Gen: stonerollers	Campostoma	MIN-T				HE	
goldfish	Carassius auratus	EX		SER	T,TCW,VT	DEM,GE,OM	
redside dace	Clinostomus elongatus	MIN-T	HW-T	MA<2,SILI	I,ICW,S,SCW	IN-T,INCYP	
lake chub	Couesius plumbeus	MIN-T		SILI	I,ICW,S,SCW	IN-T,INCYP	
grass carp	Ctenopharyngodon idella	EX		MA<2	T,TCW	DEM,HE	
red shiner	Cyprinella lutrensis		SL	MA<2,SER	T,TCW	GE	
spotfin shiner	Cyprinella spiloptera	MIN-T		MA<2,SER		DE,DEM,IN-T,INCYP	
common carp	Cyprinus carpio	EX		MA<2	T,TCW,VT	DE,DEM,GE,OM	
gravel chub	Erimystax x-punctatus	MIN-T	SL	MA<2,SILI	I,S	BI-T,DEM,IN-T,INCYP	
brassy minnow	Hybognathus hankinsoni		SL	MA<2	T,TCW	DE,DEM,HE	
Mississippi silvery minnow	Hybognathus nuchalis	MIN-T			I,S	HE	
pallid shiner	Hybopsis amnis	MIN-T			I,S	IN-T,INCYP	
bighead carp	Hypophthalmichthys nobilis	EX		SER	T,TCW,VT	DEM	
silver carp	Hypophthalmichthys molitrix	EX			T,TCW,VT		
common shiner	Luxilus cornutus	MIN-T		MA<2,SILI		DEM,GE	
redfin shiner	Lythrurus umbratilis	MIN-T	SL	MA<2	S	IN-T,INCYP	
shoal chub	Macrhybopsis hyostoma	MIN-T	SL	MA<2	S	BI-T,IN-T,INCYP	
silver chub	Macrhybopsis storeriana	MIN-T		MA<2		BI-T,IN-T,INCYP	
pearl dace	Margariscus margarita	MIN-T	HW-T, WE-T	MA<2	S,SCW	DEM,IN-T,INCYP	
hornyhead chub	Nocomis biguttatus	MIN-T		MA<2,SER	S	DEM,IN-T,INCYP	
golden shiner	Notemigonus crysoleucas	MIN-T	WE-T	MA<2,SER		GE	
pugnose shiner	Notropis anogenus	MIN-T	SL	MA>3-T	I,S	DE,HE	
emerald shiner	Notropis atherinoides	MIN-T	SL	MA<2,SILI		DEM,IN-T,INCYP	
river shiner	Notropis blennioides	MIN-T	SL	MA<2,SILI		DEM,IN-T,INCYP	
ghost shiner	Notropis buechanani	MIN-T		MA<2,SER	I,S	DE,IN-T,INCYP	
bigmouth shiner	Notropis dorsalis		SL	MA<2,SER	T,TCW,VT	DEM,INCYP	
blackchin shiner	Notropis heterodon	MIN-T		MA<2,SER	I,S	DEM,IN-T,INCYP	
blacknose shiner	Notropis heterolepis	MIN-T	SL	MA<2	I,S	IN-T,INCYP	
spottail shiner	Notropis hudsonius	MIN-T		MA<2	S	IN-T,INCYP	
Ozark minnow	Notropis nubilus	MIN-T		MA<2,SILI	I,S	DEM,HE	
carmine shiner	Notropis percobromus	MIN-T	SL	MA<2,SILI	S	DE,IN-T,INCYP	
sand shiner	Notropis stramineus		SL	MA<2,SER	T,TCW	DE,DEM,INCYP	
weed shiner	Notropis texanus	MIN-T	SL	MA<2,SER	I,S	DE,DEM,HE	

## Appendix D (continued)

Topeka shiner	Notropis topeka	MIN-T	HW-T	SL	MA<2	I,S	IN-T,INCYP
mimic shiner	Notropis volucellus	MIN-T		SL	SER	I,S	DEM,IN-T,INCYP
channel shiner	Notropis wickliffi	MIN-T		SL	MA<2	S	IN-T,INCYP
Gen: Notropis	Notropis	MIN-T					
pugnose minnow	Opsopoeodus emiliae	MIN-T		SL	MA<2,NE,SER	I,S	DE,DEM,IN-T,INCYP
suckermouth minnow	Phenacobius mirabilis	MIN-T			MA<2,SER,SILI		BI-T,DEM,IN-T,INCYP
northern redbelly dace	Phoxinus eos	MIN-T	HW-T, WE-T	SL	MA<2,SER	S	HE
southern redbelly dace	Phoxinus erythrogaster	MIN-T	HW-T	SL	MA<2,SER,SILI		DEM,HE
finescale dace	Phoxinus neogaeus	MIN-T	HW-T, WE-T		MA<2,SER	S,SCW	IN-T,INCYP
Gen: Phoxinus	Phoxinus		HW-T				
bluntnose minnow	Pimephales notatus			PI,SL	MA<2,NE,SER	T,TCW,VT	DE,DEM,GE
fathead minnow	Pimephales promelas			PI,SL	MA<2,NE,SER	T,TCW,VT	DE,DEM,GE,OM
bullhead minnow	Pimephales vigilax	MIN-T			MA<2,NE,SER		GE
flathead chub	Platygobio gracilis	MIN-T			MA<2,SER		IN-T,INCYP
blacknose dace	Rhinichthys atratulus			SL	MA<2,SILI	T	GE
longnose dace	Rhinichthys cataractae	MIN-T			SILI	I,ICW,S,SCW	BI-T,IN-T,INCYP
Gen: Rhinichthys	Rhinichthys				SILI		
creek chub	Semotilus atromaculatus			PI	MA<2	T	GE
Suckers	Catostomiidae						
river carpsucker	Carpionotus carpio				MA>3-T		DE,DEM,GE,OM
quillback	Carpionotus cyprinus				MA>3-T		DE,DEM,GE,OM
highfin carpsucker	Carpionotus velifer				MA>3-T	S	DE,DEM,GE,OM
Gen: carpsuckers	Carpionotus						GE,OM
SubFam: buffalo/carpsuckers	Ictiobinae						GE,OM
longnose sucker	Catostomus commersoni	DSS			MA>3-T,SILI	I,ICW,S,SCW	BI-T,IN-T
white sucker	Catostomus commersoni				SILI	T	DE,DEM,GE,OM
Gen: Catostomus	Catostomus				SILI		
blue sucker	Cycleptus elongatus	DSS			SILI	I,S	BI-T,IN-T
northern hogsucker	Hypentelium nigricans	DSS			MA>3-T,SILI	S	BI-T,DEM,IN-T
smallmouth buffalo	Ictiobus bubalus				MA<2		DEM,GE,OM
bigmouth buffalo	Ictiobus cyprinellus					T,VT	DEM,GE,OM
black buffalo	Ictiobus niger						DE,DEM,GE,OM
Gen: buffalos	Ictiobus						GE,OM
spotted sucker	Minytrema melanops	DSS			MA>3-T,SILI	I,S	BI-T,DEM,IN-T
silver redhorse	Moxostoma anisurum	DSS			MA>3-T,SILI		BI-T,DEM,IN-T
river redhorse	Moxostoma carinatum	DSS			MA>3-T,SILI	I,S	BI-T,IN-T
black redhorse	Moxostoma duquesnei	DSS			MA>3-T,SILI	I,S,SCW	BI-T,IN-T
golden redhorse	Moxostoma erythrurum	DSS			MA>3-T,SILI		BI-T,DEM,IN-T
shorthead redhorse	Moxostoma macrolepidotum	DSS			MA>3-T,SILI		BI-T,DEM,IN-T
greater redhorse	Moxostoma valenciennesi	DSS			MA>3-T,SILI	I,S	BI-T,IN-T
Gen: redhorses	Moxostoma	DSS			SILI		BI-T,IN-T

## Appendix D (continued)

Catfishes	Ictaluridae					
black bullhead	Ameiurus melas				T,TCW,VT	DEM,GE,OM
yellow bullhead	Ameiurus natalis	WE-T		NE,SER		DEM,GE,OM
brown bullhead	Ameiurus nebulosus	WE-T		NE,SER		DEM,GE,OM
Gen: bullheads	Ameiurus					GE,OM
blue catfish	Ictalurus furcatus			NE		PI
channel catfish	Ictalurus punctatus			MA>3-T,NE		DEM,PI
slender madtom	Noturus exilis				I,S	BI-T,DE,IN-T
stonecat	Noturus flavus			MA>3-T,SER	S	BI-T,IN-T
tadpole madtom	Noturus gyrinus	WE-T		MA<2,NE,SER		BI-T,IN-T
Gen: madtoms	Noturus					BI-T,IN-T
flathead catfish	Pylodictis olivaris			MA>3-T,NE		PI
Pikes	Esocidae					PI
northern pike	Esox lucius	WE-T		MA<2		PI
muskellunge	Esox masquinongy			MA>3-T	I,S	PI
tiger musky	Esox hybrid					PI
Mudminnows	Umbridae					
central mudminnow	Umbra limi			MA<2	T,TCW,VT	
Smelts	Osmeridae					
rainbow smelt	Osmerus mordax	EX		SER		PI
Trouts	Salmonidae					
SubFam: salmonids	Salmoninae					PI
lake herring	Coregonus artedi			MA>3-T		
lake whitefish	Coregonus clupeaformis			MA>3-T		IN-T
bloater	Coregonus hoyi			MA<2		
kiyi	Coregonus kiyi			MA>3-T		
shortjaw cisco	Coregonus zenithicus			MA>3-T		
pink salmon	Oncorhynchus gorbusha	EX	CW			PI
coho salmon	Oncorhynchus kisutch	EX	CW	MA>3-T		PI
rainbow trout	Oncorhynchus mykiss	EX	CW	MA>3-T	S,SCW	PI
chinook salmon	Oncorhynchus tshawytscha	EX	CW	MA>3-T		PI
round whitefish	Prosopium cylindraceum			MA>3-T		IN-T
Atlantic salmon	Salmo salar	EX	CW	MA>3-T		PI
brown trout	Salmo trutta	EX	CW	MA>3-T	S,SCW	PI
brook trout	Salvelinus fontinalis		CW,CWN	MA>3-T	I,ICW,S,SCW	PI
lake trout	Salvelinus namaycush		CW,CWN	MA>3-T		PI
tiger trout	Salmonidae hybrid		CW			PI
Trout-perches	Percopsidae					
trout-perch	Percopsis omiscomaycus		SL	MA<2		BI-T,IN-T
Pirate perches	Aphredoderidae					
pirate perch	Aphredoderus sayanus			MA<2		IN-T

## Appendix D (continued)

Codfishes	Gadidae					
burbot	Lota lota			MA>3-T,SILI	I,S	PI
Silversides	Atherinidae					
brook silverside	Labidesthes sicculus		SL	MA<2	S	IN-T
Killifishes	Fundulidae					
banded killifish	Fundulus diaphanus			MA<2,SER		IN-T
starhead topminnow	Fundulus dispar		HW-T	MA<2,SER	I,S	IN-T
blackstripe topminnow	Fundulus notatus			MA<2,SER		IN-T
plains topminnow	Fundulus sciadicus		SL	MA<2	S	IN-T
Gen: topminnows	Fundulus					IN-T
Sticklebacks	Gasterosteidae					
brook stickleback	Culaea inconstans		SL	MA<2,NE	T	
threespine stickleback	Gasterosteus aculeatus	EX	SL	MA<2,NE	T,TCW	
ninespine stickleback	Pungitius pungitius			MA<2,NE		IN-T
Sculpins	Cottidae					
mottled sculpin	Cottus bairdii	DS,DSS	CW,CWN,HW-T	MA<2	S,SCW	BI-T,IN-T
slimy sculpin	Cottus cognatus	DS,DSS,PERC	CW,CWN,HW-T	MA>3-T	I,ICW,S,SCW	BI-T,IN-T
spoonhead sculpin	Cottus ricei	DS,DSS		MA<2	I,S	BI-T,IN-T
Gen: sculpins	Cottus	DS,DSS	CW,CWN		S,SCW	BI-T,IN-T
deepwater sculpin	Myoxocephalus thompsonii	DS,DSS		MA>3-T	I,S	BI-T,IN-T
Temperate Basses	Moronidae					
white perch	Morone americana	EX,PERC		MA>3-T		IN-T
white bass	Morone chrysops	PERC		MA<2		PI
yellow bass	Morone mississippiensis	PERC		MA>3-T		PI
Sunfishes	Centrarchidae					
rock bass	Ambloplites rupestris	PERC		MA>3-T,NE	S	PI
green sunfish	Lepomis cyanellus	PERC	PI	MA<2,NE	T,TCW,VT	GE
pumpkinseed	Lepomis gibbosus	PERC		MA<2,NE		IN-T
warmouth	Chaenobryttus gulosus	PERC		MA<2		PI
orangespotted sunfish	Lepomis humilis	PERC		MA<2,SER	T,TCW,VT	
bluegill	Lepomis macrochirus	PERC		MA<2,NE,SER		IN-T
longear sunfish	Lepomis megalotis	PERC		MA<2,NE	I,S	IN-T
hybrid sunfish	Lepomis hybrid				T,TCW	
Gen: common sunfishes	Lepomis					IN-T
smallmouth bass	Micropterus dolomieu	PERC		MA>3-T,NE	I,S	PI
largemouth bass	Micropterus salmoides	PERC		NE		PI
Gen: Micropterus	Micropterus					PI
white crappie	Pomoxis annularis	PERC		MA<2,NE		PI
black crappie	Pomoxis nigromaculatus	PERC		NE		PI
Gen: crappies	Pomoxis					PI
Perches	Percidae					
western sand darter	Ammocrypta clara	DS,DSS,PERC	SL	MA<2,SER,SILI	I,S	BI-T,IN-T

## Appendix D (continued)

crystal darter	Crystallaria asprella	DS,DSS,PERC		SL	MA<2,SER,SILI	I,S	BI-T,IN-T
mud darter	Etheostoma asprigene	DS,DSS,PERC		SL	MA<2	S	BI-T,IN-T
rainbow darter	Etheostoma caeruleum	DS,DSS,PERC			MA<2,SER,SILI	S,SCW	BI-T,IN-T
bluntnose darter	Etheostoma chlorosomum	DS,DSS					BI-T,IN-T
Iowa darter	Etheostoma exile	DS,DSS,PERC	WE-T	SL	MA<2	S	BI-T,IN-T
fantail darter	Etheostoma flabellare	DS,DSS,PERC	HW-T		MA<2,SER	S,SCW	BI-T,IN-T
least darter	Etheostoma microperca	DS,DSS,PERC		SL	MA<2,SER	I,S	BI-T,IN-T
johnny darter	Etheostoma nigrum	DS,DSS,PERC		PI	MA<2,NE		BI-T,IN-T
banded darter	Etheostoma zonale	DS,DSS,PERC			MA<2,SER,SILI	S	BI-T,IN-T
Gen: Etheostoma	Etheostoma	DS,DSS					BI-T,IN-T
ruffe	Gymnocephalus cernuus	EX			MA<2,SER	T,TCW	
yellow perch	Perca flavescens	PERC	WE-T		MA>3-T		IN-T
logperch	Percina caprodes	DS,DSS,PERC			MA<2,SILI	I,S	BI-T,IN-T
gilt darter	Percina evides	DS,DSS,PERC			MA<2,SILI	I,S	BI-T,IN-T
blackside darter	Percina maculata	DS,DSS,PERC			MA<2,SILI		BI-T,IN-T
slenderhead darter	Percina phoxocephala	DS,DSS,PERC			MA<2,SILI	S	BI-T,IN-T
river darter	Percina shumardi	DS,DSS,PERC		SL	MA<2,SILI	I,S	BI-T,IN-T
Gen: Percina	Percina	DS,DSS			SILI		BI-T,IN-T
sauger	Sander canadensis	PERC			MA>3-T,SILI		PI
walleye	Sander vitreus	PERC			MA>3-T,SILI		PI
saugeye	Sander hybrid	PERC			SILI		PI
Gen: Sander	Sander				SILI		PI
Drums	Sciaenidae						
freshwater drum	Aplodinotus grunniens	PERC			MA>3-T		IN-T
Gobies	Gobiidae						
round goby	Neogobius melanostomus	EX			MA>3-T,NE,SER		PI
tubenose goby	Proterorhinus marmoratus	EX					IN-T