

Development of a Macroinvertebrate Index of Biological Integrity (MIBI) for Rivers and Streams of the St. Croix River Basin in Minnesota

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Development of a Macroinvertebrate Index of Biological Integrity (MIBI) for Rivers and Streams of the St. Croix River Basin in Minnesota

ABSTRACT

As part of the Minnesota Pollution Control Agency's long-term monitoring strategy, macroinvertebrates were collected from 88 streams in the St. Croix River Watershed between 1996 and 2000. The samples were collected primarily from small wadeable streams, and wadeable reaches of larger streams. The macroinvertebrate community data collected was used to develop a series of biologically meaningful measures or metrics. The resulting metrics were assigned scoring criteria, scored, and combined into a multimetric index, the Macroinvertebrate Index of Biological Integrity (MIBI). The MIBI, in conjunction with a similar index measuring the biological integrity of the fish community (Niemela and Feist 2000), was used to evaluate the biological integrity of selected stream reaches. The ability of the MIBI to discern differences between varying degrees of human influence on biological integrity was tested by evaluating streams with a wide range of upstream landuse patterns. The MIBI was capable of discerning differences between high and low levels of upstream human influence, but was not able to discern the subtle differences between reaches with moderate human influence. Due to natural topographic variation in the basin, reaches were classified as having either high or low gradient. A MIBI was developed for each morphological class because the difference in macroinvertebrate community structure between these stream classes was significant. Streams with a drainage area larger than five hundred square miles were not included in the MIBI analysis because 1) the number of large river sampling locations was small and not adequate for a rigorous statistical analysis,

2) it is uncertain if the sampling method currently used is adequate for collecting a representative sample on large systems, and 3) the variability of the data collected could not be attributed to differences in landuse, habitat, or other human influence. The samples collected in this study were located in the two ecoregions that dominate the St. Croix River Basin, the Northern Lakes and Forests and the Central Hardwood Forest, suggesting that natural differences should exist between reaches that are in different ecoregions. There were no reference sites in the Central Hardwood Forest Ecoregion making it impossible to discern natural variation between the two ecoregions. The results of this work suggest that a more robust MIBI could be developed with additional information from very small streams and additional sites in the Central Hardwood Forest Ecoregion. The MIBI developed in this study represents a single tool for the assessment of biological integrity and should be used in conjunction with other biological, chemical, hydrological, and habitat information.

I. BACKGROUND INFORMATION

Rivers and streams serve many functions in today's society including serving as a source of food and water, a mode of transportation for agricultural and manufactured goods and as a recreational and aesthetically pleasing resource for many people. The innumerable functional and aesthetic qualities of rivers and streams create pressures on the resource that are exacerbated by an expanding human population. Watersheds that were once mainly forested have been altered for the social and economic benefit of today's society. The degradation of Minnesota's

rivers comes from numerous sources including: chemical pollutants from municipal and industrial point source discharges; agricultural runoff of pesticides, nutrients, and sediment; hydrologic alteration from stream channelization, dams, and artificial drainage; and habitat alteration from agriculture and urban encroachment. To ensure the integrity of our rivers and streams we must understand the relationship between human induced disturbances and their effect on aquatic resources.

For many years we have managed human impact on stream systems by restricting the amount and kinds of chemicals that enter them. Federal and state government agencies have developed and enforced water-quality standards to ensure that chemical concentrations in our streams do not exceed certain limits. But while we have been largely successful in limiting chemical pollution sources, we have in many respects failed to recognize the effects that landscape alteration and non-point pollution have on river and stream quality. Watershed disturbances from urban, residential, and agricultural development contribute to an overall decrease in the biological integrity in many of our rivers and streams (i.e. road building, stream channelization, alteration of the stream's riparian zone, and many others). It is increasingly apparent that monitoring activities cannot focus on chemical indicators alone, but must instead focus on indicators that integrate the effects of both physical and chemical stressors. Proper management of river and stream systems must be predicated upon a comprehensive monitoring strategy that is able to detect degradation in streams due to human disturbance.

In recent years, scientists have developed methods to quantify and interpret the results of biological surveys, allowing water-quality

managers and policy makers to make informed decisions concerning rivers and streams. There are many advantages to using aquatic organisms, such as macroinvertebrates and fish, in a water quality monitoring program.

ADVANTAGES OF USING MACROINVERTEBRATES IN A WATER QUALITY MONITORING PROGRAM

Macroinvertebrates have been widely used as indicators of water quality by state and federal monitoring agencies for many years (Ohio EPA 1988, Barbour et. al. 1996, Barbour et. al. 1999). Many studies have shown them to be very useful indicators of water quality. They are ubiquitous in nearly every aquatic habitat. Many aquatic macroinvertebrates spend most of their lives in relatively small areas, making them excellent indicators of site specific ecological condition. Additionally, many macroinvertebrates have relatively long life cycles, ranging from several months to several years, and are important indicators of site condition over time. Aquatic organisms are responsive to the cumulative affects of both physical and chemical disturbances. They respond with a range of sensitivities to many kinds of stressors. Some are very tolerant of pollution while others are intolerant. Some are known to respond predictably to specific stressors and others are sensitive to a wide array of stressors. They inhabit the sediment, water column, and submerged substrates of streams, rivers, lakes, and wetlands, and thus can reflect the biological integrity of the entire aquatic ecosystem. Additionally, standardized field sampling methods and laboratory processing protocols for macroinvertebrates are well developed and taxonomic keys are available to identify most specimens to genus or species.

Macroinvertebrates are widely used by citizen monitoring groups throughout the United States (U.S. EPA 1997). However, school and community based volunteer monitoring groups tend to focus on one or two streams and typically lack a larger dataset to allow for a comparison to a regional gradient of impairment conditions. Using macroinvertebrates in a statewide monitoring program will provide a valuable source of information for volunteer monitoring groups, and lead them to a better understanding of the condition of the streams they are monitoring.

HISTORY OF THE INDEX OF BIOTIC INTEGRITY

In an effort to understand and communicate biological information in a meaningful way, Dr Jim Karr developed the Index of Biotic Integrity (IBI) in the early 80's (Karr 1981). The IBI was first developed using attributes of fish communities in moderate size wadeable streams in Illinois. It has subsequently been modified for use throughout the country for aquatic macroinvertebrates (Ohio EPA 1988, Kerans and Karr 1994, Barbour et. al. 1996), terrestrial macroinvertebrates (Kimberling and Karr 2002) and algae (McCormick and Stevenson 1998). Each metric in an IBI denotes a quantifiable attribute of a biological assemblage that changes in a predictable way with different levels of human influence. Typically, 8-12 metrics are combined to form a single index or IBI score. The metrics in a typical Macroinvertebrate Index of Biotic Integrity (MIBI) fall into four broad categories: 1) richness measures, 2) tolerance measures, 3) composition measures, and 4) trophic structure measure. A well-rounded MIBI will include one or more metrics from each of these categories.

The IBI concept has proven to be very adaptable (Karr and Chu 1999). Many of the same metrics have been used successfully throughout different regions of the country in a variety of stream types (Simon and Lyons 1995). Metrics such as the total number of taxa or the number of Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT taxa) are common to most MIBIs that have been developed for invertebrate assemblages. However, Karr and Chu (1999) emphasize that "no metric should become part of a regional multimetric index before it is thoroughly and systematically tested and its response has been validated across a gradient of human influence." This is particularly true when developing an IBI for a new region or stream type, or when considering a new or unproven metric.

Many states have begun to develop multimetric indices for rivers and streams with the ultimate goal of developing biological criteria for use within their own water-quality programs (U.S. EPA 1996). Biological criteria are narrative expressions or numerical values that describe the reference biological condition. The Ohio Environmental Protection Agency (Ohio EPA) has taken the definitive lead by developing numeric biological criteria and using the information to guide management activities. Ohio EPA uses the information from biological assessments in wastewater permitting, 305(b) assessments, 401 certification process, waste load allocation, and overall basin assessments. Other state programs in which multimetric biological assessments are integrated into water-quality programs include the programs of North Carolina, Florida, and Maine.

Most of the work in IBI development has focused on small to moderate size wadeable streams (Ohio EPA 1988, Barbour et al 1996, Barbour et al. 1999). Sampling

methods for these streams have been developed that provide reliable and reproducible results. Additionally, aquatic communities within these systems have been extensively studied, particularly macroinvertebrate and fish assemblages. Recently, promising applications of the multimetric concept have been developed to assess wetlands (Gernes and Helgen 1999; Helgen and Gernes 2001), large rivers (Simon and Emery 1995; Simon and Sanders 1999), lakes (Jennings et al. 1999; Whittier 1999; Drake and Pereira 2000), reservoirs (Jennings et al. 1995; McDonough and Hickman 1999), and terrestrial environments (Kimberling and Karr 2002). Many of these applications are still in the early stages of development.

THE MINNESOTA POLLUTION CONTROL AGENCY'S BIOLOGICAL MONITORING PROGRAM

Efforts at the state level to assess the biological health of aquatic ecosystems, largely by the Minnesota Pollution Control Agency (MPCA) and Minnesota Department of Natural Resources (MDNR), began in 1976 when the MPCA began an aquatic macroinvertebrate monitoring program (MPCA 1979, MPCA 1981). This program focused on the assessment of many of Minnesota's large rivers and streams. Unfortunately, at the time this program began there were few tools available to adequately analyze and use the data collected. After four years of data collection agency priorities changed and the program lost its funding.

The first multimetric indices for Minnesota resulted from the Minnesota River Assessment Project (MRAP conducted from 1990-1992 (Zischke et al. 1994, Bailey et al. 1994). A subsequent fish community study conducted during 1994-1995, resulted

in the development of an IBI for fish in the Lake Agassiz Plain Ecoregion (Niemela et al. 1999). In 1995 the MPCA adopted a monitoring strategy and management framework centered on the idea of managing watersheds. The strategy included a plan to monitor the condition of each basin using a random site selection process (Stevens 1997) to provide a basin-wide assessment of water quality in streams. This monitoring program was supported by long term legislative funding for biological monitoring and biological criteria development.

OBJECTIVES OF THE BIOLOGICAL MONITORING PROGRAM

The MPCA's biological monitoring program has several goals:

- to define and document statewide baseline conditions of in-stream invertebrate and fish biology
- to measure spatial and temporal variability of population and community attributes
- to develop regional indices of biological integrity based on community similarity, beginning with each of Minnesota's nine major river basins with the intent of developing statewide numeric biological criteria in the future.

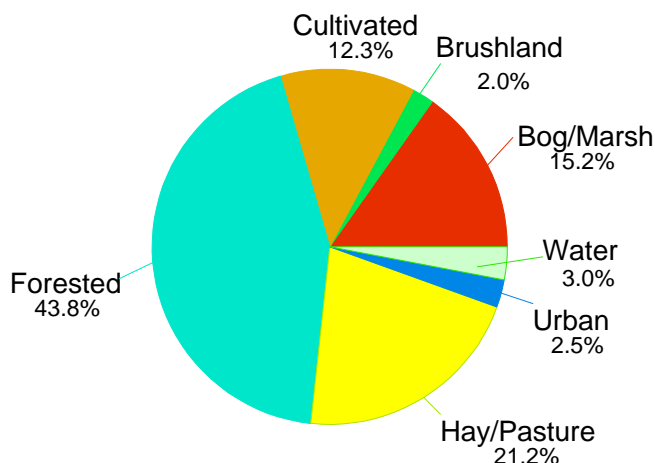
It is paramount to the development of biological criteria in Minnesota that we obtain invertebrate and fish community information statewide. There is currently a paucity of invertebrate and fish community data for streams in Minnesota, particularly those streams that have little potential to contain game fish. In fact, invertebrate and fish community information had not previously been obtained for most of the small streams sampled during the course of this study.

PURPOSE AND SCOPE

This report is the result of an effort to develop an MIBI for all permanent coolwater rivers and streams within the St. Croix River Basin in Minnesota. The report is intended to provide guidance for those interested in conducting an MIBI assessment. Readers interested in the theoretical underpinnings of multimetric indices in general should refer to Karr and Chu (1999).

II. THE ST. CROIX RIVER BASIN

The St. Croix River Basin includes 7650 mi² of flat to gently rolling terrain in Minnesota and Wisconsin (Figure 2). Historically, the basin was almost entirely vegetated by a variety of forest types including the Great Lakes pine forest which was typified by vast stands of mature white and red pines (Fago and Hatch 1993). Logging and agricultural land use practices have almost entirely eliminated large pine stands. A diverse mixture of second growth mixed-hardwood forests, open fields, and cropland now dominate the basin (Figure 1). An



ecoregional divide running roughly through

the center of the basin in an east-west direction separates the Northern Lakes and Forests ecoregion in the north from the North Central Hardwood Forest ecoregion in the south. Today the mixed forests that are found in the nutrient poor soils of the Northern Lakes and Forests ecoregion provide a contrast to the more agricultural landscape of the North Central Hardwood Forests ecoregion. The amount of forest cover within the entire basin is currently about 44% (Figure 2). However, the majority of the remaining forest is confined to the northern half of the basin. Residential development is a concern, primarily in the southern portion of the basin around the Twin Cities metropolitan area.

RIVERS AND STREAMS OF THE ST. CROIX RIVER BASIN

Rivers and streams within the St. Croix River Basin are arguably some of the most scenic in Minnesota. The federal government recognized the importance of the St. Croix system in 1968 when the Upper St. Croix River (above Taylors Falls) and its main tributary, the Namekagon River, were included as one of eight initial stream reaches in the National Wild and Scenic Rivers System. In 1972 the Lower St. Croix River (from Taylors Falls to its confluence with the Mississippi) was added to the national system (Fago and Hatch 1993).

Headwater streams within the basin often originate from peat lands, resulting in dark, tannin-stained water. These streams are usually low gradient, lack riffles, and have a glide/pool type of stream morphology. They are typically sinuous with fine substrates and have a riparian zone comprised of wetland vegetation. The Snake and Kettle Rivers, the two largest tributaries to the St. Croix River in Minnesota, originate in wetland

complexes. However, as these streams progress towards their confluence with the St. Croix River their morphology changes. Lower reaches of the Snake and Kettle Rivers, like many other larger streams in the St. Croix River Basin, have a riffle/run/pool stream morphology with a variety of substrate types and a wooded riparian zone.

THE MACROINVERTEBRATE ASSEMBLAGE

The St. Croix River Basin supports a diverse and unique invertebrate assemblage. The main stem of the St. Croix River has been the focus of two comprehensive studies done in the basin due to its status as a National Wild and Scenic Riverway. Montz et al. (1990) and Boyle et al. (1992), conducted longitudinal surveys of the St. Croix main stem and found it to support a healthy and diverse assemblage of macroinvertebrates.

The St. Croix River watershed is the premier mussel watershed of the Upper Mississippi River watershed, and one of the premier mussel watersheds of the world (U.S. FWS 2003). There are two federally listed, endangered species of mussels found in the St. Croix mainstem, the winged maple leaf (*Quadrula fragosa*) and the Higgins eye (*Lampsilis higginsii*). Two additional mussel species found in the St. Croix are candidates for federal listing, as well as several other mussel species that are on the Minnesota state list as either endangered, threatened, or special concern (Hornbach, 1996a, Hornbach et al. 1996b). The main tributaries on the Minnesota side of the St. Croix River Basin (the Kettle, Snake, and Sunrise Rivers) are also known to maintain healthy assemblages of mussels (Davis and Miller 1997).

Two dragonfly species found in the St. Croix basin are candidates for federal listing

as well. The St. Croix Snake Tail (*Ophiogomphus susbehcha*) is a candidate for endangered status, and the Extra-Striped Snaketail (*Ophiogomphus anomalus*) is a candidate for threatened status.

III. CHARACTERIZATION OF STUDY AREA AND SAMPLING LOCATIONS

If the MIBI is to detect human induced changes in resource integrity, it is necessary to identify and partition the factors that contribute to the natural variability of streams so that changes caused by humans may be detected. On a broad regional scale, differences in climate, topography, geology and other geophysical characteristics of an area dictate species distributions. Thus, an MIBI developed for predominantly agricultural areas in the Midwest should not be applied to the mountainous regions of the western U.S. The ecoregion concept (Omernik and Gallant 1988) has been the most commonly used regional framework for developing IBIs. In Minnesota, macroinvertebrate IBIs have been developed using a basin framework (Stroom and Richards 2000, Zischke et al. 1994). Fish IBIs have also been developed using a basin framework (Bailey et al. 1994, Niemela and Feist 2000), and an ecoregion framework (Niemela et al. 1999).

Rivers and streams in Minnesota are physically, chemically and biologically diverse. They range in size from small headwater streams less than one meter wide to large navigable waterways such as the main stem of the Mississippi River. The majority of streams in Minnesota are considered warm or coolwater, but coldwater streams are also present, particularly in the northeastern and southeastern regions of the state. Riffles are

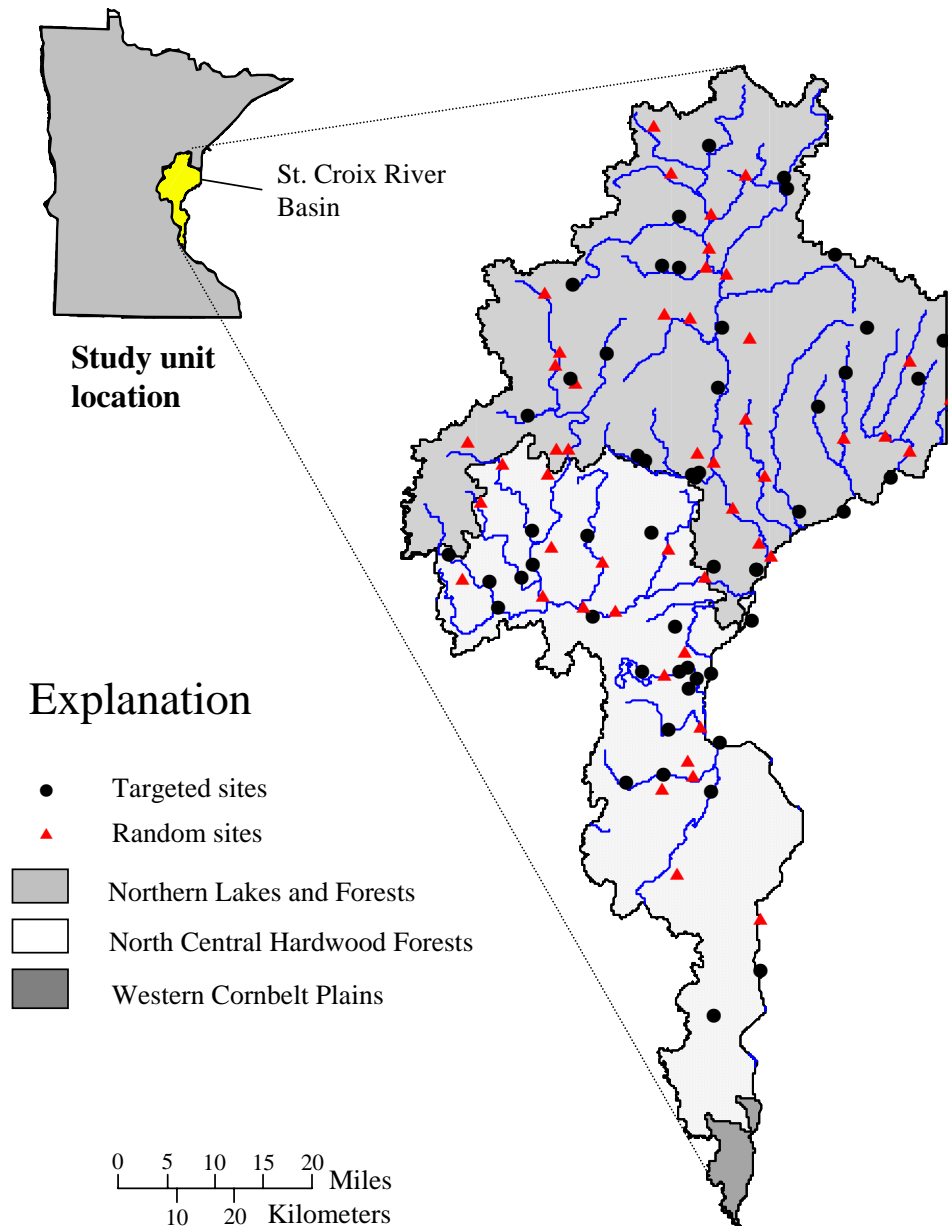


Figure 2. Map of the St. Croix River Basin in Minnesota with major rivers, ecoregional boundaries and the location of each site used to develop the St. Croix River Basin, Macroinvertebrate Index of Biological Integrity (MIBI)

an important feature of high gradient streams. However, in many of Minnesota's low gradient streams there are few or no riffles.

Within a stream reach, variables such as stream size, gradient, and habitat have a great influence on the type of aquatic assemblages present. An MIBI should account for reach level differences as well as regional differences through proper stream classification

Once a stream classification framework is developed to account for the natural variation in the invertebrate community structure, each metric within the MIBI must be selected and calibrated to account for differences in metric expectations between stream classes. For example, calibration of each metric is necessary because one would expect to find fewer stonefly taxa in a low gradient stream than a high gradient stream. It is also possible that metrics will need to be calibrated to account for stream morphological or ecoregional differences.

SITE SELECTION

The St. Croix basin MIBI was developed using data collected during the 1996 through 2000 sampling seasons. A complete list sampling stations and their corresponding MIBI scores is provided in Appendix 2.

The sites selected for development of an MIBI should focus on multiple sites within similar environments, across a range from minimal to severe human disturbance (Karr and Chu 1999). We sampled forty seven sites to represent a range of stream sizes, disturbances, and morphology types within the basin. Least disturbed sites were selected by a cursory assessment of habitat and land use within the watershed. Sites representing a condition of human influence

were selected by examining GIS landuse, point source discharge, feedlot, and stream ditching coverages to locate stream reaches where the cumulative effects of multiple stressors were likely to be the greatest. Forty additional sites were used in the analysis but were not selected specifically for the purpose of developing the MIBI. Rather, these sites were chosen randomly using an approach developed by the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) (U.S. EPA 2001) to monitor the condition of rivers and streams throughout the St. Croix basin. The randomly selected sites were important to the process of MIBI development because they allowed us to develop a better understanding of stream characteristics, the magnitude of human disturbance, and the types of human disturbance that appeared to be the most influential on biological integrity throughout the basin.

QUANTIFYING HUMAN DISTURBANCE

At any given point along a stream, resource integrity may be affected by the interaction of many human activities within the watershed. This is particularly true in a river basin like the St. Croix where a variety of land use activities occur. Human disturbances are complex and dynamic; because of this, no single variable can account for all disturbances. We explored numerous methods to define a disturbance gradient that most accurately reflected disturbance within the basin including: 1) general rankings of each site from excellent to poor based on our first hand knowledge of conditions at the site, 2) rankings based on GIS coverages for land use, ditching, point source discharges, feedlots, roadways etc., and 3) identification of variables from the habitat assessment (i.e. % fines, %

embeddedness, % of disturbed riparian area) that may reflect human disturbance. We chose a GIS-based watershed characterization of disturbance because it could be calculated easily using GIS landuse coverages, it could not be confused with naturally occurring factors (e.g., the percent fine substrate within the reach could be a reflection of human disturbance or natural geologic features within the watershed) and it is understandable conceptually. The more the watershed is altered, the higher the probability the rivers and streams within the watershed will be impaired.

Upstream land use in the watershed was characterized using 1990 vintage (MNDNR filename: lulcxy3) or 1995 vintage (MNDNR filename: lusatpy3) GIS land use coverages. The GIS land use theme was overlaid in Arcview onto the drainage area theme and clipped producing a land use theme identical in shape and size to the drainage area theme. Percentages for each land use were determined by summing land use across the entire drainage area and then dividing by the total area. The percent watershed disturbance was calculated by adding the percentages for the land use themes that were indicative of human disturbance. This included all agricultural and urban themes, grassland that was associated with pasture, and mines and open pits. Agricultural landuse was the most widely distributed disturbance within the St. Croix Basin.

HABITAT ANALYSIS

A quantitative habitat assessment was performed at each site to aid in stream classification and to help delineate excellent quality sites from poor quality sites. We used a quantitative habitat assessment procedure that was slightly modified from Simonson et al. (1993).

DISTURBANCE RATING

An a priori human disturbance rating system was developed to provide a summary of habitat data and to provide a subjective score of human disturbances upstream of the sampling location based on thorough examination of maps and aerial photography. Five variables were selected to reflect human impact at the sampling locations. Each variable was given a score of 0, 2, 4, 6, 8, or 10. Zero indicating severe impairment, 10 indicating minimal impairment. The variables included land use, riparian zone condition within and upstream of the sampling reach, permitted wastewater discharges and feedlots in close proximity to the stream upstream of the sampling location, the number of miles and proximity of ditches to the sampling location, and the habitat rating based on the quantitative habitat assessment. The rating was used to distinguish a priori differences between sampling locations, and to select and validate metrics.

STREAM CLASSIFICATION

Proper stream classification is an important component in MIBI development. With too few classes it may be difficult to distinguish between natural stream variability and human induced variability (Karr and Chu 1999). Alternatively, the limited resources available to conduct biological monitoring may be wasted with too many stream classes. We considered stream size, morphological type (riffle/run or glide/pool) and ecoregion as possible stream classification variables.

Stream temperature greatly influences the structure of the fish community and consequently, the metrics in a fish IBI. Temperature has less effect on the invertebrate community, but since the goal

Table 1. Guidelines for classifying stream reaches into a morphological type, listed in order of importance. Habitat variables used to classify streams by morphological type were collected using Wisconsin’s habitat assessment guidance (Simonson et al. 1993).

Stream Characteristics	Riffle/Run	Glide/Pool
Prevalence of riffles	Riffles present within the stream reach	No riffles within the stream reach
¹ Width-to-Depth ratio	Usually > 12	Usually 12 or less
² Stream gradient	Usually > 1.0 m/km	Usually < 1.0 m/km
Substrate type	Course substrates usually prevalent	Course substrates not a significant component of stream bottom
Riparian zone type	In least impacted streams the dominant riparian vegetation is usually forest	In least impacted streams the dominant riparian vegetation is usually wetland, grass, or shrubs.

¹Width-to-depth ratio is obtained by dividing the average stream width by the average thalweg depth in runs and pools.

²Stream gradient was obtained using 1:24,000 USGS topographic maps.

of this study was to develop fish and invertebrate IBIs concurrently, we did not include stream reaches considered to be coldwater. Data from a stream that contained a significant population of trout or based on water temperature data were considered coldwater were omitted from the data set.

The St. Croix River Basin MIBI accounts for differences in metric expectations due to morphological and size characteristics by developing scoring criteria for two stream morphological classes, and two size classes. We categorized sites as either riffle/run or glide/pool based on observational data and habitat information collected using Wisconsin’s habitat assessment guidance (Simonson et al. 1993).

The habitat features used to distinguish between different stream morphological classes were: the presence of riffles within the reach, stream gradient, width to depth ratio, and substrate type. The other important stream characteristic considered was riparian vegetation (Table 1). We further divided the riffle/run

class into two size classes; streams with a drainage area less than or equal to fifty square miles, and streams with a drainage area greater than fifty square miles.

To determine if stream morphology had a significant influence on invertebrate metric expectations, we compared least impaired

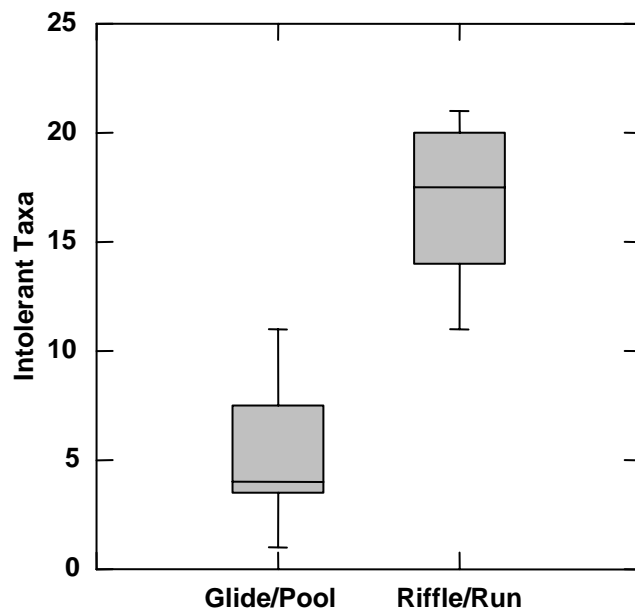


Figure 3. Box and whisker plot showing a significant difference ($p < 0.05$) between least impaired glide/pool and riffle/run sites based on the number of intolerant taxa.

sites between each morphological class. A Mann-Whitney U test was used to test for significant differences between the metrics of least impaired sites for each classification. Glide/pool and riffle/run sites were shown to be significantly different from each other ($p < 0.05$) for most of the metrics (Figure 3), verifying that using a morphological classification scheme for streams is valid for benthic macroinvertebrate community analysis.

The influence of stream size on metric expectations was determined by examining the relationship between drainage area and selected richness metrics (Total and EPT taxa) for glide/pool and riffle/run sites separately. If either relationship was significant, a scatter plot of watershed drainage area (\log_{10}) vs. the richness measures was examined to determine size classification break points (Niemela and Feist 2002). Size classes were chosen to minimize differences in maximum species richness within each size class. However, the number of size classes that could be partitioned was limited by the resulting number of sites within each class. For example, a break point may be evident at a drainage area of $< 5 \text{ mi}^2$ but only 10 sites may fall into this category, making it very difficult to develop a robust IBI with so few sites.

For the riffle/run streams, both total taxa richness ($R^2 = 0.458$, $P < 0.01$) and EPT ($R^2 = 0.603$, $P < 0.001$) exhibited a significant relationship with drainage area (\log_{10}). Since the relationship with EPT was the stronger of the two, the scatter plot of EPT vs drainage area (\log_{10}) was used to determine size classes (Figure 1). Given the number of riffle/run sites ($N = 40$), it was decided that two size classes could be delineated while allowing for an adequate number of sites in each class with a gradient of human influence for developing an IBI.

Therefore, a size classification break point that was close to bisecting the number of sites was selected. The riffle/run M-IBI accounts for differences in species richness due to stream size by developing separate scoring criteria for two stream size classes: $< 50 \text{ mi}^2$ and $> 50 \text{ & } < 500 \text{ mi}^2$.

In glide/pool streams there was no significant relationship between either total taxa richness or EPT and drainage area ($P > 0.05$). Therefore, it was not necessary to develop separate scoring criteria based on stream size for this morphological class of streams.

In addition to a graphical analysis, differences were looked at by comparing least impacted sites between stream size classes. A Mann-Whitney U test was done to test for significance. Class size breaks were examined at 20, 30, 40, 50, 60, 70, 80, 100, 150, 200 and 270 square miles of drainage. Several metrics previously shown to be strong indicators were used to examine

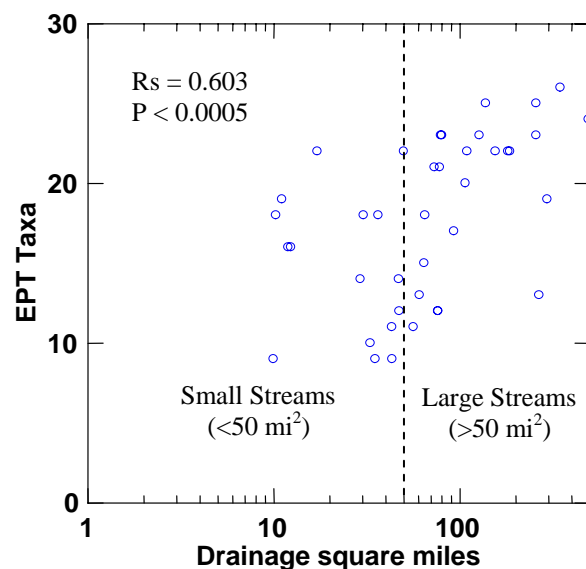


Figure 4. Number of Ephemeroptera, Trichoptera, and Plecoptera taxa (EPT) versus drainage area (mi^2) in riffle/run streams less than 500 mi^2 . Vertical line represents size classification break point.

the breaks, including total taxa richness, intolerant taxa, Ephemeroptera taxa, Plecoptera taxa, and Tricoptera taxa. Within the riffle/run class of streams the strongest differences for the greatest number of metrics occurred at 50 square miles of drainage ($p < 0.05$). Within the glide/pool class of streams no strong break was found.

Ecoregion could not be properly evaluated as a possible stream classification scheme. There were not an adequate number of least impaired sites in the North Central Hardwood Forest to make a statistically rigorous comparison between ecoregions. When a comparison was made using all sites, there was a significant difference between ecoregions. This was likely due to the current landuse patterns in the ecoregions being compared. The portion of the St. Croix River Basin that lies in the North Central Hardwood Forest ecoregion has a higher percentage of urban and agricultural landuse, making it significantly different than, but less suitable for comparison with, the less urbanized, more highly forested, Northern Lakes and Forests ecoregion.

Given a larger sample size, it is likely that more stream classes could have been found (two morphology classes, two ecoregion classes, several potential size classes). With the sample size taken, more classes would have meant fewer sites per class, and would have limited our ability to conduct statistically rigorous tests on candidate metrics.

To calculate the watershed area upstream of sampling sites, we used the Minnesota Planning Land Management Information Center's (LMIC) Upstream program. The watershed containing the site was picked from MNDNR's 1995 minor watershed file (bas95ne3) using the latitude and longitude

of the site. The MNDNR minor watershed boundaries are nearly equivalent to the 14-digit hydrologic unit code (HUC) developed by the U.S. Geological Survey.

Upstream additions were confirmed using the MNDNR's 24K streams file (dnrstln3).

It may be necessary to edit the minor watershed containing the site so that the portion of the water downstream of the site is not included in the drainage area calculation. We edited the minor watershed containing the site using Geographic Information System (GIS), Arcview coverages. However, in most cases an estimate of the minor watershed area upstream of the site may be determined using U.S. Geological Survey (USGS) standard series, 1:24,000 topographical maps. The following methods were used, in order of preference, to edit the minor watershed containing the site:

- a) using Arcview to delineate the drainage area with digital elevation models (DEM)
- b) following the contour lines on digital raster graphics (DRG) from USGS standard series topographic maps.
- c) personal experience of watershed boundaries from visiting the site

IV. BENTHIC SAMPLING AND LABORATORY METHODOLOGY

WHEN TO SAMPLE

Sampling occurs in the late summer/fall, from late August through early October. Flood and drought events can have strong effects on macroinvertebrate community structure; therefore streams should be sampled under stable, base flow conditions. Sampling should be delayed in streams following high flow events until stable

conditions return. If a stream is known to have been dry at an earlier date in the sample year, it should not be sampled.

SAMPLING REACH DETERMINATION

It is important to collect a sample representative of the stream reach selected. The reach established during site reconnaissance must be walked in its entirety to determine the presence and abundance of productive macroinvertebrate habitats. The reach length is based on what is necessary to collect an adequate fish sample, 35 times the average stream width (Lyons 1992b). It is not necessary to sample the entire reach for invertebrates. The important thing is that all major habitat types are sampled. Collecting an adequate sample normally requires walking 200 to 300 ft of stream length, although sometimes much longer distances must be covered.

BENTHIC SAMPLING TECHNIQUE

A qualitative multi-habitat sample is taken at each sampling location. The only piece of sampling gear used is the D-Frame dip-net, with a 500 micron mesh net. Care must be taken when collecting a sample to ensure that as many invertebrates are collected for each area sampled as possible. The net should always be held downstream of the area being sampled. When flow is negligible, the net must be swept repeatedly in upstream fashion to ensure that as many invertebrates are collected as possible.

Qualitative Multi-habitat sample - QMH:

The qualitative multi-habitat sample is collected to characterize the overall diversity of the sample reach. All productive habitats are sampled in proportion to their presence within the predefined stream reach. For example, if 20 percent of the reach habitat consists of riffles, then 4 of the 20 samples

collected should come from riffles. Fine sediment substrates are not sampled. Samples are collected in a downstream to upstream fashion. Twenty sampling efforts, or sweeps, are collected and composited in a 500 micron mesh sieve bucket. Samples are labeled and preserved in 100% denatured ethanol.

The 5 productive habitats to be considered when sampling include; 1) riffles or shallow, fast flowing runs, 2) undercut banks and overhanging vegetation, 3) submerged or emergent aquatic macrophytes, 4) snags and woody debris, and 5) leaf packs.

A sample effort is defined as taking two D-net samples in a common habitat. A sample is taken by placing the D-net on the substrate and disturbing the area directly upstream of the net opening equal to the square of the net width, ca. 1ft². Each effort should cover approximately .18m² of substrate. Total area sampled is ca. 3.6m².

This process becomes complicated when dealing with multi-dimensional substrates like weed beds and woody debris. Following is a description of each habitat and how to sample it.

Riffles - This category is intended to cover rocky substrates with fast flowing water. Runs and wadeable pools often have suitable rocky substrates, and should not be excluded from sampling. To sample riffles the D-net should be placed firmly and squarely on the substrate downstream of the area to be sampled. If the water is shallow enough, the area directly in front of the net should be disturbed with the hands, taking care to clean off large rocks directly into the net. If the water is too deep for this, kicking the substrate in front of the net is adequate.

Aquatic Macrophytes - Any vegetation found at or below the water surface should be considered in this category. Emergent vegetation is included because all emergent plants have stems that extend below the water surface, serving as suitable substrate for macroinvertebrates. The emergent portion of these plants should not be sampled. Submerged plants should be sampled with an upward sweep of the net. If the net fills with weeds, the weeds should be hand washed vigorously or jostled in the net for a few moments and then discarded. Emergent plants should be sampled with horizontal and vertical sweeps of the net until the area being swept has been thoroughly sampled.

Undercut Banks/Overhanging Vegetation -

This category is meant to cover shaded, in-bank or near-bank habitats, away from the main channel that typically are buffered from high water velocities.

Undercut banks can vary in how undercut they are. An additional problem is that many banks appear undercut, but when investigated prove not to be. For these reasons banks must be prodded to determine how deeply they are undercut. Overhanging vegetation should be treated the same way. Sampling should consist of upward thrusts of the net, beating the undercut portion of the bank or the overhanging vegetation, so as to dislodge any clinging organisms.

Woody Debris - Woody debris can include any piece of wood found in the stream channel. Logs, tree trunks, entire trees, tree branches, large pieces of bark, rootwads and dense accumulations of twigs should all be considered snags. Best professional judgment must be used to determine what a "sampling effort" is. Approximating the amount of sampleable surface area is a sensible method with larger tree trunks or branches. Whereas masses of smaller

branches and twigs must be given a best guess. Given their variable nature, there is not single, superior method for sampling snags. Using something akin to a toilet brush works well for large pieces of wood, whereas kicking and beating with the net works best for masses of smaller branches.

Leaf Packs - Leaf packs are dense accumulations of leaves typically present in the early spring and late fall. They are found in deposition zones, generally near stream banks, around logjams, or in current breaks behind large boulders. A leaf pack sample should be taken near the surface of the leaf pack. Sweeping to the bottom of every leaf-pack could create a disproportionately large amount of sample volume being collected for a given area. Due to the sample index period, leaf packs are generally not dominant enough to be included in a sample.

Laboratory Sample Processing

Due to the large volume of sample material, the QMH sample is subsampled using a 24 inch by 24 inch gridded screen tray divided into 144 two inch squares. The sample material is spread evenly across this grid and organisms are picked from randomly selected grid squares until a minimum of 300 organisms were collected. Following this, any large and/or rare organisms are removed from the remaining sample material on the grid. The two sub-sample components are not combined until the data is analyzed.

Ten percent of each sample is checked for picking efficiency by another biologist. If more than ten percent of organisms previously picked are found, the sample is reprocessed. Entire samples are checked for new staff until picking efficiency is 95% or better.

All organisms are identified to the genus level if possible. Five percent of all samples identified are checked for proper taxonomic characterization. An independent taxonomist resolves taxonomic discrepancies. A reference collection is maintained for taxonomic comparisons.

V. THE METRICS

When considering metrics for inclusion in the MIBI it is important to choose metrics that have been found to be biologically meaningful based on their ability to respond to human disturbance. Numerous invertebrate metrics have been used by states and agencies around the country that have been shown to respond to human disturbance, of one form or another, in predictable ways. For example, species richness of mayflies, stoneflies, and caddisflies have been shown to be reduced by agricultural impacts such as sedimentation and nutrient enrichment (Lenat 1984, Quinn and Hickey 1990a, b). See Table 2 for a list of metrics used in other studies and their predicted response to disturbance.

Most metrics can be grouped into 4 general classes: richness measure (such as total taxa), tolerance measures (such as percent tolerant taxa), composition measures (such as percent dominant two taxa), and trophic measures (such as percent shredders). Metrics which measure richness are those used most widely in multi-metric indices. Taxonomic diversity, particularly among groups known to be intolerant of pollution is a useful measurement of the degree of water quality impairment.

GRAPHICAL ANALYSIS OF CANDIDATE METRICS

In order for a metric to be selected for inclusion in the MIBI it must have been able to discriminate between known impaired and known reference sites, *or* show a significant relationship with a human disturbance gradient. Invertebrate community attributes were selected as metrics based on: 1) their ability to distinguish between the five least-impaired and five most-impaired sites; 2) a significant relationship with human disturbance ($p < 0.05$); and 3) their contribution of non-redundant information to the final M-IBI.

Box-and-whisker plots were used to evaluate how well each metric could discriminate between the five most-impaired and the five least-impaired sites. The five most impaired sites within each stream class were those that had the lowest human disturbance rating. Box and whisker plot comparisons should show a significant vertical separation. A Mann-Whitney U test was used to test for significant differences between the most and least disturbed sites (Figure 7). Metrics were considered strong discriminators of impairment if the difference between impaired and reference sites were significant (Mann-Whitney U, $p < 0.05$).

Spearman values were calculated to test for significance of the dose response relationship with human disturbance (Table 9). A metric was maintained if it was shown to have a significant relationship with watershed disturbance (Spearman r_s , $p < 0.05$). Ideally, every metric would show a response along a gradient of human disturbance, but due to the large number of sites of intermediate quality a linear response is not always attainable. In order

Table 2. Metrics used by other states.

Metric	Predicted response	Ohio EPA	Florida DEP	Oregon DEQ	Maine	RPB	Tennessee Valley
Taxa Richness							
Total taxa	Decrease	X	X	X	X	X	X
EPT taxa	Decrease	X	X	X	--	X	--
Ephemeroptera taxa	Decrease	X	--	--	--	--	X
Plecoptera taxa	Decrease	X	--	--	--	--	X
Trichoptera taxa	Decrease	X	--	--	--	--	X
Dipteran taxa	Increase	X	--	--	--	--	--
Chironomidae taxa	Decrease	--	X	--	--	--	--
Long lived taxa	Decrease	--	--	--	--	--	--
POET taxa	Decrease	--	--	--	--	--	--
Odonata taxa	Decrease	--	--	--	--	--	--
Composition							
Abundance	Variable	--	--	--	X	--	X
% Oligochaetes	Increase	--	--	--	X	--	X
% Chironomidae	Increase	--	--	X	X	--	--
% very tolerant	Increase	--	--	--	--	--	--
% legless	Increase	--	--	--	--	--	--
% Crustacea + Mollusca	Decrease	--	--	--	--	--	--
% Tanytarsini to Chironomidae	Decrease	X	--	--	--	--	--
% other Diptera and non-insect	Increase	X	--	--	--	--	--
% dominant taxa (1 or 2 taxa)	Increase	--	X	X	--	--	X
% Diptera	Increase	--	X	--	X	--	--
% Corbicula		--	--	--	--	--	X
% Ephemeroptera		X	--	--	--	--	--
% Trichoptera		X	--	--	--	--	--
Tolerance							
Intolerant taxa	Decrease	--	--	--	--	--	--
Sediment-intolerant	Increase	--	--	--	--	--	--
% tolerant	Increase	X	--	--	--	--	--
% sediment tolerant	Increase	--	--	--	--	--	--
Hilsenhoff Biotic Index (HBI)	Increase	--	--	X	--	X	--
Intolerant snail and Mussel taxa	Decrease	--	--	--	--	--	X
Trophic structure and other habits							
% predators	Decrease	--	--	--	--	--	X
% scrapers	Variable	--	--	X	--	--	X
% gatherers	Variable	--	--	--	--	--	--
% filterers	Variable		X	X	--	--	X
% omnivores	Increase	--	--	--	--	--	X
% shredders	Decrease	--	--	X	--	--	--
% mud burrowers	Increase	--	--	--	--	--	--
Clinger taxa	Decrease	--	--	--	--	--	--
Ratio of scrapper/filterer	Decrease	--	--	--	--	X	--

to attain a robust set of metrics, some metrics were retained for redundancy testing that showed a significant response in one evaluation and a strong, but not significant, response in the other.

To evaluate the redundancy in information provided from the metrics a correlation analysis was done between all candidate metrics. Metrics that are highly correlated with each other and show a graphically linear relationship contribute approximately the same information. Those with scatter in

the correlation or those that are nonlinear can still contribute useful information despite a strong correlation (Barbour et al. 1996, Karr and Chu 1999). A metric was retained if there was a non-linear, or curvilinear relationship. If the correlation coefficient was 0.85 or greater, and the relationship was linear, the two correlated metrics were analyzed further to determine if one should be disregarded. When two metrics are strongly correlated, it is not justifiable to automatically disregard one metric if it is known that the two metrics represent two different functional components of the biological community. Given a different set of environmental conditions (i.e. different types of disturbance) each metric may respond in a non-parallel manner (Niemela and Feist 2002).

SCORING METRICS

Cumulative density functions (CDF) were used to score each metric (Figure 5). A CDF distribution tells what percent of the total observations in the data are of a particular value or lower (Kachigan 1986). If natural breaks were apparent in the CDF, vertical lines were drawn at the breaks (Figure 5a) dividing the graph into three sections. In Figure 5a two distinct natural breaks are shown. If no natural breaks were apparent, lines were drawn to reflect the 33rd and 67th percentiles of the CDF (Figure 5b). CDF plots with scoring criteria and break points for all glide/pool metrics used in the St. Croix MIBI are shown in Figure 6.

V. CALCULATION AND INTERPRETATION OF THE MIBI SCORE

The MIBI is intended to be used in streams with drainage areas less than 500 square miles. The upper end of the size

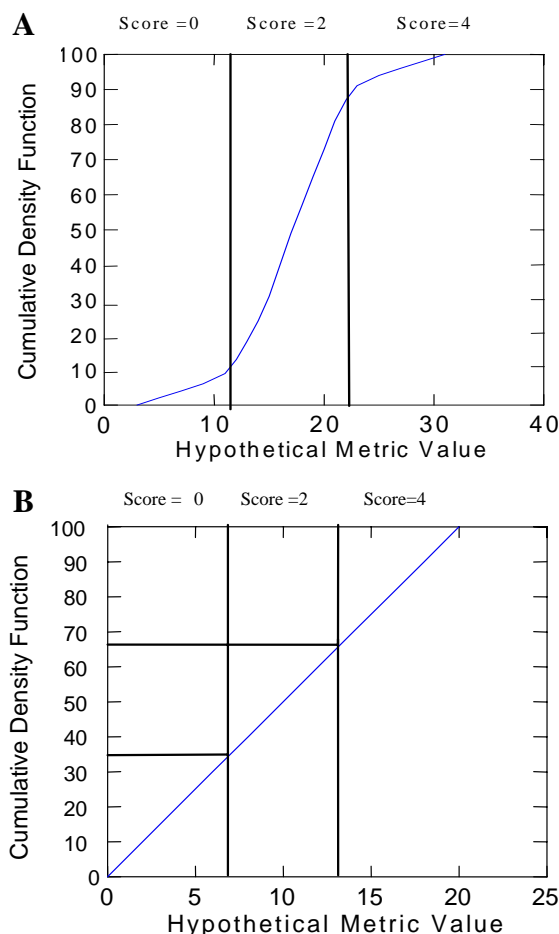


Figure 5 Hypothetical example of metric scoring based on cumulative density function (CDF) plots. Graph A depicts natural breaks in the data; Graph B depicts a linear progression, with breaks at the 33rd and 67th percentiles.

classification reflects the level beyond which we classify streams as large streams or rivers for the sake of MIBI development. Large streams and rivers are either too large for effective invertebrate sampling or are unsampleable. These streams are difficult to accurately characterize, and have not been

found suitable for inclusion in most macroinvertebrate IBIs. Morphological and size classifications were chosen because most metrics differ significantly between least impaired sites of each classification. Separate scoring criteria have been developed for glide/pool, small riffle/run,

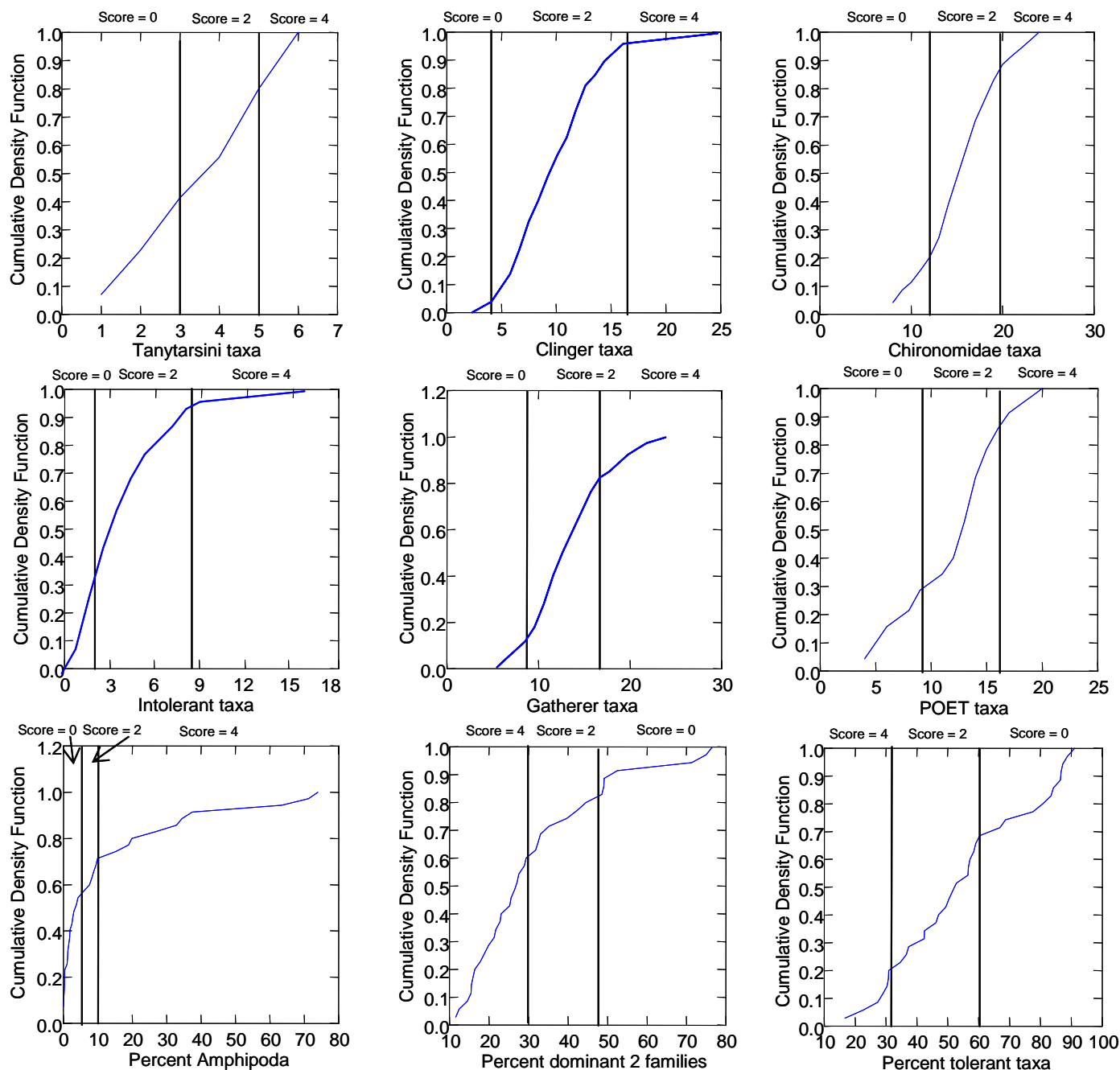


Figure 6 CDF plots with scoring criteria and break points for each metric used in the glide/pool stream class in St. Croix River Basin MIBI.

and large riffle/run streams. To be classified as riffle/run a stream had to have shallow gravel/cobble substrate, with slow to fast, non-laminar flow. Streams were classified as glide/pool if flow was slow and laminar, regardless of substrate. To be classified as large riffle/run a riffle/run site had to have a drainage area larger than 50 square miles, but less than 500. Small riffle/runs include all riffle/run streams with less than 50 square miles of drainage area.

The biological integrity of the site is determined by summing the metric scores for the appropriate stream class. Each metric in the MIBI represents a unique and important aspect of the invertebrate community. A low metric score indicates that the macroinvertebrate community deviates substantially from a minimally disturbed site. Conversely, a high metric score indicates that the macroinvertebrate community approximates that of a minimally disturbed site. Many of the same metrics are used in each MIBI. However, a few metrics are unique to a single stream class. For a list of stream metrics and corresponding scoring criteria refer to Table 4.

Scores of 0, 2, and 4 have been assigned for each metric (Table 4). Metric scores are added to produce a total MIBI score ranging from 0 to 40 for small riffle/run sites, 0 to 24 for large riffle/run sites, and 0 to 36 for glide/pool sites. Scores are then normalized to a one hundred point scale to allow for a easily understandable and comparable scoring range. Narrative descriptions of characteristics of the invertebrate community within certain MIBI scoring ranges can be used as a guideline for interpreting the MIBI score (Karr 1981) (Tables 7, 8, and 9). A list of the sampling sites and the MIBI score for each site is provided in Appendix 1.

Three factors may contribute to the variability of MIBI scores: sampling error, natural variability, and human disturbance. The first two sources of variability must be limited in order to detect the third.

Sampling error results from a failure to characterize the invertebrate community with accuracy and precision. Natural variability occurs because of climatic fluctuations, biological interactions, or any other factor that cannot be attributed to human disturbance (Lyons 1992a). Proper study design and rigorous adherence to sampling protocols can limit the effects of sampling error and natural variation on the MIBI score.

The MIBI methodology described in this report will allow the user to detect changes in environmental condition due to human disturbance with a reasonable level of certainty.

VII. RESULTS AND DISCUSSION

METRICS USED IN THE MINNESOTA MIBI

Based on metric selection criteria the 13 metrics listed in table 3 were maintained for use in the MIBI for each respective stream class.

The rationale for the usefulness of each metric is described below. All of the metrics selected have either been successfully used in MIBIs developed by other states and organizations, or have been tested and considered for inclusion in other IBIs.

The metrics selected for use in this IBI should not be considered as the only useful metrics for the St. Croix basin. This is the first attempt to develop an index of

Table 3. Metrics used for each stream class in the MIBI for St. Croix River Basin, Minnesota.

Metric Name	Glide Pool	Small Riffle-run	Large Riffle-run
# Ephemeroptera Taxa	---	X	---
# Plecoptera Taxa	---	X	---
# Trichoptera Taxa	---	X	X
# Chironomidae Taxa	X	X	---
# POET Taxa	X	---	---
# Intolerant Taxa	X	X	X
% Tolerant Taxa	X	---	X
# Clinger Taxa	X	X	X
# Tanytarsini Taxa	X	X	---
# Gatherer Taxa	X	X	---
# Filterer Taxa	---	---	X
% Amphipoda Taxa	X	X	X
% Dominant 2 Taxa	X	X	---

biological integrity for this region, and additional data could lead to an alteration in the suite of metrics selected.

Note on taxonomic richness metrics:

Taxonomic diversity is considered a good indicator of environmental quality. The usefulness of this type of metric is demonstrated by the fact that as environmental disturbance increases, and natural systems are disturbed, taxonomic diversity decreases (Lenat 1988).

The following rules are applied to counting total number of taxa for each taxonomic metric:

- 1) A family level identification with less than one taxon identified to a lower level will be counted as a separate taxon.
- 2) A family with one or more taxa identified to a lower taxonomic level will not be counted. Counts will be split amongst genera that are present.
- 3) Higher level taxonomic identifications are not counted unless they are the only representatives of that group.
- 4) Pupae are not considered.

- 5) All identifications made to the species level will be aggregated to the generic level for the purposes of taxa counting.

Taxonomic Richness Measures

Number of Chironomidae Taxa: The chironomidae, or midges, are a diverse and abundant group of aquatic insects in Minnesota. Tolerant forms have historically been known to exist in very high densities in highly polluted areas. However, the Chironomidae display a wide array of sensitivities and their diversity is a good indicator of environmental health. This metric, much like the total taxa metric, only measures diversity.

Number of Ephemeroptera Taxa:

Ephemeroptera, or mayflies, are benthic invertebrates that are sensitive to environmental disturbance. They occupy a variety of habitats including interstitial spaces between rocks, rock surfaces, sediment, and aquatic vegetation. Most mayflies are sensitive to low dissolved oxygen; some are sensitive to metals, as well as others toxicants.

Number of Trichoptera Taxa: Trichoptera, or caddisflies, are a diverse group of benthic insects that are considered good indicators of environmental disturbance. As a group, they are somewhat more tolerant to pollution than mayflies, but in the presence of significant impairment they do not persist as a diverse community. Trichopterans inhabit a wide variety of habitats, ranging from fast flowing riffles, to sparsely vegetated pools, and slow moving wetland type reaches. Because of their ability to exploit a variety of habitats, their diversity is a good indicator of habitat quality. Their ability to thrive in lentic conditions makes them excellent indicators for use in slow moving streams as well.

Number of Plecoptera Taxa: Plecoptera, or stoneflies, are among the most sensitive indicator organisms. They occupy the interstitial spaces between rocks, woody debris, and vegetation, and require a relatively high amount of dissolved oxygen in order to survive. Because they are generally absent from low gradient streams, this metric is not included in the MIBI for glide/pool streams. The absence of stoneflies in riffle/run type streams can indicate impairment resulting from low dissolved oxygen, or siltation.

Number of Plecoptera, Odonata, Ephemeroptera, and Trichoptera Taxa (POET): Stoneflies, mayflies and caddisflies are included in this low-gradient stream metric for reasons already indicated. Odonata, or dragon and damselflies, are a diverse group of organisms that display a wide array of sensitivities and life histories. They exploit most aquatic microhabitats, and their diversity is considered a good indicator of aquatic health. Because Odonata tend to be more dominant in slow moving water than stoneflies, they further supplement this EPT-like metric in low gradient streams.

Tolerance Measures

Number of Clinger Taxa: Clinger taxa are organisms that have morphological adaptations that allow them to thrive by attaching to the substrata in fast flowing water. Clinger taxa include flat bodied organisms such as stoneflies and Heptageniid mayflies; organisms that attach themselves to rocks and plants, such as blackflies and crane flies; net-spinning caddisflies that attach themselves to stationary substrates; and case-building caddisflies (Rossano 1995, Merritt and Cummins 1996). A diverse group of clinger taxa indicate that substrate has not become

embedded or covered by fine organic or inorganic material. A lack of clinger taxa can indicate siltation or substrate embeddedness that generally is the result of erosion.

Number of Intolerant Taxa: Number of Intolerant Taxa is a direct measure of taxa richness of those organisms receiving a score of two or lower in the Hilsenhoff Biotic Index (HBI) (Hilsenhoff 1987). The HBI was developed as a tool to monitor the effects of organic enrichment on the aquatic invertebrate community. An organism with a high score has been defined by Hilsenhoff to be tolerant of organic pollution. An organism with a low score is considered sensitive to organic pollution. The presence of moderate numbers of intolerant taxa is an indicator of good aquatic health.

Percent Tolerant Taxa: This metric looks at relative abundance of tolerant taxa. Tolerant taxa are those that receive a rating of eight or higher in the HBI. Tolerant invertebrates are often found to thrive in areas known to have low dissolved oxygen, high turbidity, or heavy siltation. Unlike intolerant taxa, tolerant organisms occur at all sites but tend to dominate in relative abundance as conditions are degraded (Fore et al. 1996).

Number of Tanytarsini Taxa: This metric was developed as an additional way to express intolerance in the invertebrate community. The tribe Tanytarsini is generally considered to be intermediate in pollution tolerance, and can decline under moderate pollution stress (DeShon 1995).

Composition Measures

Percent Dominant 2 Taxa: The relative abundance of the two most dominant taxa tends to increase in degraded streams. Healthy aquatic ecosystems tend to have

diverse invertebrate communities in which no one or two taxa dominate the community. An uneven distribution of organisms or a population dominated by one or a few taxa, can be indicative of disturbance.

Percentage of Amphipoda: Amphipoda are considered to be tolerant of organic pollution, and can become very abundant in conditions of low dissolved oxygen. Their abundance has been shown to be a good indicator of impairment across a range of stream classes and condition.

Trophic Structure Measures

Number of gatherer taxa: The number of gatherer taxa represents the number of different taxa that collect their food by gathering it from the substrate.

Number of filterer taxa: The number of filterer taxa represents the number of different taxa that collect their food by filtering it out of the water column. The filtering is typically done one of two ways: 1) by using physical adaptation such as a filamentous antennal structure or 2) by constructing a net which filters the water and gathering filtered material from the net.

GLIDE POOL SITES (0 TO 500 MI² DRAINAGE AREA)

37 Glide pool sites were sampled in the late summer/fall of 1996 and 1997. 42 sites were visited, but five were dry and not sampleable.

Low gradient, laminar flow, and a lack of riffle habitat characterize glide pool sites. Glide pool sites are not necessarily devoid of rocky areas, but they lack the flow to create the turbulent, well oxygenated habitat that riffle dwelling organisms prefer. Many of these sites lacked measurable flow and

the productive invertebrate habitats were dominated by woody debris, vegetated/ undercut banks, and aquatic vegetation.

Metric Selection

Of the 30 metrics tested for glide pool streams, 16 were either significantly correlated with disturbance or the range of values of the five most disturbed and five least disturbed sites for each stream morphology class were significantly different. Of these, 9 were chosen that showed either a significant response in both tests, or a significant response in one and a strong response in the other. The glide pool metrics selected include total Chironomidae taxa, total Clinger Taxa, total Plecoptera, Odonata, Ephemeroptera, and Tricoptera taxa (POET taxa), total Tanytarsini taxa, number of intolerant taxa, number of gatherer taxa, percentage of Amphipoda, percentage of tolerant taxa, and percent dominant 2 taxa.

Six of the glide/pool metrics used in the MIBI were significantly correlated with disturbance (Spearman r , $p < 0.05$) (Table 6). Ideally, every metric would show a response along a gradient of human influence, but due to the large number of sites of intermediate quality a linear response is not always attainable.

In order to determine if metrics were responding to human disturbance independent of a linear dose response relationship, box plots of most impaired and least impaired sites were examined to determine if there was significant vertical separation between the interquartile ranges of the corresponding conditions. Box plots indicated that all of the metrics tested, including those that did not show a significant correlation with disturbance,

Table 4. Range, predicted response to disturbance, and scoring criteria for metrics used in each stream class of the MIBI for the St. Croix Basin, Minnesota.

Metrics	Range	Predicted Response to disturbance	Score		
			0	2	4
<u>Glide/Pool Sites (<500 mi²)</u>					
Plecoptera + Odonata + Ephemeroptera + Tricoptera Taxa	4-20	decrease	<9	9-16	>16
Chironomidae Taxa	8-24	decrease	<13	13-20	>20
Clinger Taxa	2-20	decrease	<4	5-16	>18
Intolerant Taxa	0-11	decrease	<2	2-6	>6
Tanytarsini Taxa	1-6	decrease	<3	3-5	>5
Gatherer Taxa	8-26	decrease	<9	10-19	>17
Percent Tolerant Taxa	16.8-91.1	increase	>60	31-60	<31
Percent Dominant 2 Taxa	11.5-76.6	increase	>49	30-49	<30
Percent Amphipoda	0-74	increase	>10	6-10	<6
<u>Riffle/Run Sites (<50 mi²)</u>					
Ephemeroptera Taxa	1-6	decrease	<3	3-5	>5
Plecoptera Taxa	0-4	decrease	<2	2-3	>3
Trichoptera Taxa	3-12	decrease	<6	6-9	>9
Chironomidae Taxa	10-21	decrease	<13	13-18	>18
Clinger Taxa	6-23	decrease	<7	7-16	>16
Intolerant Taxa	2-20	decrease	<7	7-14	>14
Tanytarsini Taxa	1-7	decrease	<3	3-6	>6
Gatherer Taxa	10-27	decrease	<14	14-21	>21
Percent Dominant 2 Taxa	13.2-67	increase	>36	26-36	<26
Percent Amphipoda	0-62	increase	>10	5-10	<5
<u>Riffle/Run Sites (>50 mi² and <500 mi²)</u>					
Tricoptera Taxa	5-16	decrease	<7	7-13	>13
Clinger Taxa	11-36	decrease	<18	18-28	>28
Intolerant Taxa	3-21	decrease	<7	9-16	>16
Filterer Taxa	3-16	decrease	<7	7-13	>13
Percent Tolerant Taxa	1.5-24.9	increase	>10	4-10	<4
Percent Amphipoda	0-13.8	increase	>8	2-8	<2

showed a significant difference between impaired and reference sites (Mann-Whitney U, $p < 0.05$) (Figure 7). None of the 36 possible glide/pool metric pairs were highly correlated with each other (Spearman $r_s > 0.8$). See table 5 for the most highly correlated metrics in each stream class.

Testing the MIBI for glide pool sites

The 9 metric MIBI developed for glide pool streams showed a significant negative correlation with percent disturbed landuse (Spearman $r_s = -0.470$, $p < 0.01$). The glide/pool MIBI also showed a significant difference between impaired and reference sites, (Mann-Whitney U, $p = 0.014$). This

demonstrates that the MIBI is able to discern the difference between an impaired and an unimpaired stream reach in the St. Croix River Basin in streams classified as glide/pool, smaller than 500 square miles in drainage area.

SMALL RIFFLE RUN SITES (0 TO 50 MILES² DRAINAGE AREA)

15 small riffle run sites were sampled in the late summer and early fall of 1996 and 1997. 18 sites were visited but 3 were dry and not sampleable.

Measurable flow and a sampeable area of course, rocky substrate, characterized riffle/run sites. Riffles had flow sufficient to create turbulent water. Most riffle/run sites also included bank vegetation, in-stream vegetation or woody debris.

Metric Selection

Of the 30 metrics tested for small riffle/run streams, 16 were either significantly correlated with disturbance or the range of values for the five most disturbed and five least disturbed sites for each stream morphology class were significantly different. Of the 16 metrics that demonstrated a response to disturbance, 10 were chosen that showed either a significant

response in both tests, or a significant response in one and a strong response in the other. The small riffle/run metrics chosen include total Chironomidae taxa, total Clinger Taxa, Ephemeroptera taxa, Plecoptera Taxa, Tricoptera taxa, Tanytarsini taxa, number of intolerant taxa, number of gatherer taxa, percentage of Amphipoda, and percent dominant 2 taxa.

Nine of the small riffle/run metrics used in the MIBI were significantly correlated with disturbance (Spearman r , $p < 0.05$) (Table 6).

Box plots indicated that 9 of the metrics tested, including one that did not show a significant correlation with disturbance, showed a significant difference between impaired and reference sites (Mann-Whitney U, $p < 0.05$).

Three of the 45 possible small riffle/run metric pairs were highly correlated with each other (Spearman $r_s > 0.8$) (Table 5). The highly correlated metrics were retained because they relate different functional components of the biological community.

Testing the MIBI for small riffle run sites

The 10 metric MIBI developed for small riffle run streams showed a significant negative correlation with percent disturbed landuse (Spearman $r_s = -0.884$, $p < 0.001$). The small riffle run MIBI also showed a significant difference between impaired and reference sites, (Mann-Whitney U, $p = 0.008$). This demonstrates that the MIBI is able to discern the difference between an impaired and an unimpaired stream reach in the St. Croix River Basin in streams classified as riffle run, smaller than 50 square miles in drainage area.

Table 5. Metrics with the highest Spearman Rank Correlation Coefficients.

Class	Metrics	r_s
Glide	Gatherer/Chironomidae	.762
Pool	% Tolerant/% Amphipoda	.792
Small	Clinger/Plecoptera	.936
Riffle	Tanytarsini/Gatherer	.808
Run	Tanytarsini/Amphipoda	.828
Large	Tricoptera/Clinger	.834
Riffle	Intolerant/Tricoptera	.737
Run		

Table 6. Spearman rank correlation coefficients and significance values for each metric and total IBI score against percent watershed disturbance within glide/pool and riffle/run streams.

Metric	<u>Glide/pool streams (0-500 mi²)</u>		<u>Riffle/run streams (0-50 mi²)</u>		<u>Riffle/run streams (50-500 mi²)</u>	
	correlation coefficient (r _s)	significance value (p)	correlation coefficient (r _s)	significance value (p)	correlation coefficient (r _s)	significance value (p)
<u>Taxa richness metrics</u>						
Number of ephemeroptera taxa			-.490	<.10		
Number of plecoptera taxa			-.635	<.05		
Number of trichoptera taxa			-.635	<.05	-.591	<.005
Number of chironomidae taxa	-.427	<.02	-.621	<.01		
Number of POET taxa (plecoptera, odonata, ephemeroptera, trichoptera,)	-.451	<.01				
<u>Tolerance metrics</u>						
Number of intolerant taxa	-.321	<.01	-.946	<.0005	-.716	<.001
Percent tolerant taxa	.315	<.01			.464	<.05
Number of clinger taxa	-.213	<.5	-.675	<.02	-.574	<.01
Number of Tanytarsini taxa	-.489	<.005	-.729	<.01		
<u>Trophic Metrics</u>						
Number of gatherer taxa	-.347	<.05	-.612	<.05		
Number of filterer taxa					-.475	<.05
<u>Community composition metrics</u>						
Percentage of Amphipoda	.405	<.02	.739	<.01	.481	<.05
Percent of the dominant two taxa	.352	<.05	.786	<.005		
<u>Total IBI score</u>	-.470	<.01	-.884	<.001	-.661	<.002
	n=34		n=13		n=22	

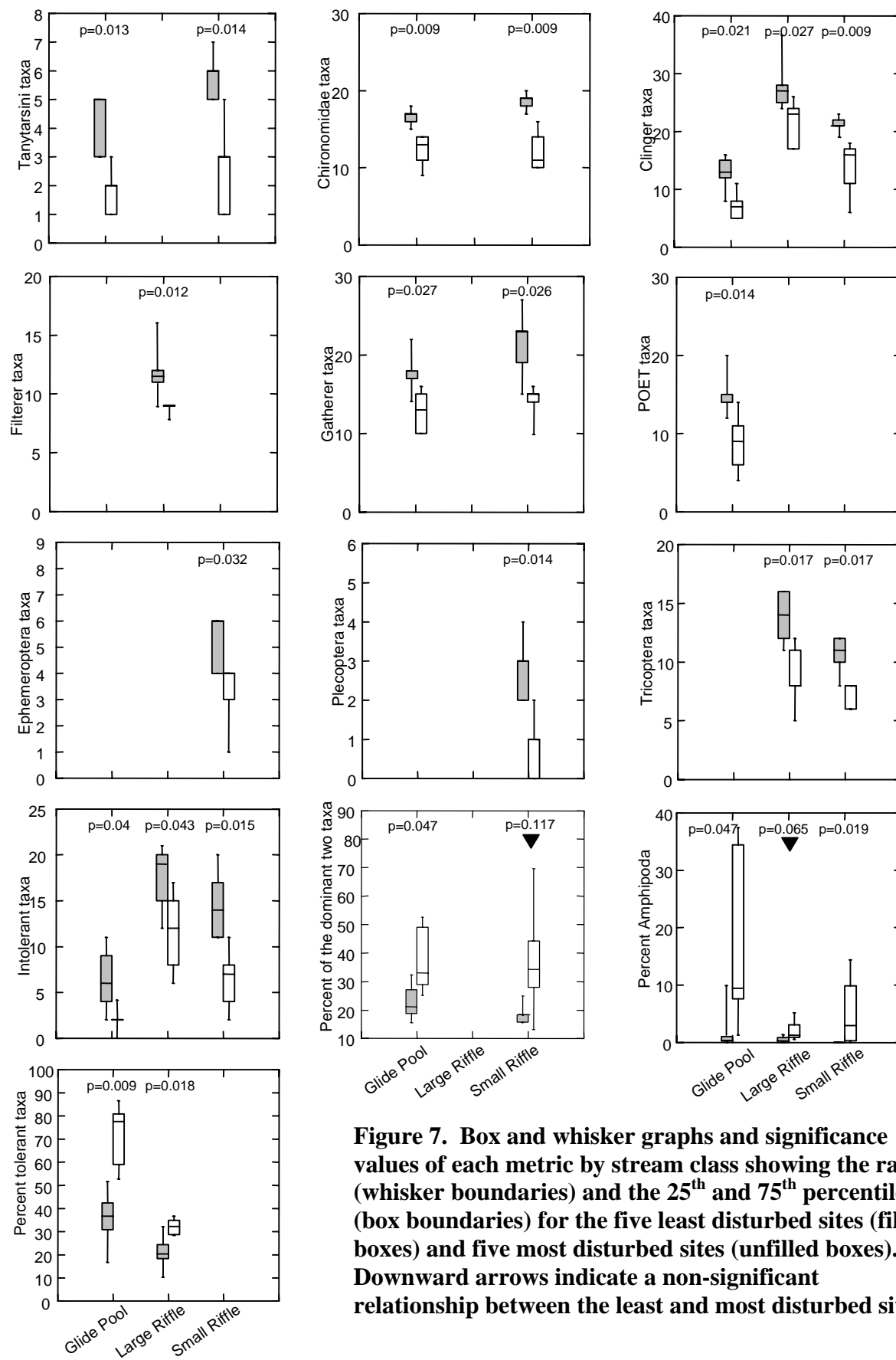


Figure 7. Box and whisker graphs and significance values of each metric by stream class showing the range (whisker boundaries) and the 25th and 75th percentiles (box boundaries) for the five least disturbed sites (filled boxes) and five most disturbed sites (unfilled boxes). Downward arrows indicate a non-significant relationship between the least and most disturbed sites.

LARGE RIFFLE RUN SITES (0 TO 500 MILES² DRAINAGE AREA)

25 large riffle run sites were sampled in the late summer and early fall of 1996 and 1997. 27 sites were visited, but 2 were dry and not sampleable.

A drainage area greater than 50 square miles and less than 500 square miles, measurable flow and a sampleable area of course, rocky substrate, characterized large riffle/run sites. Riffles had flow sufficient to create turbulent water. Most riffle/run sites also included bank vegetation, instream vegetation or woody debris.

Metric Selection

Of the 30 metrics tested for large riffle/run streams, 14 were either significantly correlated with disturbance or the range of values for the five most disturbed and five least disturbed sites for each stream morphology class were significantly different. Of the 14 metrics that demonstrated a response to disturbance, 6 were chosen that showed either a significant response in both tests or a significant response in one and a strong response in the other. The large riffle run metrics chosen include Clinger Taxa, Tricoptera taxa, number of intolerant taxa, number of filterer

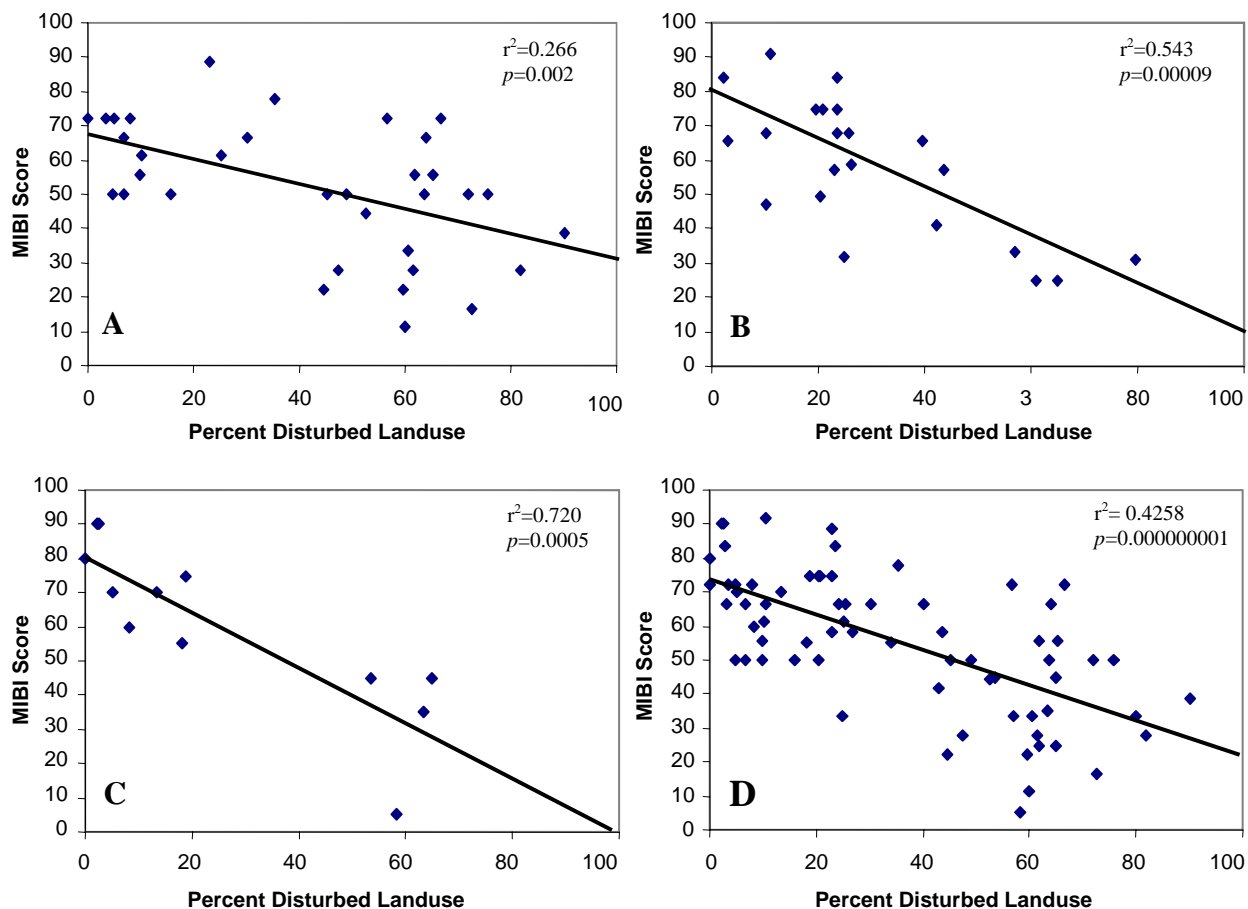


Figure 8. Macroinvertebrate index of biological integrity (MIBI) scores plotted against percent disturbed landuse in the upstream watershed for A) glide/pool streams (0-500mi² drainage area), B) large riffle/run streams (50-500 mi² drainage area), C) small riffle/run streams (0-50 mi² drainage area), and D) a composite of all MIBI scores throughout the basin.

taxa, percentage of Amphipoda, and percentage of tolerant taxa. All of the metrics used in the large riffle run MIBI were significantly correlated with disturbance (Spearman r_s , $p < 0.05$) (Table 6).

Box plots revealed that 5 of the metrics chosen showed a significant difference between impaired and reference sites (Mann-Whitney U, $p < 0.05$).

Of the 15 possible large riffle/run metric pairs one was highly correlated (Spearman $r_s > 0.8$) (Table 5). The highly correlated metrics were retained because they relate different functional components of the biological community.

Testing the MIBI for large riffle run sites

The 6 metric MIBI developed for large riffle run streams showed a significant negative correlation with percent disturbed landuse (Spearman $r_s = -0.661$, $p < 0.002$). The large riffle run MIBI also showed a significant difference between impaired and reference sites, (Mann-Whitney U, $p = 0.012$). This demonstrates that the MIBI is able to discern the difference between an impaired and an unimpaired stream reach in the St. Croix River Basin in streams classified as riffle run, greater than 50 square miles and less than 500 square miles in drainage area.

LARGE RIVER SITES (>500 MILES² DRAINAGE AREA)

10 large river sites were sampled in the St. Croix River Basin. Due to the limited number of sampling locations and the size of these rivers, it was decided that it was not appropriate to develop an independent IBI for large rivers. Additionally, we were not confident that our methods could accurately characterize the diverse nature of these large systems and thus we were not comfortable

in attempting to develop a tool for comparing one river reach to another.

ECOREGIONAL DIFFERENCES

Using the MIBI scores, it was found that there was a significant difference between streams in the NLF ecoregion and NCHF ecoregion for each stream class. In order to understand the nature of these differences it would be best to compare reference streams between the ecoregions. Unfortunately this was impossible as there were no reference sites sampled in the NCHF ecoregion. The lack of reference conditions, along with the fact that there was a significant difference between the amount of disturbed landuse between the ecoregions (Mann-Whitney U, $p < 0.005$), suggests that differences being detected between the ecoregions in the St. Croix basin are due to changes in the landscape rather than natural background conditions. As we continue to expand our sampling throughout the state, and work to further define a geographical framework for MIBI development, it will be necessary to find reference streams in the NCHF ecoregion in order to do a more rigorous statistical comparison of the ecoregions.

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APPENDIX 1 – NARRATIVE GUIDELINES FOR INTERPRETING MIBI SCORES.

Table 7. Narrative guidelines for interpreting overall MIBI scores for glide/pool streams in the St. Croix River Basin, Minnesota (modified from Karr 1981)

Overall IBI Score	Biotic Integrity Rating	Invertebrate Community Attributes
100-80	Excellent	Comparable to the best situations with minimal human disturbance; all regionally expected taxa for habitat and stream class, including the most intolerant forms, are present; balanced trophic structure
79-60	Good	Taxonomic richness somewhat below expectations, 34-64 taxa possible, but more commonly 51-55; decreased numbers of intolerant taxa, typically 5 or 6 present; typically less than 40% of the sample is comprised of tolerant forms; the dominant 2 families comprise 11 to 35% of all individuals, but more commonly 20%; balanced trophic structure with slightly elevated numbers of individuals of gatherer taxa.
59-40	Fair	Signs of additional deterioration include decreased taxa richness, 28-60 possible, but more commonly 44-46; decreased numbers of intolerant taxa, 1 to 9 taxa possible, typically 3 or 4 present; typically less than 55% of the sample is comprised of tolerant forms; the dominant 2 families comprise 14 to 55% of all individuals, but more commonly 30%; gatherer taxa beginning to dominate trophic structure, typically 35% of all individuals.
39-20	Poor	Community becoming dominated by tolerant forms, comprising up to 90% of the community, but typically 70 to 75%; decreased numbers of intolerant taxa, 0 to 8 taxa possible, typically 2 or 3 present; the dominant 2 families comprise 23 to 75% of all individuals, but more commonly 45%; gatherer taxa dominate the trophic structure, typically 48% of all individuals; taxa richness remains stable, 32-56 possible, but more commonly 44-45.
19-0	Very Poor	The community is indicative of an environment that is severely modified by human disturbance. Signs of additional deterioration include decreased taxa richness, up to 35 possible taxa; decreased numbers of intolerant taxa, 1 or 2 taxa possible; tolerant forms dominate the community, comprising up to 95% of the community, but typically 85 to 90%; the dominant 2 families comprise 44 to 88% of all individuals, but more commonly 70%; gatherer taxa dominate the trophic structure, typically 68% of all individuals, greatly reduced numbers of filterers.
No Score		Thorough sampling finds few or no invertebrate; impossible to calculate IBI.

APPENDIX 1 (continued)**Table 8. Narrative guidelines for interpreting overall MIBI scores for small riffle/run streams (0 to 50 mi² drainage area) in the St. Croix River Basin, Minnesota (modified from Karr 1981)**

Overall IBI Score	Biotic Integrity Rating	Invertebrate Community Attributes
100-80	Excellent	Comparable to the best situations with minimal human disturbance; 55 to 73 taxa possible, but more commonly 60 to 62; 14-20 intolerant taxa possible, typically 18; typically less than 40% of the sample is comprised of tolerant forms; the dominant two families comprise 11 to 26 of all individual, but more commonly 17%; balanced trophic structure
79-60	Good	Taxonomic richness somewhat below expectations, 44 to 67 taxa possible, but more commonly 54 to 56; decreased numbers of intolerant taxa, 8 to 18 taxa possible, typically 12 or 13 present; typically less than 40% of the sample is comprised of tolerant forms; the dominant 2 families comprise 15 to 35% of all individuals, but more commonly 23%; balanced trophic structure with slightly elevated numbers of individuals of gatherer taxa.
59-40	Fair	Signs of additional deterioration include decreased taxa richness, 38 to 56 possible, but more commonly 49 to 51; decreased numbers of intolerant taxa, 7 to 14 taxa possible, typically 8 or 9 present; typically less than 40% of the sample is comprised of tolerant forms; the dominant 2 families comprise 13 to 47% of all individuals, but more commonly 30%; balanced trophic structure with slightly elevated numbers of individuals of gatherer taxa.
39-20	Poor	Signs of additional deterioration include decreased taxa richness, 29 to 54 possible, but more commonly 45 to 50; decreased numbers of intolerant taxa, 2 to 7 taxa possible, typically 3 or 4 present; typically less than 40% of the sample is comprised of tolerant forms; the dominant 2 families comprise 27 to 47% of all individuals, but more commonly 30%; balanced trophic structure with slightly elevated numbers of individuals of gatherer taxa.
19-0	Very Poor	The community is indicative of an environment that is severely modified by human disturbance. Signs of additional deterioration include decreased taxa richness, up to 46 possible taxa; decreased numbers of intolerant taxa, up to 4 taxa possible; tolerant forms dominate the community, comprising up to 86% of the community; the dominant 2 families comprise 50 to 70% of all individuals; gatherer taxa dominate the trophic structure, typically 67% of all individuals.
No Score		Thorough sampling finds few or no invertebrate; impossible to calculate IBI.

APPENDIX 1 (continued)**Table 9. Narrative guidelines for interpreting overall MIBI scores for large /run streams (50 to 500 mi² drainage area) in the St. Croix River Basin, riffle Minnesota (modified from Karr 1981)**

Overall IBI Score	Biotic Integrity Rating	Invertebrate Community Attributes
100-80	Excellent	Comparable to the best situations with minimal human disturbance; all regionally expected taxa for habitat and stream class, including the most intolerant forms, are present; balanced trophic structure
79-60	Good	Taxonomic richness somewhat below expectations, 44 to 67 taxa possible, but more commonly 55 to 60; decreased numbers of intolerant taxa, 14 to 21 taxa possible, typically 17 or 18 present; typically less than 25% of the sample is comprised of tolerant forms; the dominant 2 families comprise 18 to 35% of all individuals, but more commonly 23%; balanced trophic structure.
59-40	Fair	Signs of additional deterioration include decreased numbers of intolerant taxa, 6 to 18 taxa possible, typically 13 or 14 present; tolerant forms begin to increase in number, typically comprising 32 to 35% of the community; the relative abundance of the dominant two families remains stable at around 23%; taxa richness remains stable, 41-74 possible, but more commonly 55 to 60; balanced trophic structure.
39-20	Poor	Signs of additional deterioration include decreased taxa richness, 29 to 49 possible, but more commonly 43 to 48; decreased numbers of intolerant taxa, 4 to 9 taxa possible, typically 3 or 4 present; tolerant forms become a larger part of the community, comprising 32 to 80% of all individuals, more typically 53%; the relative abundance of the dominant 2 families increases, comprising 25 to 72% of all individuals, but more commonly 38%; balanced trophic structure
19-0	Very Poor	The community is indicative of an environment that is severely modified by human disturbance. Signs of additional deterioration include decreased numbers of intolerant taxa, 2 to 8 possible, typically 4 present; tolerant forms become a larger part of the community, comprising 43 to 61% of all individuals, more typically 58; the relative abundance of the dominant two families remains stable at around 38%; gatherer taxa dominate the trophic structure, typically 34% of all individuals; taxa richness remains stable
No Score		Thorough sampling finds few or no invertebrate; impossible to calculate IBI.

APPENDIX 2 ST. CROIX RIVER BASIN SAMPLING SITES

Stream Name	Sample Date	Drainage Area (mi ²)	Field Number ¹	County	Location	Latitude ²	Longitude	IBI Score ³	Land Use % ⁴
Glide Pool Streams (<500 mi² drainage area)									
tributary to Burnam Creek	09/05/96	1.5	96SC044	Pine	2 mi. S. of Ellson	46.28567	-92.98710	78	7.81
tributary to Chelsey Brook	09/10/96	1.5	96SC051	Aitkin	Near C.S.A.H. 23, 3 mi. S.W. of Giese	46.17338	-93.17530	83	8.77
West Fork Redhorse Creek	09/04/96	1.5	96SC073	Pine	@ Chengwatana State Forest	45.85730	-92.76871	78	0.25
County ditch #7	08/21/96	2	96SC027	Chisago	1.5 mi. S. of North Branch	45.49064	-92.99110	61	53.95
tributary to Snake River	09/05/96	2.4	96SC049	Aitkin	3.5 mi. S. of McGrath	46.20014	-93.25390	56	13.12
Squib Creek	09/11/96	2.7	96SC080	Pine	Rd. btn. S 28/33, 2.5 mi. W. of Cloverton	46.17207	-92.37464	67	14.33
Wolf Creek	08/27/96	4	96SC075	Pine	2 mi. N. of Sandstone	46.16223	-92.86000	83	41.84
Deer Creek	09/18/96	5.5	96SC054	Pine	4 mi. N.E. of Hinckley	46.05324	-92.88170	89	17.36
Hay Creek	09/17/98	5.9	98SC007	Chisago	4.5 mi. NE of North Branch	45.53085	-92.87723	56	75.90
Hay Creek	10/09/98	5.9	98SC007	Chisago	4.5 mi. NE of North Branch	45.53085	-92.87723	39	75.90
Bear Creek	09/04/96	6.5	96SC068	Pine	@ C.S.A.H. 10, 4 mi. N.E. of Pine City	45.85945	-92.86947	83	52.93
Bear Creek	9/9/1996	6.5	96SC068	Pine	@ C.S.A.H. 10, 4 mi. N.E. of Pine City	45.85945	-92.86947	83	52.93
Bear Creek	08/26/97	6.5	96SC068	Pine	@ C.S.A.H. 10, 4 mi. N.E. of Pine City	45.85945	-92.86947	61	52.93
tributary to Rock Creek	09/16/98	7.2	98SC014	Pine	@ railroad bridge in town of Rock Creek	45.75742	-92.96370	28	69.02
tributary to Kettle River	09/15/98	7.8	98SC012	Pine	@ CSAH 33 bridge, 1 mi. E. of Rutledge	46.25970	-92.84663	56	26.78
Cane Creek	08/29/96	10.7	96SC045	Pine	@ C.S.A.H. 33, 4 mi. N. of Askov	46.24627	-92.78090	72	18.46
Judicial ditch #4	09/17/98	11.2	98SC006	Isanti	@ CSAH, 8 mi. SE of Cambridge	45.49890	-93.07842	56	55.88
West Fork Crooked Creek	08/29/96	11.3	96SC064	Pine	@ C.S.A.H. 30, 5 mi. W. of Duxbury	46.12927	-92.61719	56	6.51
Hay Creek	09/16/98	11.6	98SC016	Pine	@ CSAH 5, 9 mi. NW of Rock Creek	45.77863	-93.13241	17	55.91
Spring Brook	08/22/96	12.1	96SC078	Kanabec	1 mi. E. of Mora	45.86176	-93.27390	61	67.60
Browns Creek	09/16/96	13.6	96SC066	Washington	@ C.R. 68, 4 mi. N.W. of Stillwater	45.10778	-92.87444	39	77.49
Knife River	09/04/96	13.9	96SC008	Mille Lacs	C.S.A.H. 27, 5 mi. S. of Isle	46.07000	-93.46440	44	37.92
Gillespie Brook	08/28/96	14.5	96SC042	Carlton	Near C.R. 135, 5 mi. N. of Moose Lake	46.52123	-92.79180	61	7.82
Keene Creek	09/12/96	14.5	96SC059	Pine	2.5 mi. N.E. of Duxbury	46.15933	-92.47710	39	5.71
Redhorse Creek	09/04/96	15.9	96SC072	Pine	@ Chengwatana State Forest	45.85687	-92.76659	56	1.96
Snake River	09/05/96	16.5	96SC069	Aitkin	C.S.A.H 2, 2.5 mi. E. of Pliny	46.33351	-93.21024	50	5.98
S. Branch Grindstone River	08/27/96	26.5	96SC063	Pine	Rd. btn. S 17/18, 4 mi. N.W. of Hinckley	46.03819	-93.03452	78	27.61
East Fork Crooked Creek	09/12/96	27.7	96SC058	Pine	4 mi. S.W. of Duxbury	46.07920	-92.55500	78	6.56
Mission Creek	08/27/96	29.3	96SC013	Pine	1 mi. S.W. of Beroun	45.89314	-92.98040	44	44.37
Mission Creek	09/17/96	29.3	96SC013	Pine	1 mi. S.W. of Beroun	45.89314	-92.98040	28	44.37
Mission Creek	09/16/98	29.3	96SC013	Pine	1 mi. S.W. of Beroun	45.89314	-92.98040	28	44.37
Mission Creek	09/02/99	29.3	96SC013	Pine	1 mi. S.W. of Beroun	45.89314	-92.98040	50	44.37
Mission Creek	10/05/00	29.3	96SC013	Pine	1 mi. S.W. of Beroun	45.89314	-92.98040	44	44.37

APPENDIX 2 (continued)

Stream Name	Sample Date	Drainage Area (mi ²)	Field Number ¹	County	Location	Latitude ²	Longitude	IBI Score ³	Land Use % ⁴
<u>Glide Pool Streams (>500 mi² drainage area)</u>									
Mud Creek	09/16/98	29.6	98SC018	Kanabec	@ SH 23 on SE side of Quamba	45.91266	-93.17566	33	35.34
Pokegama Creek	09/15/98	44.4	98SC015	Pine	Near CR 130, 3.5 mi. W. of Beroun	45.91702	-93.02131	22	33.78
Rush Creek	09/11/96	45.9	96SC015	Chisago	I 35 @ Rush City	45.68060	-92.99010	22	47.73
Rush Creek	09/23/96	45.9	96SC015	Chisago	I 35 @ Rush City	45.68060	-92.99010	17	47.73
Rush Creek	08/26/97	45.9	96SC015	Chisago	I 35 @ Rush City	45.68060	-92.99010	11	47.73
South Fork Groundhouse River	09/16/98	51.2	98SC011	Kanabec	Near unnamed road, 4 mi. S.E. of Ogilvie	45.78992	-93.38871	28	51.40
Rush Creek	08/26/96	52.3	96SC081	Chisago	@ C.S.A.H. 5, 2 mi. E. of Rush City	45.67386	-92.91122	44	55.23
Rush Creek	09/15/99	52.3	96SC081	Chisago	@ C.S.A.H. 5, 2 mi. E. of Rush City	45.67386	-92.91122	33	55.23
Rush Creek	10/04/00	52.3	96SC081	Chisago	@ C.S.A.H. 5, 2 mi. E. of Rush City	45.67386	-92.91122	22	55.23
Rush Creek	09/25/01	52.3	96SC081	Chisago	@ C.S.A.H. 5, 2 mi. E. of Rush City	45.67386	-92.91122	28	55.23
Mud Creek	08/22/96	52.7	96SC011	Pine	Near C.S.A.H. 11, 1 mi. W. of Henriette	45.87187	-93.13500	56	37.82
N. Branch Sunrise River	09/22/98	61	98SC008	Chisago	@ SH 95, .5 mi E of North Branch	45.51322	-92.96385	56	57.17
N. Branch Sunrise River	10/09/98	61	98SC008	Chisago	@ SH 95, .5 mi E of North Branch	45.51322	-92.96385	28	57.17
Snake River	09/10/96	65.2	96SC050	Aitkin	Near C.S.A.H. 2, 1 mi. S.W. of Pliny	46.32371	-93.27620	72	8.04
Snake River	09/15/98	65.2	96SC050	Aitkin	Near C.S.A.H. 2, 1 mi. S.W. of Pliny	46.32371	-93.27620	56	8.04
Snake River	09/14/99	65.2	96SC050	Aitkin	Near C.S.A.H. 2, 1 mi. S.W. of Pliny	46.32371	-93.27620	61	8.04
Snake River	09/25/00	65.2	96SC050	Aitkin	Near C.S.A.H. 2, 1 mi. S.W. of Pliny	46.32371	-93.27620	44	8.04
Ann River	09/16/98	72.3	98SC019	Kanabec	Near CSAH 14, 4 mi. SW of Mora	45.84157	-93.33088	61	27.71
N. Branch Sunrise River	09/16/96	74.5	96SC025	Chisago	S.H. 95, 4 mi. E. of North Branch	45.51293	-92.89320	67	59.14
Sunrise River	09/17/96	114.6	96SC024	Chisago	Near C.R. 84, 1 mi. E. of Wyoming	45.34657	-92.95970	11	64.36
<u>Small Riffle Run Streams (<50 mi² drainage area)</u>									
Trout Brook	09/16/96	5.8	96SC092	Washington	@ C.S.A.H. 21 @ Afton State Park	44.86360	-92.79971		86.61
Lawrence Creek	08/27/96	10	96SC026	Chisago	Near U.S. 8, near Taylors Falls	45.38493	-92.69430	35	85.84
Chelsey Brook	09/04/96	10.3	96SC077	Aitkin	@ S.H. 18, 1 mi. W. of Giese	46.21754	-93.13024	70	4.06
East Fork Crooked Creek	09/04/96	11.1	96SC079	Pine	@ C.S.A.H. 32, 11 mi. E. of Askov	46.18695	-92.54955	95	6.25
East Fork Crooked Creek	09/09/96	11.1	96SC079	Pine	@ C.S.A.H. 32, 11 mi. E. of Askov	46.18695	-92.54955	100	6.25
East Fork Crooked Creek	08/27/97	11.1	96SC079	Pine	@ C.S.A.H. 32, 11 mi. E. of Askov	46.18695	-92.54955	80	6.25
East Fork Crooked Creek	09/22/98	11.1	96SC079	Pine	@ C.S.A.H. 32, 11 mi. E. of Askov	46.18695	-92.54955	85	6.25
East Fork Crooked Creek	09/14/99	11.1	96SC079	Pine	@ C.S.A.H. 32, 11 mi. E. of Askov	46.18695	-92.54955	80	6.25
East Fork Crooked Creek	09/27/00	11.1	96SC079	Pine	@ C.S.A.H. 32, 11 mi. E. of Askov	46.18695	-92.54955	80	6.25

APPENDIX 2 (continued)

Stream Name	Sample Date	Drainage Area (mi ²)	Field Number ¹	County	Location	Latitude ²	Longitude	IBI Score ³	Land Use % ⁴
Small Riffle Run Streams (<50 mi² drainage area)									
Cowan's Brook	09/05/96	12	96SC061	Aitkin	5.5 mi. S.W. of Giese	46.17407	-93.21583	70	12.25
Lower Tamarack River	08/29/96	17.2	96SC082	Pine	Rd. btn. S 28/33, 8.5 mi. S.E. of Bruno	46.26003	-92.49655	100	4.70
Birch Creek	09/05/96	29.3	96SC074	Pine	Rd. btn. S 21/22, 2 mi. W. of Denham	46.36697	-92.99243	65	9.87
Birch Creek	10/02/97	29.3	96SC074	Pine	Rd. btn. S 21/22, 2 mi. W. of Denham	46.36697	-92.99243	80	9.87
Birch Creek	09/02/98	29.3	96SC074	Pine	Rd. btn. S 21/22, 2 mi. W. of Denham	46.36697	-92.99243	75	9.87
Birch Creek	08/30/99	29.3	96SC074	Pine	Rd. btn. S 21/22, 2 mi. W. of Denham	46.36697	-92.99243	75	9.87
Birch Creek	09/27/00	29.3	96SC074	Pine	Rd. btn. S 21/22, 2 mi. W. of Denham	46.36697	-92.99243	70	9.87
McDermott Creek	09/02/96	30.5	96SC038	Pine	C.S.A.H. 32, 4.5 mi. N.W. of Cloverton	46.20651	-92.39440	85	1.43
Birch Creek	09/02/98	33.2	98SC020	Pine	@ CSAH 40 in town of Denham	46.36224	-92.95082	50	13.73
Rush Creek	09/17/98	35.3	98SC001	Chisago	upstream of S 19, 1.5 mi W of Rush City	45.68372	-93.01373	5	46.22
Rush Creek	10/09/98	35.3	98SC001	Chisago	upstream of S 19, 1.5 mi W of Rush City	45.68372	-93.01373	5	46.22
Rush Creek	08/30/99	35.3	98SC001	Chisago	upstream of S 19, 1.5 mi W of Rush City	45.68372	-93.01373	15	46.22
Rush Creek	09/25/01	35.3	98SC001	Chisago	upstream of S 19, 1.5 mi W of Rush City	45.68372	-93.01373	0	46.22
Willow River	08/28/96	36.6	96SC083	Pine	@ C.S.A.H. 48, 1 mi. N.W. of Durquette	46.38127	-92.57215	65	11.38
Rush Creek	09/28/98	43.3	98SC002	Chisago	Near CR 55 .2 mi E of Rush City	45.68540	-92.95420	35	48.80
Rush Creek	09/15/99	43.3	98SC002	Chisago	Near CR 55 .2 mi E of Rush City	45.68540	-92.95420	20	48.80
Rush Creek	10/04/00	43.3	98SC002	Chisago	Near CR 55 .2 mi E of Rush City	45.68540	-92.95420	30	48.80
Rush Creek	09/25/01	43.3	98SC002	Chisago	Near CR 55 .2 mi E of Rush City	45.68540	-92.95420	25	48.80
Bear Creek	08/29/96	43.5	96SC034	Pine	@ S.H. 48, @ Cloverdale	46.01327	-92.74480	65	24.23
Bear Creek	09/11/96	43.5	96SC034	Pine	@ S.H. 48, @ Cloverdale	46.01327	-92.74480	70	24.23
Bear Creek	08/27/97	43.5	96SC034	Pine	@ S.H. 48, @ Cloverdale	46.01327	-92.74480	85	24.23
Bear Creek	09/28/98	43.5	96SC034	Pine	@ S.H. 48, @ Cloverdale	46.01327	-92.74480	85	24.23
Bear Creek	08/30/99	43.5	96SC034	Pine	@ S.H. 48, @ Cloverdale	46.01327	-92.74480	60	24.23
Bear Creek	10/05/00	43.5	96SC034	Pine	@ S.H. 48, @ Cloverdale	46.01327	-92.74480	50	24.23
Rush Creek	09/17/98	47.2	98SC003	Chisago	@ CR 55, .8 mi E. of Rush City	45.68958	-92.93439	45	52.20
Rush Creek	09/15/99	47.2	98SC003	Chisago	@ CR 55, .8 mi E. of Rush City	45.68958	-92.93439	35	52.20
Rush Creek	10/04/00	47.2	98SC003	Chisago	@ CR 55, .8 mi E. of Rush City	45.68958	-92.93439	35	52.20
Rush Creek	09/25/01	47.2	98SC003	Chisago	@ CR 55, .8 mi E. of Rush City	45.68958	-92.93439	30	52.20
Goose Creek	08/21/96	47.5	96SC084	Chisago	@ C.S.A.H. 30 in Harris	45.58751	-92.97638	15	43.26
Goose Creek	10/03/97	47.5	96SC084	Chisago	@ C.S.A.H. 30 in Harris	45.58751	-92.97638	40	43.26
Goose Creek	09/17/98	47.5	96SC084	Chisago	@ C.S.A.H. 30 in Harris	45.58751	-92.97638	50	43.26
Goose Creek	9/2/1999	47.5	96SC084	Chisago	@ C.S.A.H. 30 in Harris	45.58751	-92.97638	60	43.26
Goose Creek	10/04/00	47.5	96SC084	Chisago	@ C.S.A.H. 30 in Harris	45.58751	-92.97638	55	43.26

APPENDIX 2 (continued)

Stream Name	Sample Date	Drainage Area (mi ²)	Field Number ¹	County	Location	Latitude ²	Longitude	IBI Score ³	Land Use % ⁴
<u>Large Riffle Run Streams (>50 and <500 mi² drainage area)</u>									
Split Rock River	09/11/96	50.1	96SC086	Carlton	C.S.A.H. 17, 9 mi. W. of Moose Lake	46.44727	-92.95045	67	14.69
Rush Creek	09/17/98	56.6	98SC004	Chisago	Near C.R. 56, 3 mi S.E. of Rush City	45.65457	-92.90075	42	55.23
Rush Creek	10/07/99	56.6	98SC004	Chisago	Near C.R. 56, 3 mi S.E. of Rush City	45.65457	-92.90075	42	55.23
Rush Creek	10/04/00	56.6	98SC004	Chisago	Near C.R. 56, 3 mi S.E. of Rush City	45.65457	-92.90075	25	55.23
Rush Creek	09/25/01	56.6	98SC004	Chisago	Near C.R. 56, 3 mi S.E. of Rush City	45.65457	-92.90075	50	55.23
Groundhouse River	09/16/98	60.9	98SC005	Kanabec	Upstream of SH 23, .1 mi E of Ogilvie	45.83268	-93.40956	17	15.56
Rock Creek	08/26/96	64.6	96SC022	Chisago	Near C.S.A.H. 3, 3 mi. N.E. of Rush City	45.71850	-92.91020	50	58.82
Ann River	09/18/96	65.2	96SC021	Kanabec	Near C.S.A.H. 12, 2 mi. W. of Mora	45.87211	-93.34390	42	20.15
Kettle River	08/28/96	73.4	96SC085	Carlton	@ C.S.A.H. 14, 6 mi. N. of Kettle River	46.56601	-92.88022	58	18.29
Goose Creek	09/16/96	76.5	96SC023	Chisago	@ Wild River State Park	45.59438	-92.90090	42	48.19
Knife River	09/04/96	76.8	96SC006	Kanabec	Near C.S.A.H. 15, 6 mi. S.W. of Warman	46.03534	-93.38000	33	17.79
Grindstone River	09/03/98	78.3	98SC009	Pine	N. side of C.R. 140, 1 mi. E. of Hinckley	46.01487	-92.92397	58	29.17
Grindstone River	09/03/98	79.4	98SC010	Pine	N. side of C.R. 140, 2 mi. E. of Hinckley	46.01733	-92.90616	58	29.32
Grindstone River	09/28/98	80.4	98SC013	Pine	Downstream at SH 48, 3 mi. E. of Hinckley	46.01062	-92.88681	58	29.41
Knife River	09/11/96	107.6	96SC097	Kanabec	@ C.R. 77, 3 mi. N. of Mora	45.92043	-93.30816	58	17.43
Pine River	09/23/96	109.9	96SC043	Pine	3 mi. N.W. of Rutledge	46.28033	-92.92780	42	23.25
Lower Tamarack River	09/10/96	128	96SC056	Pine	@ St. Croix State Forest	46.07923	-92.42780	75	6.46
Sand Creek	09/11/96	138.5	96SC090	Pine	@ St. Croix State Park	45.95387	-92.66688	92	23.63
Snake River	09/05/96	155.9	96SC052	Aitkin	Near S.H. 18, 2 mi. S.E. of McGrath	46.22269	-93.24180	50	8.11
Snake River	09/10/96	155.9	96SC052	Aitkin	Near S.H. 18, 2 mi. S.E. of McGrath	46.22269	-93.24180	67	8.11
Lower Tamarack River	09/10/96	182.3	96SC029	Pine	@ St. Croix State Forest	46.05375	-92.39670	67	6.42
Kettle River	08/28/96	187	96SC040	Carlton	@ S.H. 27 & 73, 5 mi. W. of Moose Lake	46.45581	-92.87360	67	19.75
Snake River	08/27/96	258.3	96SC002	Kanabec	Near C.S.A.H. 24, 3 mi. E. of Warman	46.06186	-93.21950	92	9.18
Sunrise River	08/21/96	268	96SC065	Chisago	Downstream of Kost Dam County Park	45.48178	-92.87413	8	48.69
Sunrise River	09/17/96	268	96SC065	Chisago	Downstream of Kost Dam County Park	45.48178	-92.87413	33	48.69
Sunrise River	08/26/97	268	96SC065	Chisago	Downstream of Kost Dam County Park	45.48178	-92.87413	33	48.69
Sunrise River	09/17/98	268	96SC065	Chisago	Downstream of Kost Dam County Park	45.48178	-92.87413	58	48.69
Sunrise River	09/20/99	268	96SC065	Chisago	Downstream of Kost Dam County Park	45.48178	-92.87413	25	48.69
Sunrise River	10/10/00	268	96SC065	Chisago	Downstream of Kost Dam County Park	45.48178	-92.87413	42	48.69
Kettle River	08/25/96	296.2	96SC047	Pine	C.S.A.H. 46, 3 mi. N.W. of Sturgeon Lake	46.39814	-92.87970	58	21.58
Kettle River	08/28/96	348.5	96SC046	Pine	Near C.S.A.H. 52, 3 mi. N. of Willow River	46.36701	-92.86100	75	22.68
Kettle River	09/10/96	348.5	96SC046	Pine	Near C.S.A.H. 52, 3 mi. N. of Willow River	46.36701	-92.86100	58	22.68
Kettle River	10/02/97	348.5	96SC046	Pine	Near C.S.A.H. 52, 3 mi. N. of Willow River	46.36701	-92.86100	67	22.68
Kettle River	09/22/98	348.5	96SC046	Pine	Near C.S.A.H. 52, 3 mi. N. of Willow River	46.36701	-92.86100	67	22.68
Kettle River	08/25/96	493.6	96SC048	Pine	Near C.S.A.H. 52, 2 mi. N. of Willow River	46.35320	-92.84020	83	19.76

APPENDIX 2 (continued)

Stream Name	Sample Date	Drainage Area (mi ²)	Field Number ¹	County	Location	Latitude ²	Longitude	IBI Score ³	Land Use % ⁴
<u>Large Streams (>500 mi² drainage area)</u>									
Snake River	09/09/96	545	96SC018	Kanabec	3.5 mi. S. of Mora	45.81297	-93.28070		19.50
Snake River	09/09/96	803.2	96SC019	Kanabec	2 mi. W. of Grasston	45.79365	-93.18110		26.42
Snake River	09/03/96	824.2	96SC010	Pine	2 mi. E. of Grasston	45.78951	-93.10690		27.10
Snake River	09/03/96	978.8	96SC012	Pine	4 mi. E. of Pine City	45.84351	-92.88970		29.59
Kettle River	08/29/96	1049.9	96SC033	Pine	@ Kennedy Brook in St. Croix State Park	45.90111	-92.73090		21.65
Kettle River	09/10/96	1049.9	96SC033	Pine	@ Kennedy Brook in St. Croix State Park	45.90111	-92.73090		21.65
St. Croix River	09/19/96	2886	96SC030	Pine	Kettle River Slough	45.88046	-92.72960		13.40
St. Croix River	10/03/96	2886	96SC030	Pine	Kettle River Slough	45.88046	-92.72960		13.40

¹ Field number assigned to each station to designate a unique sampling location.

² Latitude and longitude are formatted in WGS84 decimal degrees.

³ IBI score is the overall IBI score assigned to the site. Scores range from 0 (lowest biological integrity) to 100 (highest biological integrity).

⁴ Land use expressed as a percent of the watershed upstream of the sampling location that has been altered by humans. It includes disturbance from agricultural, residential, urban, and mining land usage.

* Sites that were designated as being of excellent quality based on land use and habitat.

** Sites that were designated as poor quality based on land use and habitat.