2010

Temporal Variability of Bioassessment Indices Used to Evaluate Three Midwestern Streams

Nia M. Haller
Loyola University Chicago

LOYOLA UNIVERSITY CHICAGO

TEMPORAL VARIABILITY OF BIOASSESSMENT INDICES USED TO EVALUATE THREE MIDWESTERN STREAMS

A THESIS SUBMITTED TO

THE FACULTY OF THE GRADUATE SCHOOL

IN CANDIDACY FOR THE DEGREE OF

MASTER OF SCIENCE

PROGRAM IN BIOLOGY

BY

NIA M. HALLER

CHICAGO, ILLINOIS

MAY 2010

ACKNOWLEDGEMENTS

I would like to extend my sincere thanks to everyone who made a contribution to this project. Most of all, I would like to thank my graduate advisor, Marty Berg. Marty was the first to spark my interest in stream ecology. He has been a constant source of guidance and encouragement. I have learned from his incredible wisdom and patience; having Marty as a role model has been invaluable. I appreciate the time and energy he put forth to help me develop as a biologist and as a person.

My graduate committee has been an intricate part of my growth throughout graduate school. They've helped me to weave my project into its final product, a very comprehensive study that has very practical applications. Nancy has given me a wonderful educational base in aquatic ecology that has been the backbone of my research. Chris offered the wonderful idea to address bioassessment indices in my study, which became the central theme of the project. I appreciate the time he gave to the project, offering much guidance with the statistical analysis. I would also like to thank the following people for their aid: Nicole Vidales, Grace Kilbane, Stephanie Carman, Kirby Wolfe, Ramona Zak and Julie Ryan.

An amazing thanks has to go to my lab partner and wonderful friend, Nicole Vidales. If it weren't for Nicole, I may have thrown in the towel long ago. Nicole was there to pick me up when I was down and push me forth when I needed a nudge. Her new and fresh ideas always sparked me, making for great conversations regarding our projects. Nicole is the most giving and compassionate person I’ve ever met. Words can’t express my thanks for her support throughout our journey together.

The most important thanks goes to my husband and family for enduring this process with me. For all of those weekends spent on my thesis instead of with you, thank you for your understanding and patience. Most of all, thank you for your encouragement and reminders of the light at the end of the tunnel.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Population, community and functional measures, or metrics, in rapid bioassessment programs aid in establishing biological criteria for streams and rivers. Each metric measures different aspects of community structure and is important in detecting changes in macroinvertebrate community structure that are influenced by changes in water quality. In this study, temporal variation of nine commonly used bioassessment indices was examined in three midwestern streams. The indices were calculated for each of nine replicate benthic macroinvertebrate samples collected monthly for one year from Cowpie Creek (CC), Nippersink Creek (NC) and Lawrence Creek (LC), McHenry County, Illinois. In practice, the habitat sampled for bioassessments often is limited to riffle sites in an attempt to reduce the effects of spatial variability on indices, and midges (Diptera: Chironomidae) often are omitted to remove error associated with sampling, level of identification and variable life histories. Where appropriate, indices in this study were calculated using all sites or only riffles areas, and using all macroinvertebrates or all macroinvertebrates exclusive of the Chironomidae. Sites were ordinated by Detrended Correspondence Analysis (DCA) to reveal temporal trends among index ratings. In assessments using all sites, biotic indices reflected different temporal changes in macroinvertebrate community structure than the multimetric index and taxa richness metrics. When only riffle sites were included in assessments, the ability of indices and metrics to reflect macroinvertebrate community structure was

dependent on the community structure of the stream assessed, improving in some streams but not in others. Similarly, the omission of Chironomidae from assessments resulted in differing abilities of the indices to reflect macroinvertebrate community structure. Because the indices showed poor performance in some streams when assessing riffle sites or omitting midges from assessments, it was concluded that all habitats and all macroinvertebrates should be included in assessment protocols. Although multimetric indices provide more information about stream communities than biotic indices, the use of ordination analyses are helpful in verifying accuracy of water quality assessments.

CHAPTER I.

INTRODUCTION

Rapid Bioassessment

Land-use adjacent to agricultural streams can strongly impact stream communities, causing a wide variety of stresses to macroinvertebrates. Erosion from fields can alter macroinvertebrate habitat by increasing suspended sediments sediments on substrates and in interstitial spaces. Runoff of fertilizers increases nutrient levels in streams, causing algal and macrophyte blooms that subsequent die-off, resulting in oxygen-depleted environments (Lenat and Crawford, 1994). Runoff of pesticides can have toxic effects on macroinvertebrates, ultimately affecting functionality of the community. Because macroinvertebrates vary in their responses to these different impacts, biomonitoring of macroinvertebrate communities is a useful tool to determine long term effects of land-use on stream ecosystems (Rosenberg *et al*., 1986).

Biomonitoring can be defined as "the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control program" (Matthews *et al*., 1982). Biomonitoring of stream ecosystems was developed for two main purposes: surveillance and compliance. Surveillance can be used to determine before and after effects of pollutant introductions, as well as determine if water resource and conservation management attempts are successful. Compliance is used to ensure that long term water quality meets statutory requirements (Rosenberg and

Resh, 1993). Rapid bioassessment was developed with these goals in mind, but adopted techniques that intended to reduce the overall effort and cost of the assessments while efficiently identifying sources of point and nonpoint pollution and documenting long term regional changes in water quality (Resh and Jackson, 1993). Benthic macroinvertebrate communities have become one of the most commonly used biological communities in rapid bioassessment of streams (Rosenberg and Resh, 1993) due to ease of collection and relatively long life cycles that allows for long-term exposure to changes in the environment (Szczytko, 1989). Rapid bioassessment has combined and employed several approaches to reduce effort, cost and complexity of macroinvertebrate monitoring: 1) a large composite sample consisting of several collections from different habitats is used rather than many individual replicate samples, 2) a subsampling method is used to reduce the number of organisms sorted and identified and to standardize the effort in sample processing, and 3) results of the analysis are presented in a simplified form so that they may be understood by nonbiologists (Resh *et al*., 1995). As a result, more water resources can be assessed in a shorter period of time. Unfortunately, because of the subjective nature of rapid assessments due to the lack of statistical testing, use of rapid bioassessment programs for the purpose of compliance in regional water quality monitoring programs has been questioned. Many biologists agree that rapid bioassessment approaches should be used only as preliminary screening tools to detect initial impairment and rank sites according to the need for further study (Hannaford and Resh, 1995).

Bioassessment Metrics

Since its development in the U.S. during the mid-1980's, rapid bioassessment has been widely accepted by state water monitoring programs designed for water resource management (U.S. EPA, 1996). Many states have incorporated a variety of population, community and functional measures, or metrics, into their programs to establish biological criteria. Although each metric measures different aspects of community structure, each one is important in detecting changes in macroinvertebrate community structure that are influenced by changes in water quality. Metrics that have been commonly used include: taxa richness, enumerations (individuals from single taxa), community diversity and similarity indices, biotic indices and functional or trophic measures (ratios of functional feeding groups) (Resh and Jackson, 1993). Taxa richness, which is the total number of taxa, has been shown to decrease with decreasing water quality. Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa richness is commonly used and considers only clean water indicator taxa. Taxa richness and enumerations of organisms within taxa groups both form the basic principles of diversity, similarity and biotic indices. Community diversity and similarity indices were initially used to measure changes in macroinvertebrate community structure. However, their ability to respond to impacts of pollution due to differences in natural variability among differing stream macroinvertebrate assemblages has been criticized (Washington, 1984). Biotic indices, the most recently introduced index of the three, were developed out of the need for a more robust measure of community structure that reflected changes in water quality but was not sensitive to natural variability of macroinvertebrate assemblages or the effects of naturally changing environmental factors (Fore *et al*. 1996, Lenat 1990). To accomplish this, biotic indices use the 'indicator species' concept by assigning pollution tolerance values to each taxon. Functional feeding group ratios incorporate changes in food availability as a measure of change in the macroinvertebrate community structure without regard to taxa richness or indicator species. All previously mentioned indices have been used to reflect some aspect of community structure, but not without some criticism (Washington, 1984). One common criticism has been the lack of accuracy and precision of the metrics due to temporal, spatial and replicate sample variability. In spite of the largely unpredictable and undefined amounts of variability inherent within individual metrics, multimetric indices, which incorporate a variety of individual metrics into a single index value, recently have been developed. The goal of multimetric indices is to integrate many aspects of community structure and function while reducing the amount of data for the purpose of simplifying the interpretation of water quality conditions (Gerritsen, 1995). The multimetric approach has received substantial criticism in the literature. Variation in the overall multimetric assessment results from additive variation of the component metrics (Hannaford and Resh, 1995) and changes in the individual metrics might be masked by changes presented by a single index value (Polls, 1994). The preferred approach is to conduct regional field studies to determine which individual metrics or group of metrics best measure community health (Polls, 1994).

Temporal Variability of Indices and Metrics

Temporal variability of bioassessment indices or metrics is a major concern for water quality monitoring programs. Distinguishing anthropogenic impacts from natural variability of the macroinvertebrate community is important in preventing the misinterpretation of changes in water quality (Polls, 1994). The added variability due to natural changes in macroinvertebrate communities can obscure any true changes in water quality due to anthropogenic impacts thereby confounding stream assessments (Lenat, 1990, Stark 1993). A potential source of error for most indices is that they rely on the number of species present in a sample. The number of species present can be dependent on a variety of fluctuating environmental conditions, thus making macroinvertebrate assemblage structure highly variable throughout the year (Murphy, 1978).

Factors affecting macroinvertebrate communities can range from large scale conditions, such as stream order, to small scale conditions such as microhabitat. Minshall et al. (1985) showed that taxa richness increased with stream order, an occurrence attributed to more stable environments in higher order streams. They found that first and second order streams tended to be more responsive than higher orders streams to temporal changes in local weather patterns, thus showing more dramatic effects of stream discharge and temperature. Stream flow can directly affect the abundance of macroinvertebrates through changes in microhabitat. Chutter (1970) suggested that low macroinvertebrate abundances were more closely related to scouring by floods than to insect emergence. Scouring can result in a complete loss of microhabitat and/or dislodgment of organisms resulting in a decrease in macroinvertebrate densities. However, when refugia are present, macroinvertebrate densities can withstand moderate changes in flow (Lenat, 1990). Water quality of an unpolluted stream can appear to be in decline if habitat quality is poor (Hannaford and Resh, 1995). For this reason, habitat assessments are an important component in determining water quality. Stream flow also is important in directly regulating water quality (Lenat, 1990). Fluctuations in flow can alter nonpoint source runoff as well as change the amount of a pollutant from point source discharges by dilution (Lenat, 1988). Lenat (1990) found that EPT taxa increased with decreased flow in areas affected by nonpoint source runoff, whereas EPT taxa decreased with decreased flow when affected by point source runoff.

Sources of Variability in Macroinvertebrate Community Structure

Effects of flow on nonpoint source runoff are especially important in agricultural areas where runoff and bank erosion bring high levels of sediment, nutrients and pesticides into streams (Lamberti and Berg 1995, Lenat 1984). Lenat (1984) suggested that the effects of agricultural runoff on benthic densities are not easily predicted. The addition of organic particulates and nutrients can increase invertebrate densities while toxic substances, low oxygen and sediment can decrease densities. Marsh and Waters (1980) concluded that taxa richness for intolerant taxa (EPT) declined in agricultural streams while taxa richness for more tolerant groups increased. In a study comparing taxa richness across forested, agricultural, and urban North Carolina stream sites, Lenat and Crawford (1994) found that taxa richness was highest in the forested stream, slightly lower in the agricultural stream and lowest in the urban stream. Despite differences in richness between forested and agricultural streams, they found higher invertebrate abundances in the agricultural stream compared to either the forested or urban streams. They attributed these differences to nutrient enrichment. Using a North Carolina biotic index adapted from Hilsenhoff's biotic index, Lenat and Crawford (1994) found that the index followed patterns in taxa richness, suggesting least impact in the forested stream and the most impact in the urban stream.

Increased flow, resulting in increased sediment loading, also can alter stream benthic macroinvertebrate communities. Lamberti and Berg (1995) examined functional feeding groups in a northern Indiana stream and found a large increase in collecting-gathering taxa due to an increase in organic particulates on the stream bottom. They also found that increases in fine sediments on benthic substrata reduced the amount of stable substrate for which filter-feeding taxa could attach and disturbed their feeding mechanism. Although functional feeding groups are clear indicators of how environmental changes affect resources in streams, studies have found them to be highly variable in determining water quality (Hannaford and Resh, 1995). Because seasonal changes in stream flow result in temporal variability in sediment inputs, nutrients and pesticides in agricultural streams, it is important to consider how macroinvertebrate communities respond, both structurally and functionally, to these inputs to accurately assess water quality.

Another source of temporal variability inherent in bioassessment indices is the phenology of aquatic insect life histories. Life cycles of aquatic insects are strongly governed by seasonal temperature changes. The accumulation of degree-days can affect densities of univoltine species throughout the year by influencing the time of insect emergence (Hilsenhoff, 1988b). Many species have life histories that coincide with particular food resources, which may result in their being absent from the stream during certain times of the year when those resources are limited (Hutchens *et al*., 1998). In addition, as a result of natural mortality, macroinvertebrate densities steadily decrease from the period of egg hatching to the time of emergence (Schwenneker and Hellenthal, 1984). This combination of factors, in conjunction with the varying densities associated with multivoltine life cycles, can alter macroinvertebrate densities drastically throughout the year (Hutchens *et al*., 1998). Berg and Hellenthal (1990) stressed the importance of considering life histories in pollution studies, cautioning that the absence of a particular species does not necessarily indicate an environmental impact.

Reference Conditions

Natural changes in macroinvertebrate assemblages can be so variable that impacts on water quality can not be deciphered (Norris and Georges, 1993). The ecoregion concept was developed in an effort to reduce environmental variability associated with a given geographic region (Omernik, 1987). The concept suggests that streams in a relatively uniform geographic area will have a comparable fauna and similar macroinvertebrate community structure. Factors used to distinguish ecoregions include: elevation, soil type and permeability, geology, vegetation and land use (Lenat, 1990, Omernik 1987). The intended design of biomonitoring programs is to compare assessments from reference sites that are assumed to be unpolluted to those from test sites within a given ecoregion to determine the level of environmental impact at the test site. It has been shown, however, that macroinvertebrate assemblage structure at reference sites varies considerably within ecoregions (Spindler, 1996). This could be due to the subjective nature of selecting reference sites. Although reference sites may be similar in macroinvertebrate community structure, differences in geomorphology and hydrology can occur within the ecoregion. A problem for many biomonitoring programs is that often only one reference site is used and careful consideration is not taken to ensure that the sites are similar in other aspects (Polls 1994, Wallace *et al*. 1996). In response to these problems, many researchers have employed the use of multivariate analyses to determine reference conditions. With this approach, environmental characteristics related to invertebrate communities at reference sites are used to predict invertebrate communities at test sites (Resh and McElravy, 1993). Multivariate techniques are useful because they reduce the multidimensionality of data caused by the accumulation of sources of variability without losing information about the community.

Error Associated with Biotic Indices

The objective of most bioassessment indices is to present changes in community structure for use in interpreting water quality, but few have set specific goals. Biotic indices, on the other hand, are one of the few bioassessment tools that have been specifically designed to detect differing dissolved oxygen (D.O.) levels due to organic pollution. The concept of the biotic index was initiated in Europe with the development of the Saprobien System (Kolkwitz and Marsson, 1909). Chutter (1972) was the next to develop a biotic index, assigning tolerance values to invertebrates in South African streams and rivers. Hilsenhoff (1977) was the first to use the biotic index (BI) in the U.S., adjusting the tolerance values for use in Wisconsin. Since 1977, Hilsenhoff has made several revisions to the tolerance values (Hilsenhoff, 1987), expanded the scale of the index (Hilsenhoff, 1987) and suggested seasonal corrections (Hilsenhoff, 1988b). In 1979, the Wisconsin Department of Natural Resources began using the BI statewide. In 1988, Hilsenhoff developed a family-level biotic index (FBI) to enable a more rapid field assessment. By 1996, the BI had been modified and employed in at least 29 states, while the FBI had been modified and employed in only 6 states (U.S. EPA, 1996). The BI was re-evaluated in 1998 by Hilsenhoff (1998) to account for seasonal variability.

The goal of the biotic index is to reflect changes in water quality that are influenced by the pollution tolerance of different taxa in the community. Pollution tolerance values represent, in part, macroinvertebrate sensitivity to dissolved oxygen levels caused by the loading of organic waste. Unfortunately, there are many disadvantages to assigning tolerance values to organisms. First and foremost, tolerance values are developed in a subjective manner, based on the best professional judgment of the biologist (Lenat, 1990). Because tolerances of particular species vary from region to region, subjective opinions of many different scientists are involved in creating regional-based values. Constant revisions to tolerance values are needed as new species assignments are made and new pollutants are discovered. How biotic indices react to non-organic pollutants alone, such as synthetic fertilizers, is not well known (Norris and Georges 1993, Chessman and McEvoy 1998). Most often streams are affected by a suite of organic and non-organic pollutants. Another disadvantage of biotic indices is the synthesis of data into one value. Although a single value aids in the understanding of assessments, it can also oversimplify the data and result in the loss of information about community structure (Norris and Georges, 1993). Most macroinvertebrate assemblages are dominated by taxa that are neither highly sensitive nor highly tolerant, therefore there is a tendency to lose important information provided by rare taxa (Fore *et al.*, 1996). Also, shifts in community structure will not be observed if the taxa in transition have similar tolerance values. Finally, the subjectivity of biotic indices and the complications of using a single value is paralleled with problems associated with the wide ranges of tolerance values within a given taxonomic level. Identification of organisms to family level is much faster and can be done easily in the field, however, due to the wide range of species tolerances within given families, sensitivity to changes in the community is lost. Wright *et al*. (1995) suggested the use of family level assessments only to detect gross disturbances in the macroinvertebrate community. Although species level identifications have been shown to better discriminate polluted sites (Hilsenhoff 1988a, Wright 1995), identifying macroinvertebrates to species can be difficult (Resh and Unzicker, 1975). Often, there also are discrepancies as to the correct tolerance values at the species level (Hilsenhoff, 1982).

In addition to the error associated with biotic index tolerance values, th use of different macroinvertebrate sampling methods contribute largely to biotic index variability. There has been much disagreement as to the best macroinvertebrate sampling method to use; one that accurately represents the macroinvertebrate community or one that does not result in large amounts of sampling variability. Hilsenhoff (1987) suggested sampling only riffle areas when using his biotic index. He found that differences in microhabitat (pools vs. riffles) can influence the pollution tolerance values of macroinvertebrates collected due to varying amounts of substrate and levels of dissolved oxygen (Hilsenhoff, 1990). Although limiting the sampling habitat can reduce variability among samples, many studies have shown that multi-habitat sampling is most representative of community structure (Resh *et al*.1995, Kerans *et al*., 1992). Kerans *et al*. (1992) recommended taking replicate quantitative samples from both pools and riffles. They suggested that measurement of human impact can be biased if a particular habitat that is more highly affected by pollution is not sampled (Kerans *et al*., 1992). Many multimetric indices have exploited qualitative composite samples as alternatives to quantitative multi-habitat replicate samples. The use of composite samples, where all habitats are represented in the composite, is a more rapid and cost-saving approach to multi-habitat sampling because macroinvertebrates from only one subsample are sorted and identified (Resh *et al*., 1995). Due to the lack of replicate samples, however, statistical power is lost (Kerans *et al*., 1992). Those who support qualitative sampling argue that statistical methods are not always helpful in interpreting ecological meaning (Fore *et al*., 1996). Often, statistically significant differences between sampling sites do not express differences in water quality as established by the index (Norris and Georges 1993, Stone and Wallace 1998). Another disadvantage to quantitative sampling is that a large number of replicates is usually necessary to collect a sufficient proportion of the taxa present in the stream. Stark (1993) reported needing 12 replicates to accurately calculate the Macroinvertebrate Community Index, whereas Resh and McElravy (1993) reported that only 3 to 5 replicates are commonly used in bioassessment studies due to time and cost constraints.

Yet another source of variability in biotic indices is due to the fixed count method of subsampling. Hilsenhoff (1977) suggested that the first 100 organisms removed from a sample would constitute an adequate sample. However, Courtemanch (1996) argued that when using fixed count methods, estimates of taxa density per sampled unit is lost and that the fraction of the community that has been sampled is unknown. The probability of collecting more and rare taxa increases as the sampling effort increases. Because macroinvertebrate densities per unit area vary as stress to the community increases, the first 100 organisms in reference streams and polluted streams represent different sampling efforts and thus different portions of the macroinvertebrate community. For instance, taxa richness per unit area may be greater in one stream than the other, but the first 100 organisms selected may not exhibit that difference. Also, the area sampled should be the same when comparing sites, otherwise taxa richness will be incorrectly estimated and erroneous interpretations will result (Courtemanch, 1996).

A source of variability in biotic indices that most studies have been reluctant to address is error involved in collecting and identifying macroinvertebrates from the family Chironomidae (midges). Midges are important components of stream macroinvertebrate communities, especially communities subjected to environmental impact, but are often omitted from pollution studies. Berg and Hellenthal (1990) reported that a majority (over 80%) of the total stream insect secondary production in Juday Creek, IN, a stream impacted by sedimentation, was accounted for by midges. Because midges have such an important energetic role in the macroinvertebrate community and are usually present in stressed environments, their omission could limit conclusions about water quality. Calle-Martinez and Casa (2006) found 6 species of chironomids that responded directly to water quality impairment along a wide gradient of impairments. There taxa increased in density with increasing impairment, thereby showing potential improvement in index sensitivity.

Error Associated with Chironomidae in Assessments

When midges are included in water quality studies, however, they are often inappropriately sampled. Many of the sampling devices used in rapid biomonitoring programs use nets with too coarse mesh sizes, resulting in underestimates of midge species richness and densities (Berg and Hellenthal, 1990). Although they occur in high densities, the small size of midges often results in their being overlooked during the sorting process. Identifications are difficult and tedious and require making slide mounts of the head capsule. In addition to these sources of error in midge data, the diverse array of life history patterns in the Chironomidae (univoltine to asynchronous) can add to data variability. Berg and Hellenthal (1990) suggested the use of regional preliminary studies to gain insight into life history patterns. However, the overlapping of cohorts can make different life cycles indistinguishable. Because of this, densities can vary greatly over time, introducing large amounts of variability into a biotic index throughout the year. Lenat (1983) concluded that Chironomidae taxa richness was not a dependable indicator of water quality, reporting much higher taxa richness of Chironomidae in moderately stressed sites than in severely polluted or unpolluted sites. He also found that taxa richness of Chironomidae was more dependent on stream size and flow than EPT richness, resulting in a poor correlation between the two metrics.

Variability Comparisons among Indices and Metrics

A few attempts have been made to describe the temporal, spatial and replicate sample variability exhibited by many bioassessment indices (Ballogh *et al*. 1976, Barbour *et al*. 1992, Hannaford and Resh 1995, Hilsenhoff 1977, 1988b, Lenat and Crawford 1994, Szczytko 1989, Wallace 1996, Zamora-Munoz *et al*. 1995). Several studies have addressed in detail the variability associated with taxa richness and EPT richness (Lenat and Crawford 1994, Wallace *et al*. 1996), however relative to its widespread use, only a few detailed studies have considered the temporal variability of biotic indices. In 1977, Hilsenhoff compared his biotic index to Margalef's index of diversity (Margalef, 1957). He found that the biotic index gave a more accurate assessment of stream quality than did the diversity index, which ranked some clean streams as polluted due to naturally low diversity.

Since the adoption of Plafkin's (1989) rapid bioassessment protocols by the U.S. EPA shortly after their development, Barbour *et al*.(1992) examined the protocol for redundancy and variability of its metrics, which includes Hilsenhoff's BI. Of the 8 metrics examined, only 4 (taxa richness, EPT index, Hilsenhoff's BI and ratio of shredders to total organisms) yielded low enough variability to distinguish different sites yet did not relay redundant information (i.e., were not correlated). High variability can indicate a metric's inability to demonstrate differences in water quality between sites. Lenat and Crawford (1994) conducted a study comparing the ability of taxa richness, a modified Hilsenhoff biotic index and presence of unique species to discriminate forested, agricultural and urban sites. They found that the biotic index confirmed site rankings of taxa richness and unique taxa, ranking the forested sites with the best water quality and urban sites with the poorest. Studies comparing the FBI to other biotic indices have shown it to be site discriminatory as well. Hannaford and Resh (1995) demonstrated the FBI's ability to distinguish between reference, unrestored and restored stream sites. However, in 1988, when Hilsenhoff compared his biotic index to his newly developed family-level biotic index (FBI), he found the FBI to underestimate pollution levels in polluted streams and overestimate pollution levels in cleaner streams. He attributed the suppressed ability to distinguish between various levels of pollution to the highly variable tolerance levels of taxa within a given family.

Hilsenhoff (1988b) addressed temporal changes exhibited by the biotic index by suggesting that temperature changes throughout the year strongly influenced index values. Hilsenhoff (1988b) found index values to increase in summer when warm water, plant respiration and decomposition of organic matter contributed to low dissolved oxygen levels. Many organisms collected in the summer were very tolerant to low dissolved oxygen and were found to have higher tolerance values. In warmwater streams, he found that index values began to increase in June, whereas values in coldwater streams did not increase until July or August. He found that index values in warmwater streams also increased in October or November when water temperatures were lower. However, he found considerable interannual variability, confounding development of an interannual correction factor. He attributed the year-to-year differences to accelerated emergence and recruitment times during warmer years. Thus, Hilsenhoff (1988b) suggested sampling during spring and fall (except for October and November in warmwater streams) to avoid unreasonably high index values. Winter sampling by Hilsenhoff was not conducted due to stream freezing. Many states have determined their specific sampling seasons, most of which are in the summer months.

Although a handful of studies have compared biotic indices, fewer studies have attempted to compare the temporal variability of biotic indices to other bioassessment indices. The majority of studies comparing the temporal variability of biotic indices have been conducted in Europe (Ballogh *et al*. 1976, Camargo 1992, Murphy 1978, Zamora-Munoz *et al*. 1995). The general conclusion was that some indices were more sensitive to changes in community structure than others. A few studies in the U.S supported these findings as well. Szczytko (1989) compared 6 single indices (BI, FBI, EPT, species richness, generic richness and Margalef's Diversity Index) with 6 paired community comparisons for Wisconsin streams and found that variability among replicate samples (5) for the single indices to be much less than variability of community comparison metrics, with the exception of the EPT index. Szczytko (1989) suggested high variability of the EPT was due to the use of enumerations rather than taxa richness. The FBI exhibited the lowest variability of the single metrics, followed closely by the BI. Seasonal changes in variability were not observed for any of the metrics, however only 2 to 3 months were sampled each year of the two-year study. The greatest overall mean variation of the single metrics was displayed in June. Wallace *et al*. (1996) addressed temporal metric variability associated with different levels of stress. Their study compared the ability of the the North Carolina Biotic Index (NCBI), an adaptation of Hilsenhoff's biotic index, and the EPT index to track changes in a macroinvertebrate community that was subjected to an insecticide treatment. Both indices were found to significantly differ temporally in the insecticide treated stream, but not in the reference stream.

Using Ordination Analyses to Describe Index Variability

Several recent studies have used ordination and cluster analyses to determine accuracy and precision of biotic indices. These types of analyses in bioassessment were first attempted in Europe. A study by Zamora-Muñoz and Alba-Tercedor (1996) compared water quality results generated by the Biological Monitoring Working Party (BMWP), a biotic index applied in the U.K. and adapted for the Iberian Peninsula, with results obtained by two multivariate methods, Twinspan and CCA (Canonical

Correspondence Analysis). They found that Twinspan, which classified sites according to macroinvertebrate community structure, was closely related to water pollution levels indicated by the biotic index. In addition, the CCA analysis showed that nutrient levels and water hardness, which can be characteristics indicative of pollution, were the main environmental factors explaining most of the variation in the macroinvertebrate distribution. A large portion of the variability was explained by the biotic index as well. Zamora-Munoz and Alba-Tercedor (1996) concluded that seasonal groupings of sampling sites were probably due to the effects of seasonal inputs of pollution on macroinvertebrate composition rather than life histories of macroinvertebrates. Linke *et al.* (1999) conducted a three-month study (June, July and November) showing the importance of time of year that invertebrates are sampled. Linke *et al*. (1999) used a cluster analysis to show that sites sampled at the same time of year clustered together, rather than reference and test sites or two sampling times of the same site. Both taxa richness and the FBI varied temporally, indicating better water quality in November than in June or July. These results were consistent with studies conducted by Lenat (1987), who found peaks in taxa richness in October and November, and Hilsenhoff (1988b), who found that winter BI values were lower in warmwater streams and higher in coldwater streams due to differences between the two stream types in numbers of indicator taxa.

The Problem

Despite the widespread use of biotic indices, there is a lack of information regarding how different indices respond to temporal variability in community structure.

So far, the solution to eliminate high biotic index variability has been to limit their application to specific types of pollution and to specific time periods throughout the year. However, knowledge of an index's sensitivity and how it compares to other indices are important in determining an index's widespread applicability (Diamond *et al*. 1996, Murphy 1978). The use of different bioassessment methods and the misapplication or misinterpretation of similar methods can make data comparisons between water resource agencies quite difficult. The U.S.E.P.A. tried eliminating the problem of using several different bioassessment methods by introducing the Rapid Bioassessment Protocols (RBP) (Plafkin *et al*., 1989). However, Diamond *et al*. (1996) argued that standardization of a single method (study design, sampling and laboratory protocols and data analysis) would not be practical due to differences among overall goals of the bioassessment. They suggested an alternative approach, although possibly more time consuming, of documenting the quality and comparability of data acquired from different bioassessment methods. If the variability of different bioassessment indices is defined, it is possible that a national network of bioassessment data eventually can be established and compared.

Goals of the Project

The overall goal of my research is to compare and contrast the influence of temporal variability of benthic stream macroinvertebrates on 9 commonly used bioassessment indices in the midwestern U.S. The indices to be examined are: the Biotic Index (BI) (Hilsenhoff, 1987), the Family-Level Biotic Index (FBI) (Hilsenhoff, 1988a), the Illinois Macroinvertebrate Biotic Index (MBI) (IEPA, 1987), the Great Lakes and Environmental Assessment Section Procedure 51 (P51) (MDEQ, 1996), the

Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa richness index (Lenat, 1988), % EPT taxa richness, Chironomidae taxa richness, non-insect taxa richness and total taxa richness (Table 3). The FBI and MBI are modifications of the Biotic Index, which use tolerance values for the specific purpose of determining levels of organic pollution. The suggested sampling protocol for the biotic indices, including the BI, MBI and FBI, requires quantitative collections, replicated if possible, from uniform habitats. Riffle collections are recommended, when such areas are present, to eliminate unreasonably high index values and to reduce variability among samples. It is recommended that sample sorting and the random selection of 100 invertebrates from each sample be carried out in the laboratory. Index values are then calculated for each sample. P51 is a multimetric index used in the state of Michigan that was developed to facilitate Best Management Practices by more rigorously monitoring nonpoint source impacts statewide. Sampling protocols for P51 suggest the use of qualitative collections from all habitats, which are combined into a composite sample. A total of 100 organisms are randomly chosen from the sample and identified in the field. Ephemeroptera-Plecoptera-Trichoptera and % EPT are metrics that monitor changes in the clean-water indicator taxa in the community structure. Chironomidae taxa richness, non-insect taxa richness, and total taxa richmess reflect the number of taxa in the respective groups. Sampling protocols for richness suggest the collection of replicated quantitative samples (Vinson and Hawkins, 1996). Percent abundance of EPT, Chironomidae, non-insects and *Gammarus* also were examined.

The questions addressed in this study are: 1) Do stream ratings according to bioassessment indices reflect overall differences in stream macroinvertebrate community structure? 2) Do temporal patterns in bioassessment indices reflect temporal changes in macroinvertebrate community structure? And if so, how similar are temporal patterns among the indices? 3) Do the bioassessment indices display similar amounts of temporal variability? 4) Does the restriction of using riffle sites in bioassessments change an index's overall performance and 5) Does the omission of Chironomidae in bioassessments change an index's overall performance?

CHAPTER II.

METHODS

Study Sites

This study was conducted in two first-order streams, Cowpie Creek (CC) (42°25.8'N 88°20.29'W) and Nippersink Creek (NC) (42°28.63'N 88°28.62'W), and one second-order stream, Lawrence Creek (LC) (42°26.46'N 88°38.98'W), all of which are located in McHenry County, in north-central Illinois. The streams flow through small woodlands with a canopy cover of willow (*Salix* sp.), cottonwood (*Populus deltoides*) and white oak (*Quercus alba*). The streams also flow through open agricultural fields containing mostly reed canary grass (*Phalaris arundinacea*). To reduce variability among samples, sampling sites were limited to the canopied stream reaches, where habitat and substrates were diverse and similar among sites. Physical and chemical parameters of the streams are shown in Table 1.

Cowpie Creek meanders through a 1.2 kilometer forested buffer zone located approximately 1.2 kilometers downstream of the headwaters. Because CC runs through some agricultural areas upstream of the forested area, it was not considered a reference stream, although water quality was expected to be better than in the other streams. The sampled reach of CC flows east, directly into Glacial Park (McHenry County Conservation District), which represents a post agricultural area. A riparian zone consisting mainly of *P. arundinacea* lies on both sides of the stream, extending 91 meters

Table 1. Mean annual physical and chemical measurements (± SE) of Cowpie Creek, Nippersink Creek and Lawrence Creek in 1997-1998.

	Cowpie	**Nippersink**	**Lawrence**
Width (m)	3.0 (±0.2)	4.1 (±0.3)	5.2 (±0.3)
Depth (m)	0.1 (±0.0)	0.1 (±0.0)	0.2 (±0.0)
Velocity (m/sec)	0.3 (±0.0)	0.3 (±0.0)	0.4 (±0.0)
Discharge (m^3/sec)	0.1 (±0.0)	0.2 (±0.0)	0.3 (±0.1)
Water Temperature (°C)	9.7 (±1.9)	10.4 (±2.4)	11.0 (±1.972)
Substrate Composition (%)Cobble/Gravel/Sand	92 / 5 / 3	86 / 10 / 4	20 / 45 / 35
Nitrate-Nitrogen (NO3-N) (mg/l)	5.1 (±0.2)	6.3 (±0.0)	3.5 (±0.2)
Orthophosphate (PO_4^{-3}) (µg/l)	58.1 (±10.7)	171.9 (±34.4)	4.2 (+0.6)
Dissolved Silica (SiO2)(mg/l)	17.7 (±0.3)	8.9 (±0.4)	8.3 (±0.4)
Length of Canopied Riparian Zone (km)	1.6	0.4	0.4

to the south of the stream and 30 meters to the north of the stream. A farmhouse resides .25 kilometers north of the stream.

The sampled reach of Nippersink Creek is located approximately 13 kilometers northwest of CC. NC originates several miles north of Hebron, IL and flows southeast into Wonder Lake. Much of the creek is channelized and runs through open agricultural fields. The sampled reach, which is located just northwest of Hebron, is an unchannelized forested portion, less than 300 meters long, near a road overpass that is bordered by corn and soybean fields. A 15-meter wide riparian canopy buffer strip separates the reach from the agricultural field on the east bank and open prairie on the west bank.

Lawrence Creek, which originates near Walworth, WI, is a channelized stream running through mostly agricultural fields. The sampled reach of LC is located in unincorporated Lawrence, IL, approximately 15 kilometers west of the NC site. The study site is located in a channelized portion of the creek that has a riparian area less than 300 meters long just east of a road overpass. The 10-meter wide riparian buffer strip separates the creek from horse and cattle pastures located on the north and south banks.

Macroinvertebrate Collections

To study the effect of temporal patterns of invertebrate assemblages on bioassessment indices, benthic samples were collected monthly for one year from riffle areas of the three streams. Nine benthic samples were collected monthly from each stream using a 0.09m^2 Hess sampler with a 243 μm mesh. Samples were collected from transects arranged perpendicular to stream flow. Three samples were collected along each of three transects in each stream. With the aim to represent the macroinvertebrate community structure across the width of the channel, one sample was collected from mid-channel areas and one sample from each margin. After collection, the samples were immediately preserved in 80% ethanol and returned to the laboratory for processing. Patterns in assemblages were expected to differ between streams due to differences in flow regime and agricultural influences.

Physical and Chemical Measurements

On each collection date, channel width was recorded at each transect and current velocity, depth, water temperature and substrate composition were recorded at each replicate sample location. Current velocity and depth measurements were used to calculate discharge for each transect, obtaining three replicate discharge values per month for each stream. Mean monthly discharge was calculated from the three replicate values and mean annual discharge was calculated from the 12 mean monthly values. Mean monthly and annual temperatures were obtained in the same manner. Water samples for nutrient analysis were collected in July 1998 from three replicate riffle areas in each stream. The samples were filtered in the field, frozen, and shipped on dry ice to the University of Michigan Biological Station for analysis. Levels of nitrate-nitrogen, ortho-phosphorate, and dissolved silica were measured using a Technicon II Dual-Channel Autoanalyzer.

Sample Processing

In the laboratory, macroinvertebrates were sorted from samples using sugar flotation (Lind, 1979) and identified to the lowest taxonomic level possible using Merritt and Cummins (1996) and several species-level keys (Bergman and Hilsenhoff 1978, Mackay 1978, Morihara and McCafferty 1979, Schuster and Etnier 1978). After sorting macroinvertebrates from the samples, those samples containing high numbers of macroinvertebrates were subsampled before identifications were made. To create a subsample, samples were placed into a gridded tray and split into halves until each portion contained at least 100 organisms and no more than 300 organisms. Organisms from one subsample were separated from the rest and identified. For indices requiring 100 individuals per sample, a random sample, consisting of identified individuals from the subsamples, was generated using Microsoft Excel to create a subset of macroinvertebrates. This was accomplished by assigning a random number to each macroinvertebrate in the sample and sorting the random numbers in ascending order. The first 100 randomly sorted macroinvertebrates were chosen for use in index calculations. Four independent random samples were generated from each complete sample, two containing individuals from all taxa, including Chironomidae, and two containing individuals from all taxa except Chironomidae. Within each of those two groups, all taxa including Chironomidae and all taxa except Chironomidae, one random sample included only individuals from the class Insecta and Amphipoda and Isopoda from the class Crustacea. The other random sample included all individuals from other non-insect groups in addition to the class Insecta and orders Amphipoda and Isopoda. Amphipoda and Isopoda were separated from other non-insects for the purpose of calculating the BI, which does not incorporate other non-insect groups.

Biotic Index Calculations

The overall goal of this research was to compare and contrast the influence of benthic macroinvertebrate temporal variability on three biotic indices, a multimetric index, and 5 richness indices used in the Great Lakes region of the U.S. The biotic indices examined were: the Biotic Index (BI) (Hilsenhoff, 1987), the Family-Level Biotic Index (FBI) (Hilsenhoff, 1988a) and the Illinois Macroinvertebrate Biotic Index (MBI) (IEPA, 1987) (Table 2). The BI, which was tested for use in the state of Wisconsin in 1977 and adopted shortly thereafter, incorporates only those invertebrates in the class Insecta, with the exception of individuals from the orders Isopoda and Amphipoda. The FBI and MBI are both modifications of the BI (Hilsenhoff 1977, 1982, 1987), however the MBI was adapted for use in Illinois and includes macroinvertebrates from non-insect groups. The BI, FBI and MBI incorporate tolerance values assigned to each taxon. The BI and the MBI use species-level assignments, whereas the FBI assigns values to the family level. In this study, when only species-level index values of a particular genus were available and either the value for the species in question was unavailable or genus was the lowest taxonomic level identified, available species-level values within the genus were averaged to obtain a mean value for the unidentified species. Also, if a particular generic-level value was unavailable, all generic-level values available for the family were averaged to obtain a mean for unidentified genera. The MBI includes family-level index values, which were used when genus-level values were unavailable. Two independent values

Table 2. Calculations and descriptions of equations used for the 9 indices or metrics.

INDEX/METRIC	CALCULATION	DESCRIPTION
Biotic Index (BI) (Hilsenhoff, 1987)	$BI=\Sigma(n_i a_i)/N$	n_i = no. of individuals in each taxon (genus/species) a_I = tolerance value for taxon N = total no. of individuals in sample Tolerance values: 1 - 10
Family Biotic Index (FBI) (Hilsenhoff, 1988)	$FBI=\Sigma(n_i a_i)/N$	n_i = no. of individuals in each family a_i = tolerance value for family N = total no. of individuals in sample Tolerance values: 1 - 10
Illinois Macroinvertebrate Biotic Index (MBI) (IEPA, 1987)	$MBI=\Sigma(n_i t_i)/N$	n_i = no. of individuals in each taxon (genus/species) t_I = tolerance value for taxon N = total no. of individuals in sample Tolerance values: 1 - 11
Procedure 51 (P51) (Michigan DEQ, 1996)	Σ of 9 metrics • total taxa • mayfly taxa • caddisfly taxa • stonefly taxa • % mayfly •%caddisfly •%dominance •%isopod,snail& leech •%surface dependent	Multimetric: each metric given score of –1 (low abundance), 0, or +1 (high abundance) Σ of metrics is total score (scale ranges from -9 to +9)
% EPT	$\% EPT=\sum(EPT)/N$	E = no. of taxa in the order Ephemeroptera P = no. of taxa in the order Plecoptera T = no. of taxa in the order Trichoptera N = total no. of taxa in sample
EPT	$EPT=\sum(EPT)$	E = no. of taxa in the order Ephemeroptera P = no. of taxa in the order Plecoptera T = no. of taxa in the order Trichoptera

Table 2. (cont.)

INDEX/METRIC	CALCULATION	DESCRIPTION
Chironomidae Taxa Richness (CTR)	CTR=∑C	C = total no. of Chironomidae taxa in sample
Non-Insect Taxa Richness (NITR)	NITR=∑NI	NI = total no. of non-insect taxa in sample
Total Taxa Richness (TR)	TR=∑N	N = total no. of taxa in sample

were calculated per index for each sample, one value derived from the randomly generated sample including Chironomidae and one derived from the randomly generated sample omitting Chironomidae. MBI index values were derived from randomly generated samples including non-insect groups. To calculate the indices, the product of each taxon abundance and tolerance value was summed to obtain the final index value. Values of biotic indices increase with degradation in water quality.

The Great Lakes and Environmental Assessment Section Procedure 51 (P51) (MDEQ, 1996) (Table 2), a multimetric index developed and used in the state of Michigan, groups nine taxonomic metrics into one of three categories, with each metric representing an invertebrate group at the order level. The categories are assigned values of 1, 0 , or -1 and are based on the number of families present in a given order and the percentage of individuals in a given order representing the total individuals in the sample. Stream channel width also influences the assignment of the categorical value. The categories are then summed to obtain the final index value. In contrast to biotic indices, P51 index values increase with improving water quality.

The richness metrics examined were: EPT taxa richness, % EPT taxa richness, Chironomidae taxa richness, non-insect taxa richness and total taxa richness (Table 1). The EPT index represents the total number of taxa in the orders Ephemeroptera, Plecoptera and Trichoptera, whereas %EPT indicates the percentage that EPT comprise of the total number of taxa in a sample. Chironomidae taxa richness and non-insect taxa richness are calculated by tallying the total number of chironomid and non-insect taxa, respectively, in a sample. Total taxa richness is calculated by tallying the total number of macroinvertebrate taxa, preferably at the species level, in a sample. As with P51, values of the three richness metrics increase with improvements in water quality. Percent abundance of EPT, Chironomidae, non-insect taxa and *Gammarus* sp. also were calculated by tallying the percentage that individuals in each group represented of the total number of individuals in the sample (Table 3).

Data Analysis

Index calculations resulted in 12 monthly values and one annual value for each index. Values were calculated for each of the nine replicate samples collected per month and were averaged to obtain a mean monthly index value. To facilitate comparisons with other studies, months were assigned to specific seasons (September - November = Fall; December - February = Winter; March - May = Spring; June - August = Summer). Annual values were calculated as a mean of the 12 monthly values. The change in monthly and annual ratings was examined and annual taxa richness and percent abundance metrics were tested for differences between streams using a 1-way ANOVA and a Tukey's Multiple Comparison Test due to the lack of a rating scale for those metrics. For these analyses, twelve samples served as replicates.

Because Hilsenhoff (1977, 1982, 1987, 1988, 1988b, 1990) suggested using only riffle samples to calculate biotic index values, monthly BI, MBI and FBI values also were calculated using only the three samples collected from the center of the stream, which represented riffle areas. Riffle sites were compared to all sites (including riffles and margins) to examine differences in index ratings. All taxa richness and percent abundance metrics were tested for differences between sites each month and annually

Table 3. Calculations and descriptions of equations used for percent metrics.

PERCENT METRICS	CALCULATION	DESCRIPTION
Percent EPT Abundance (% EPT-A)	% EPT-A=∑(EPT)/N	E = no. of individuals in the order Ephemeroptera P = no. of individuals in the order Plecoptera T = no. of individuals in the order Trichoptera N = total no. of individuals in sample
Percent Chironomidae Abundance (% Chir-A)	% Chir-A=∑C	C = total no. of individuals in the family Chironomidae
Percent Non-Insect Abundance (% NI-A)	% NI-A=∑NI	NI = total no. of individuals in non-insect groups
Percent *Gammarus* Abundance (% Gam-A)	% Gam=∑G	G = total no. of individuals in the genus *Gammarus*

using a 1-way ANOVA. To obtain replicates for statistical analysis, the three all site samples were calculated as an average of 2 margin sites and a riffle site along a transect.

To determine if index values differed temporally, monthly values were assigned water quality ratings with the index's corresponding rating system (Table 4). Annual values also were assigned water quality ratings to assess the overall health of the streams. To determine if similar temporal patterns were present among the different bioassessment indices, Pearson product-moment correlation coefficients were calculated for each pairwise combination of indices using mean monthly values.

To assess whether temporal variability in bioassessment indices reflected temporal changes in macroinvertebrate community structure, macroinvertebrate samples were ordinated by Detrended Correspondence Analysis (DCA) (Hill and Gaugh, 1980) and analyses were conducted for samples including all macroinvertebrates, excluding Chironomidae and including only riffles sites. Species used in the analysis comprised at least 5% of total macroinvertebrate annual abundance in any one stream. This is because rare taxa add little information to the data set and can make the data set more variable, making interpretation difficult (Norris, 1995) For both all-macroinvertebrate and chironomid omission assessments, the percentage of total macroinvertebrate monthly abundance comprised by each taxa was ordinated. Coefficients of variation (CV) were calculated for the 12 monthly ordination values in each stream to evaluate natural variation in the macroinvertebrate community throughout the year. Physical variables, such as stream discharge, depth, velocity, width and temperature, as well as each index, were tested for correlation with each axis. To determine the ability of the indices to reflect temporal changes in macroinvertebrate communities, and thus potential changes in water quality of each stream, temporal patterns in the index values were compared to temporal changes in stream macroinvertebrate community structure depicted by the ordination analysis. This was accomplished by overlaying ordination values with rating clusters so that similarity of ratings over time along the different axis gradients could be detected. To distinguish if bioassessment indices differed in their sensitivity to temporal changes in stream insect assemblages, annual CV's, obtained from the 12 monthly index values from each stream, were compared to CV's of the ordination values of each stream. To compare annual P51 CV's, a new scale was devised that assigned values 1 through 18 to the original scale of –9 through +9.

Each question addressed in this study also was examined by omitting Chironomidae from index calculations to assess whether exclusion of the family altered the performance of the indices. Of the taxa richness and percent abundance metrics, recalculation of EPT taxa richness, Chironomidae taxa richness and non-insect taxa richness does not affect final index values and therefore were not calculated without chironomids.

Table 4. Water quality ratings and corresponding index values for the BI, MBI, FBI and P51. The * denotes opposite scale compared to other indices. NA indicates a rating not applicable to the index.

Water Quality Rating	*BI*	*MBI*	*FBI*	*P51**
Excellent	0.00 – 3.50	0 – 5.0	0.00 – 3.75	≥ +5.00
Very Good	3.51 – 4.50	5.0 – 6.0	3.76 – 4.25	NA
Good	4.51 – 5.50	6.0 – 7.5	4.26 – 5.00	NA
Acceptable, tending towards excellent	NA	NA	NA	0.00 – 5.00
Acceptable, tending towards poor	NA	NA	NA	-5.00 – -0.01
Fair	5.51 – 6.50	6.0 – 7.5	5.01 – 5.75	NA
Fairly Poor	6.51 – 7.50	NA	5.76 – 6.50	NA
Poor	7.51 – 8.50	7.5 – 10.0	6.51 – 7.25	≤ -5.00
Very Poor	8.51 – 10.00	>10.0	7.26 – 10.00	NA

CHAPTER III.

RESULTS

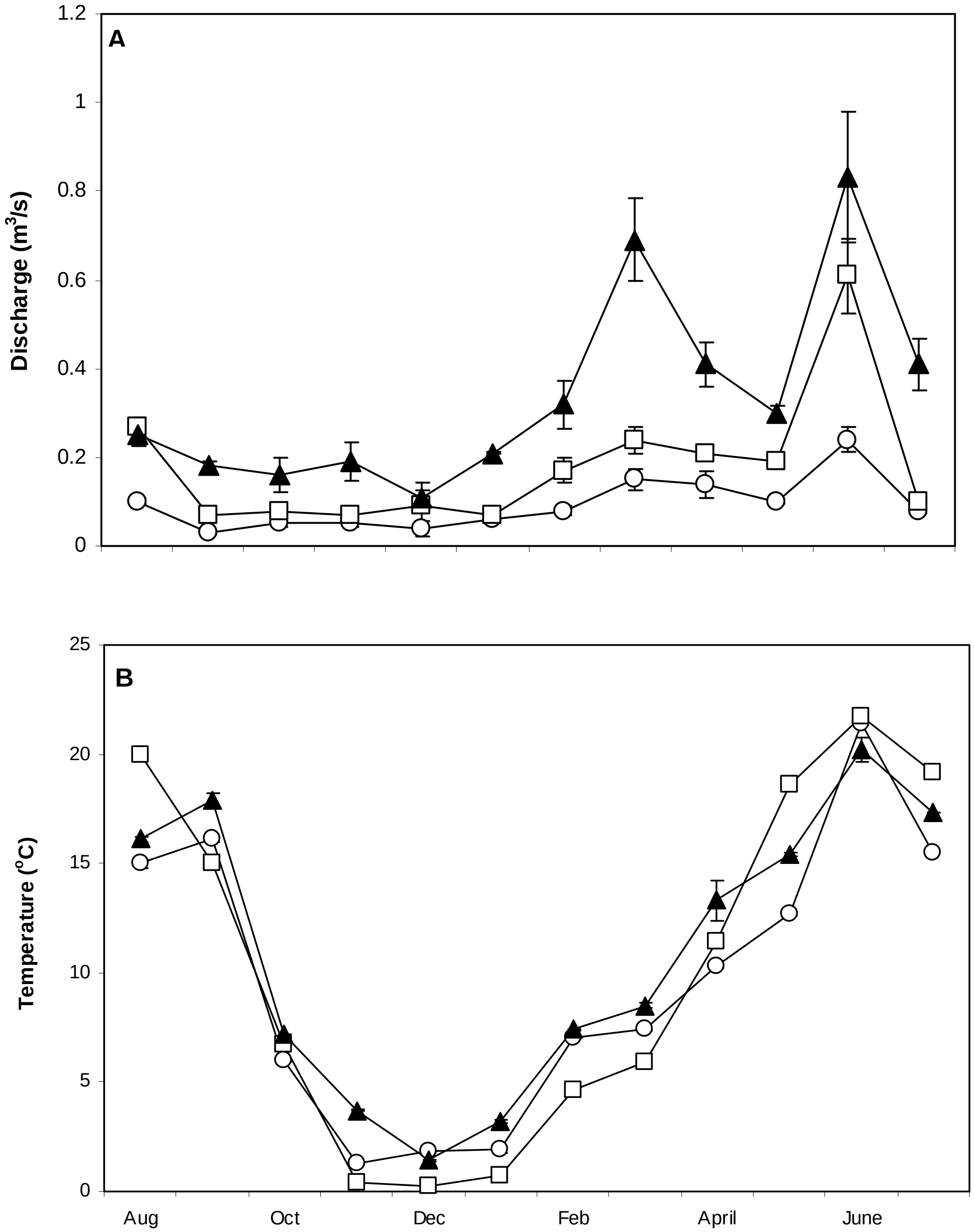
Physical Stream Characteristics

Lawrence Creek (LC) had the highest and most variable annual discharge of the three streams (Table 1) and exhibited the highest discharge in each month except August (Figure 1), whereas Cowpie Creek (CC) had the lowest annual and monthly discharge and least variable discharge throughout the year. Monthly trends in discharge were similar in all three streams, except that discharge in LC increased sharply in March, in contrast to CC and Nippersink Creek (NC), which increased only slightly during the same time period.

Although mean annual temperatures were highest in LC and lowest in CC (Table 4), mean monthly temperatures of the three streams (Figure 1) were similar throughout the year.

Nitrate-nitrogen and orthophosphate concentrations were very high in all three streams. Both were highest in NC and lowest in LC (Table 1). Dissolved silica concentrations were highest in CC. Overall, nutrient concentrations in LC were the lowest of the three streams.

Figure 1. Mean monthly discharge (A) and water temperature (B) (±1 SE) in Cowpie Creek (o), Nippersink Creek (□) and Lawrence Creek (▲) in 1997-1998.



Characterization of Macroinvertebrate Assemblages

All Sites

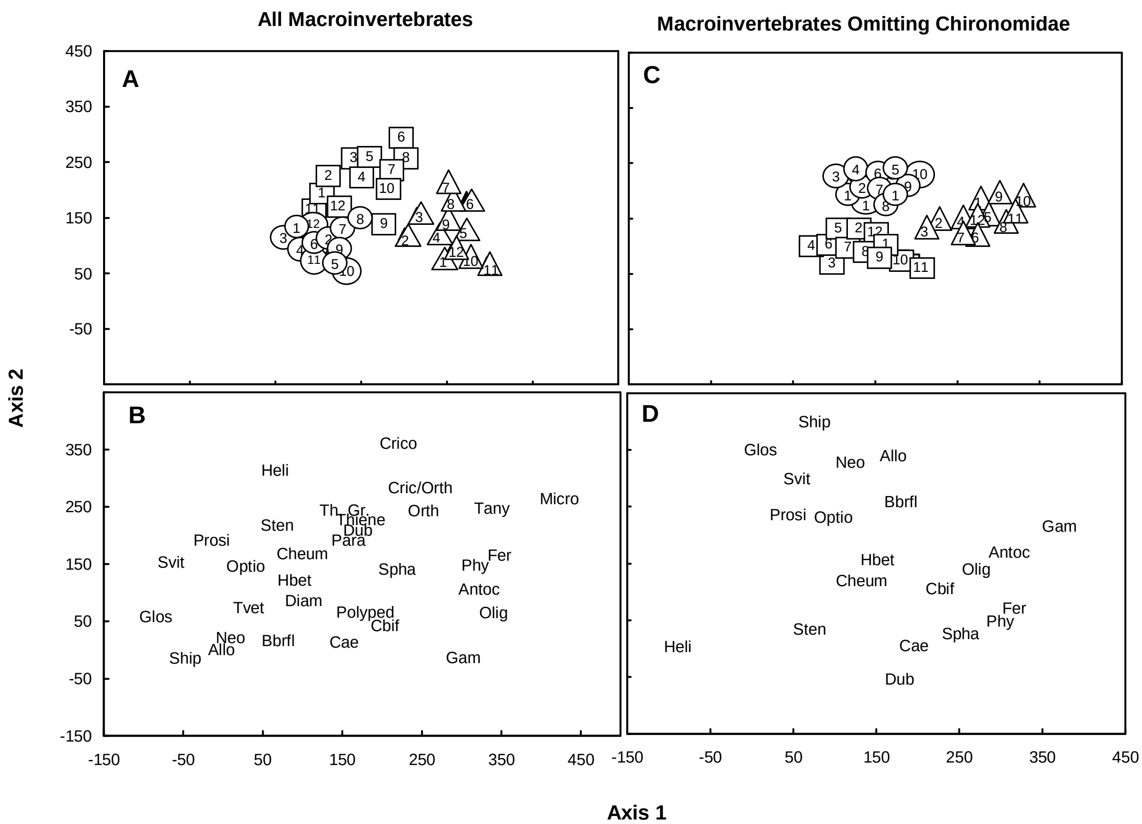
Mean annual macroinvertebrate densities (number/m^2 $\pm$SE) were significantly greater in NC (6755 $\pm$ 839) and LC (6469 $\pm$ 724) than in CC (3397 $\pm$ 425) (1-way ANOVA, $F_{2,11}$=14.1, Tukey p$\leq$0.05). Mean annual taxa richness for all sites also was significantly greater in NC and LC compared to CC (1-way ANOVA, $F_{2,11}$=40.0, Tukey p$\leq$0.05)(Table 5). Mean annual EPT (1-way ANOVA, $F_{2,11}$=38.5) and mean annual %EPT (1-way ANOVA, $F_{2,11}$=93.3) were significantly different between streams. Cowpie Creek had the highest mean annual %EPT at all sites (Tukey p$\leq$ 0.05), although mean annual EPT taxa was not significantly different between CC and NC (Tukey p>0.05). Lawrence Creek had significantly fewer EPT and %EPT taxa than the other two streams when all sites were included (Tukey p$\leq$0.05)(Table 5). Mean annual chironomid taxa richness using all sites was significantly different in all streams (1-way ANOVA, $F_{2,11}$=43.4, Tukey p$\leq$0.05)(Table 5). Lawrence Creek had the highest number of chironomid taxa (11 taxa) and CC the lowest (6 taxa), however, midge taxa richness was greater than other taxa collected in CC. Mean annual non-insect taxa richness from all sites also was significantly different in the three streams, with Lawrence Creek having the highest number of non-insect taxa (7 taxa) and CC the lowest (4 taxa)(1-way ANOVA, $F_{2,11}$=35.7, Tukey p$\leq$0.05)(Table 5).

Streams formed distinct clusters on the DCA ordination plot (Figures 2A and C), suggesting each stream supported a taxonomically distinct macroinvertebrate community. The first ordination axis accounted for 42.5% of the variation in the macroinvertebrate

Table 5. Mean annual values (± SE) for total macroinvertebrate density, Ephemeroptera,-Plecoptera-Trichoptera (EPT and %EPT), Taxa Richness (TR), Chironomid Taxa Richness (CTR) and Non-Insect Taxa Richness (NITR) in Cowpie Creek (CC), Nippersink Creek (NC) and Lawrence Creek (LC) in McHenry County, IL in 1997 and 1998. Values are presented for all sites combined (stream margins and riffles) and for riffle sites only. Means with different letters indicate significant differences (1-way ANOVA, Tukey $p \leq 0.05$) between streams.

		CC	**NC**	**LC**
All Sites	**Total Macroinvertebrate Density (#/m^2)**	a 3397 ± 425	b 6755 ± 425	b 6469 ± 742
	EPT	a 5.4 ± 0.2	a 5.4 ± 0.3	b 3.5 ± 0.3
	%EPT	a 26.3 ± 0.9	b 21.8 ± 1.3	c 13.3 ± 0.9
	TR	a 20.6 ± 0.6	b 25.2 ± 0.9	b 26.6 ± 0.5
	CTR	a 6.4 ± 0.5	b 9.3 ± 0.7	c 11.1 ± 0.3
	NITR	a 4.4 ± 0.3	b 5.5 ± 0.3	c 7.0 ± 0.3
Riffle Sites	**Total Macroinvertebrate Density(#/m^2)**	a 3814 ± 534	b 7031 ± 599	b 7243 ± 727
	EPT	a 5.6 ± 0.4	a 5.8 ± 0.4	b 4.1 ± 0.3
	%EPT	a 28.1 ± 1.1	b 23.9 ± 1.7	c 15.0 ± 1.1
	TR	a 19.9 ± 0.8	b 24.8 ± 0.8	c 27.5 ± 0.5
	CTR	a 6.1 ± 0.3	b 9.4 ± 0.7	c 10.6 ± 0.4
	NITR	a 3.6 ± 0.5	b 4.8 ± 0.5	c 6.9 ± 0.4

Figure 2. DCA values for monthly all site samples (A,C) and species loadings (B,D) for CC (○), NC (□) and LC (Δ) for all macroinvertebrates (A,B) and macroinvertebrates omitting Chironomidae (C, D). The numbers within the symbols correspond to the month of August through July, respectively. Axis 1 was positively correlated with stream discharge, width, FBI, non-insect taxa richness and total taxa richness ($p<0.05$) and negatively correlated with P51, EPT, %EPT and % EPT abundance ($p<0.05$). Axis 2 was positively correlated with the BI, MBI, FBI and % *Gammarus* abundance ($p<0.05$). Excluding Chironomidae, axis 1 also was positively correlated with current velocity ($p<0.05$), but not taxa richness or the FBI ($p>0.05$). Taxa abbreviations are provided in Table 8. Taxa shown represent at least 5% of total macroinvertebrate abundance. Each symbol in the upper panel represents a mean DCA score of 9 replicates.



community, whereas the second axis explained 31.6% of the variation. All sites were used in the DCA.

Total macroinvertebrate community structure in CC and NC was more similar to one another than to LC, as indicated by DCA values along axis 1 (Figure 2A). Annual coefficients of variation (CV) of ordination values on axis 1 for CC and NC were higher than those for LC (Figure 3). In contrast, the CV in LC was greater than in CC and NC along DCA axis 2, resulting in axis values similar to both CC and NC. Cowpie Creek and NC showed relatively little similarity among axis 2 values (Figure 2A). This indicated greater similarity in community structures of CC and NC to LC than to each other on the second axis. Annual CVs of the ordination values along DCA axis 2 (Figure 3) were higher in LC.

DCA axis 1 was positively correlated ($p \leq 0.05$) with stream width and discharge (Table 6), reflecting the greater width and discharge in LC compared to CC and NC (Figure 2A). None of the physical variables were significantly correlated with axis 2. The first axis also was positively correlated with the FBI (Table 6), TR, CTR and NITR (Table 7)($p \leq 0.05$) and significantly negatively correlated with P51 (Table 6), EPT, %EPT and percent EPT abundance (Table 7)($p \leq 0.05$), suggesting CC had the best water quality and LC the poorest (Figure 2A). The BI, MBI, and FBI were positively correlated with axis 2 (Table 6), suggesting CC had the best water quality and NC the poorest (Figure 2A). Percent *Gammarus* abundance was negatively correlated with axis 2 (Table 7)($p \leq 0.05$).

Species loadings (Figure 2B, Table 8) revealed that clean water taxa, including *Shipsa* (Plecoptera), *Glossosoma* (Trichoptera), *Allocapnia* (Plecoptera) and *Neophylax*

(Trichoptera), highly influenced community structure at sites with low values on both axes and, therefore, strongly influenced community structure of CC. *Microtendipes* (Diptera) and *Gammarus* (Amphipoda), as well as the tolerant non-insect taxa *Ferrissia* (Pelecypoda) and *Physa* (Gastropoda), strongly influenced the community structure of sites with high axis 1 values, i.e., LC. Sites with high axis 2 values were highly influenced by a variety of midges including *Cricotopus*, *Orthocladius* and the *Thienemannimyia* spp. group as well as *Helicopsyche* (Trichoptera) and therefore, strongly influenced the community structure in NC.

Riffle Sites

Mean annual macroinvertebrate densities (number/m^2) in riffle sites were significantly greater in NC (7031 $\pm$ 599) and LC (7243 $\pm$ 727) compared to CC (3814 $\pm$ 534) (1-way ANOVA, $F_{2,11}$=13.1, Tukey $p \leq 0.05$) and were similar in significance to density results from all sites. There were no significant differences in mean annual densities between all sites and riffle sites within the same stream ($p>0.05$). Although NC and LC had similar taxonomic richness in all site comparisons, taxa richness in riffle sites differed significantly among all three streams ($p \leq 0.05$), with LC having the highest taxa richness (28 taxa) and CC the lowest (20 taxa). No significant differences were found in mean annual taxa richness between riffle sites and all sites (Table 5). Similar to all site comparisons, Lawrence Creek had significantly fewer EPT taxa than the other two streams in riffle sites (1-way ANOVA, $F_{2,11}$=13.2, Tukey $p \leq 0.05$)(Table 5) and CC contained the greatest percentage of EPT taxa.

Table 6. Pearson Product-Moment correlation coefficients of ordination axes and physical parameters and biotic/ multimetric indices for all macroinvertebrates (all sites and riffle sites) and omitting Chironomidae (all sites). The * denotes $p \leq 0.05$.

Physical Parameters		**Discharge**	**Velocity**	**Depth**	**Width**	**Temp**
All Macroinvertebrates	**Axis 1**	0.674*	0.461	0.463	0.728*	0.053
	Axis 2	-0.040	-0.084	0.139	-0.041	-0.350
Riffle Sites	**Axis 1**	0.680*	0.448	0.473	0.748*	0.073
	Axis 2	0.057	-0.008	0.199	0.081	-0.371
Omitting Chironomidae	**Axis 1**	0.670*	0.542*	0.458	0.654*	0.240
	Axis 2	-0.204	-0.118	0.133	-0.334	-0.073

Indices		**BI**	**MBI**	**FBI**	**P51**
All Macroinvertebrates	**Axis 1**	0.519	0.393	0.606*	-0.874*
	Axis 2	0.805*	0.834*	0.694*	-0.318
Riffle Sites	**Axis 1**	0.686*	0.532	0.674*	NA
	Axis 2	0.615*	0.691*	0.531	NA
Omitting Chironomidae	**Axis 1**	-0.155	-0.036	0.102	-0.673*
	Axis 2	-0.898*	-0.881*	-0.768*	0.363

Table 7. Pearson Product-Moment correlation coefficients of ordination axes and taxa richness and percent abundance measures for all macroinvertebrates (all sites and riffle sites) and omitting Chironomidae (all sites). The * denotes $p \leq 0.05$.

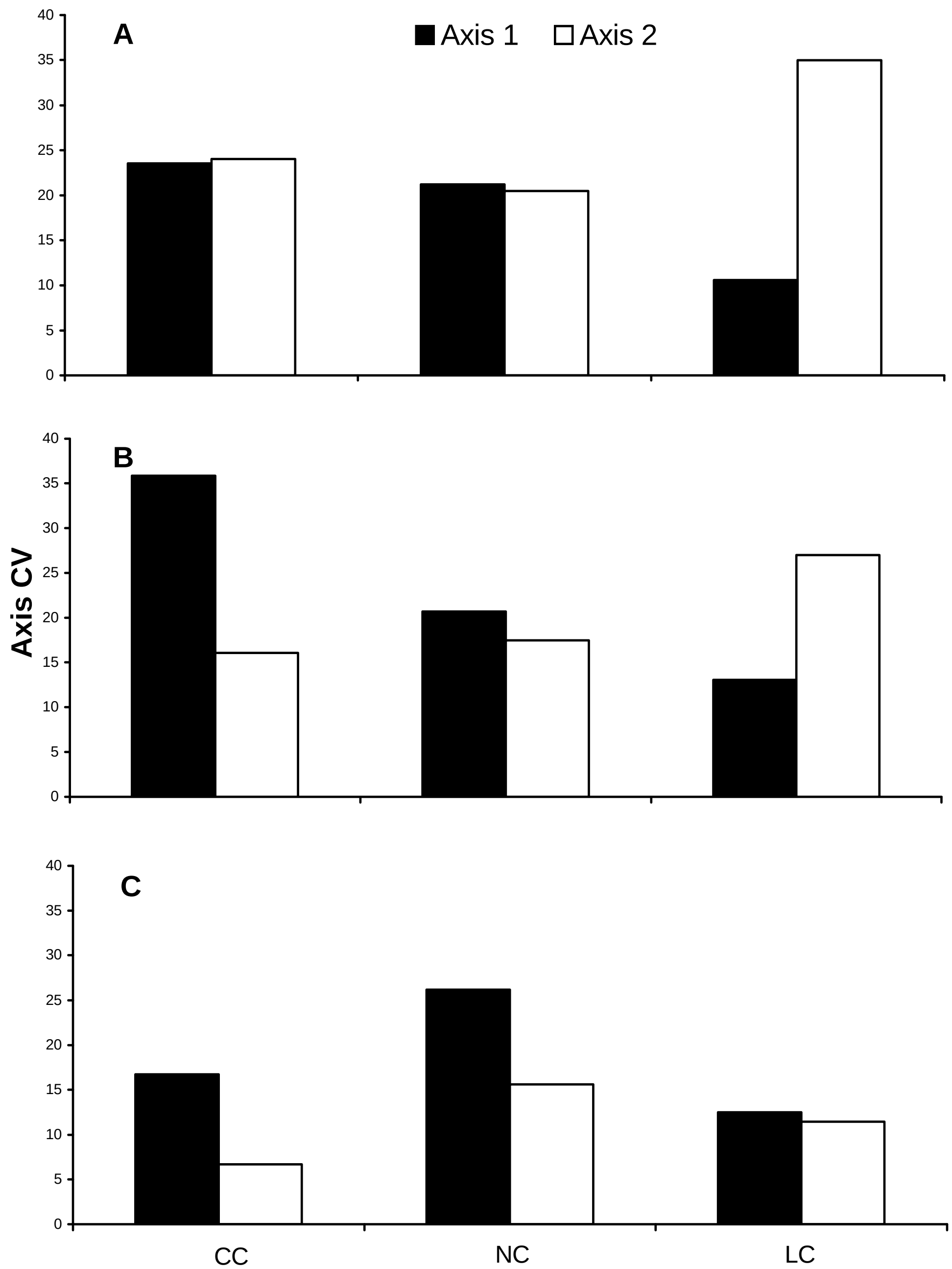
		EPT	%EPT	TR	CTR	NITR
All Macroinvertebrates	Axis 1	-0.786*	-0.844*	0.563*	0.745*	0.648*
	Axis 2	-0.057	-0.224	0.383	0.357	0.007
Riffle Sites	Axis 1	-0.447	-0.719*	0.678*	0.634*	0.596*
	Axis 2	-0.005	-0.253	0.426	0.515	0.085
Omitting Chironomidae	Axis 1	-0.696*	-0.758*	0.006	NA	0.629*
	Axis 2	0.117	-0.515	0.264	NA	-0.282

		%EPT-A	%Chir-A	%NI-A	%Gam
All Macroinvertebrates	Axis 1	-0.573*	0.508	0.412	0.419
	Axis 2	-0.162	0.424	-0.035	-0.591*
Riffle Sites	Axis 1	-0.370	0.531	0.590*	-0.518
	Axis 2	-0.078	0.258	0.184	-0.518

Table 8. Species comprising ≥5% (●) and <5% total abundance (○) in CC, NC and LC. Species that did not comprise at least 5% of total abundance in any stream are not listed.

Order	Genus/species	Abbrev.	CC	NC	LC
Ephemeroptera	*Baetis brunneicolor/flavistriga*	Bbrfl	●	●	○
Plecoptera	*Allocapnia*	Allo	●		
	Shipsa	Ship	●		
Trichoptera	*Ceratopsyche bifida*	Cbif	○	○	●
	Cheumatopsyche	Cheum	●	●	●
	Glossosoma	Glos	●		
	Helicopsyche	Heli	○	●	○
	Hydropsyche betteni	Hbet	●	●	●
	Neophylax	Neo	●		
Coleoptera	*Dubiraphia*	Dub	○	●	○
	Optioservus	Optio	●	●	●
	Stenelmis	Sten	●	●	●
Diptera	*Antocha*	Antoc	○		●
	Prosimulium	Prosi	●	○	○
	Simulium vittatum	Svit	●	○	○
	Chironomidae:				
	Cricotopus	Crico	○	●	●
	Cricotopus/ Orthocladius	Cric/Ortho	●	●	●
	Diamesa	Diam	●		
	Microtendipes	Micro		○	●
	Orthocladius	Orth	●	○	●
	Parametriocnemus	Para	●	○	●
	Polypedilum	Polyped	●	●	●
	Tanytarsus	Tany	○	○	●
	Thienemanniella	Thiene	○	●	○
	Thienemannimyia	Th. Gr.	○	●	○
	Tvetenia	Tvet	●	○	○
Amphipoda	*Gammarus*	Gam	●	●	●
Isopoda	*Caecidotea*	Cae		●	
Non-arthropods	*Ferrissia*	Fer	○	○	●
	Oligochaeta	Olig		○	●
	Physa	Phy	○	○	●
	Sphaerium	Spha	○	●	○

Figure 3. Annual coefficients of variation (CV, %) in community structure of Cowpie Creek (CC), Nippersink Creek (NC) and Lawrence Creek (LC) calculated for (A) all macroinvertebrates in all sites, (B) all macroinvertebrates in riffle sites and (C) macroinvertebrates in all sites omitting Chironomidae.

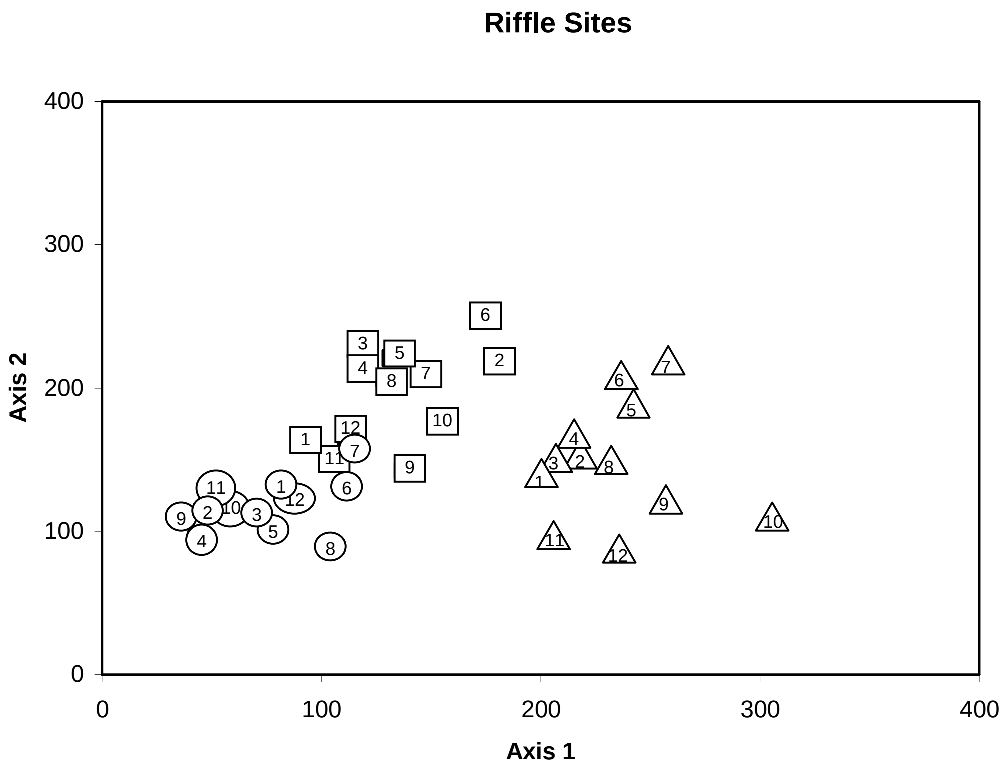


Similar to all site comparisons, chironomid taxa richness was highest in LC (11 taxa) and lowest in CC (6 taxa)(1-way ANOVA, $F_{2,11}$=43.4, Tukey $p \leq 0.05$). Mean annual chironomid taxa richness in all streams was similar between all sites and riffles sites. Non-insect taxa richness also was highest in LC (7 taxa) and lowest in CC (4 taxa)(1-way ANOVA, $F_{2,11}$=13.4, Tukey $p \leq 0.05$), which also was the trend with all site comparisons. Non-insect taxa richness in all streams was similar between riffle sites and all sites.

Streams formed distinct clusters on the DCA ordination plot of riffle samples (Figure 4), suggesting riffles supported a macroinvertebrate community that differed among streams. The first ordination axis accounted for 50.0% of the variation in the macroinvertebrate community, whereas the second axis explained 29.0%. Annual coefficients of variation (CVs) of ordination values on axis 1 for CC were higher compared to those for NC and LC (Figure 3). Annual CVs of the ordination values along DCA axis 2 (Figure 3) were higher in LC than CC or NC. Overall, CVs were less for riffle sites than for all sites, exhibiting a tighter clustering of samples within streams along the axis.

Similar to all site assessments, DCA axis 1 was positively correlated ($p \leq 0.05$) with stream width and discharge (Table 6), reflecting the greater width and discharge in LC compared to CC and NC (Figure 4). None of the physical variables were significantly correlated with axis 2. The first axis was also positively correlated with the BI, FBI (Table 6), CTR, NITR, percent NITR abundance and TR and negatively correlated with %EPT (Table 7) ($P \leq 0.05$), suggesting CC had the best water quality and

Figure 4. DCA values for monthly samples of CC (○), NC (□) and LC (Δ) in riffle sites. Axis 1 was positively correlated with stream discharge, width, BI, FBI, Chironomidae taxa richness, non-insect taxa richness, percent non-insect abundance and total taxa richness ($p<0.05$) and negatively correlated with %EPT ($p<0.05$). Axis 2 was positively correlated with the BI and MBI ($p<0.05$). Symbols represent mean DCA scores of 3 replicates.



LC the poorest (Figure 4). The BI and MBI were positively correlated with axis 2, suggesting, on average, CC had the best water quality and NC the poorest (Figure 4).

Temporal Variation in Macroinvertebrate Community Structure

All Macroinvertebrates

All Sites

Overall temporal patterns in DCA values were similar in CC and NC along the first axis (Figure 2). Again, axis 1 was positively correlated with stream discharge, width, FBI, NITR and total taxa richness, and negatively correlated with P51, EPT, %EPT and percent EPT abundance. Although lowest ordination values occurred in late summer/early fall in each stream, patterns in high DCA values were similar only for CC and NC, displaying highest values in the spring. Highest DCA axis 1 values in LC occurred in the summer. Temporal patterns in axis 2 DCA values demonstrated more similarities. All streams were found to have lowest values in late spring (May) and highest values in late winter (February). Axis 2 values were positively correlated with the three biotic indices and percent *Gammarus* abundance.

Riffle Sites

Overall temporal patterns in DCA ordination of riffle sites differed from all sites on the first axis (Figure 4). Axis 1 was positively correlated with stream discharge, width, BI, FBI, CTR, NITR, percent non-insect abundance and total taxa richness and negatively correlated with %EPT. Lowest axis 1 ordination values occurred in late summer in NC and LC, however, lowest values in CC were in late winter. Highest DCA axis 1 values occurred in late winter in CC, early fall in NC and late spring in LC. Whereas all streams were found to have lowest axis 2 values in late spring in all sites, LC displayed lowest axis 2 values in July in riffle sites. Nippersink Creek displayed highest axis 2 values in October. Axis 2 was positively correlated with the BI and MBI ($p<0.05$).

Omitting Chironomidae

The omission of chironomids resulted in patterns similar to when midges were included with the presence of distinct clusters on the DCA ordination plot (Figure 2), suggesting each stream supported a taxonomically distinct macroinvertebrate community, exclusive of chironomids. Cowpie Creek and NC macroinvertebrate community structure appeared more similar to one another than to LC on DCA axis 1, which was indicated by greater overlap of DCA values between CC and NC along the axis (Figure 2). In comparison to all macroinvertebrate assessments, the omission of midges allowed for less distinction in community structure between CC and NC along axis 1. On axis 2, LC values overlapped those of CC and NC to similar extents , whereas CC and NC showed relatively little overlap (Figure 2). In comparison to all macroinvertebrate assessments, the omission of midges allowed for more distinction in community structure between all three streams on axis 2.

All Macroinvertebrate and Non-Chironomid Comparisons

Correlations of physical parameters and indices to both axes were similar regardless of whether Chironomidae was included or omitted from assessments. An additional parameter, current velocity, also was positively correlated ($P \leq 0.05$) with DCA axis 1 when midges were omitted (Table 6).

Comparisons of the CVs along the first ordination axis when chironomids were included and omitted from assessments showed that community structure of NC was more variable when Chironomidae was removed from the assessment data (Figure 3). However, all streams showed less variable DCA values along the second axis when midges were omitted from analyses. With respect to variability across streams, annual CVs of both DCA axes (Figure 3) were higher in NC than the other two streams when midges were omitted, compared to higher axis 1 values in CC and higher axis 2 values in LC when midges were included. This suggested that the inclusion of midges resulted in less variable community structure in NC compared to the other streams, whereas the inclusion of midges resulted in greater variation in community structure of CC (first axis) and LC (second axis) compared to the other streams.

Species loadings (Figure 2) revealed that clean water taxa, including the stoneflies *Allocapnia* and *Shipsa* and the caddisflies *Glossosoma*, and *Neophylax*, highly influenced community structure of sites with low axis 1 values; this also was the case when chironomids were included in the analysis. *Gammarus* and other tolerant non-insect groups most strongly influenced community structure at sites with high axis 1 values, primarily from LC. This was similar to the assessment when midges were included. When all macroinvertebrate taxa were included in the analysis, midges were the most influential group along the second axis. When midges were omitted, *Helicopsyche*, *Stenacron* and *Dubiraphia* most heavily influenced community structure along the second DCA axis (Figure 2).

Overall temporal patterns in DCA values when midges were omitted from analyses were different than patterns observed when midges were included. The omission of midges resulted in similar patterns in each stream along the first axis (Figure 2), whereas patterns differed in LC from the other two streams when midges were included. When midges were omitted, lowest ordination values along DCA axis 1 occurred in October in each stream, whereas highest values occurred in late spring and early summer. Along the second axis in the chironomid omission assessments, the distribution of samples exhibited little to no overlap, whereas distribution of samples from all streams overlapped to some degree when midges were included. In non-chironomid assessments, patterns in DCA axis 2 values in LC were similar to those of NC from fall to winter, at which point patterns in DCA axis 2 values in LC were more similar to those of CC.

Monthly Index Values and Ratings

All Macroinvertebrates

All Sites

The indices exhibited similar trends in water quality ratings of the streams throughout the year (Figures 5A-8A). A general pattern among biotic indices (BI, MBI, FBI) among streams indicated that CC had the best water quality in most months, and either NC and LC or NC alone had the poorest (Figures 5-7A). The multimetric index, P51, showed a slightly different trend, indicating LC as having lowest index values (poor

Figure 5. Mean monthly Biotic Index (BI) values (+1 SE) calculated for all macroinvertebrates (A,C), omitting Chironomidae (B,D), using all sites (A,B) and using riffle sites (C,D) in Cowpie Creek(o), Nippersink Creek(□), and Lawrence Creek(▲).

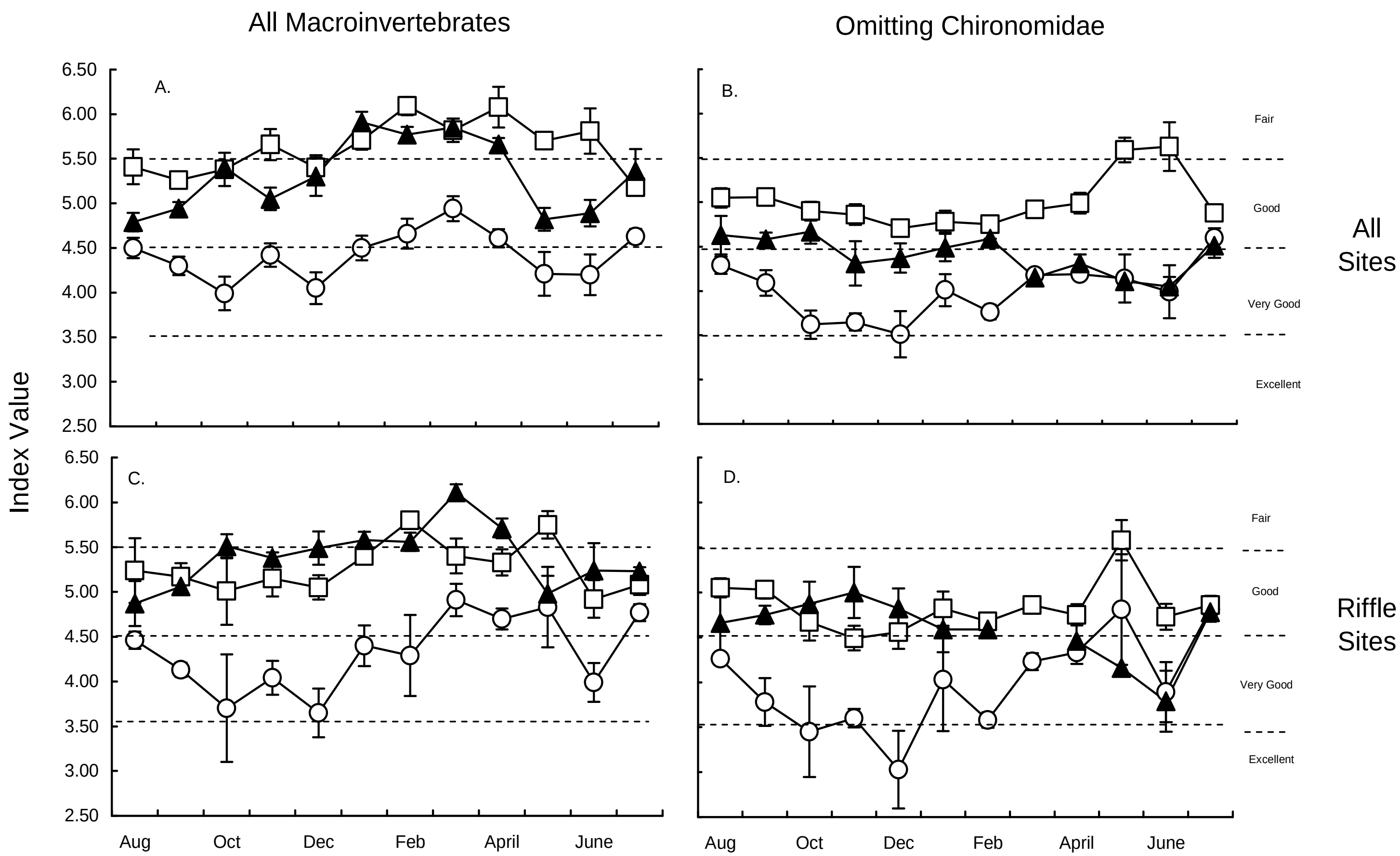


Figure 6. Mean monthly Macroinvertebrate Biotic Index (MBI) values (+1 SE) calculated for all macroinvertebrates (A,C), omitting Chironomidae (B,D), using all sites (A,B) and using riffle sites(C,D) in Cowpie Creek(o), Nippersink Creek(□), and Lawrence Creek(▲).

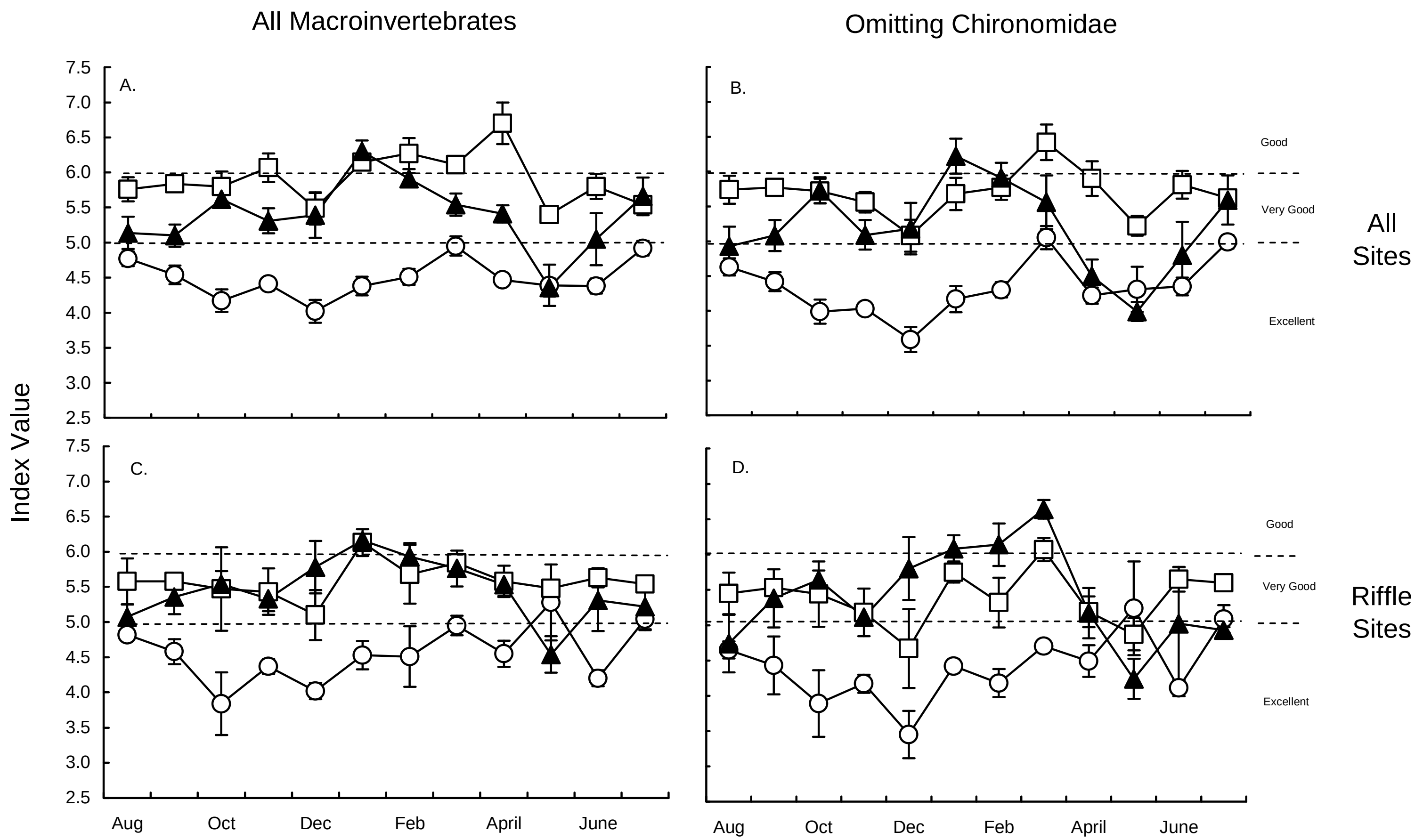


Figure 7. Mean monthly Family-Level Biotic Index (FBI values) (+1 SE) calculated for all macroinvertebrates (A,C), omitting Chironomidae (B,D), using all sites (A,B) and using riffle sites (C,D) in Cowpie Creek(o), Nippersink Creek(□), and Lawrence Creek(▲).

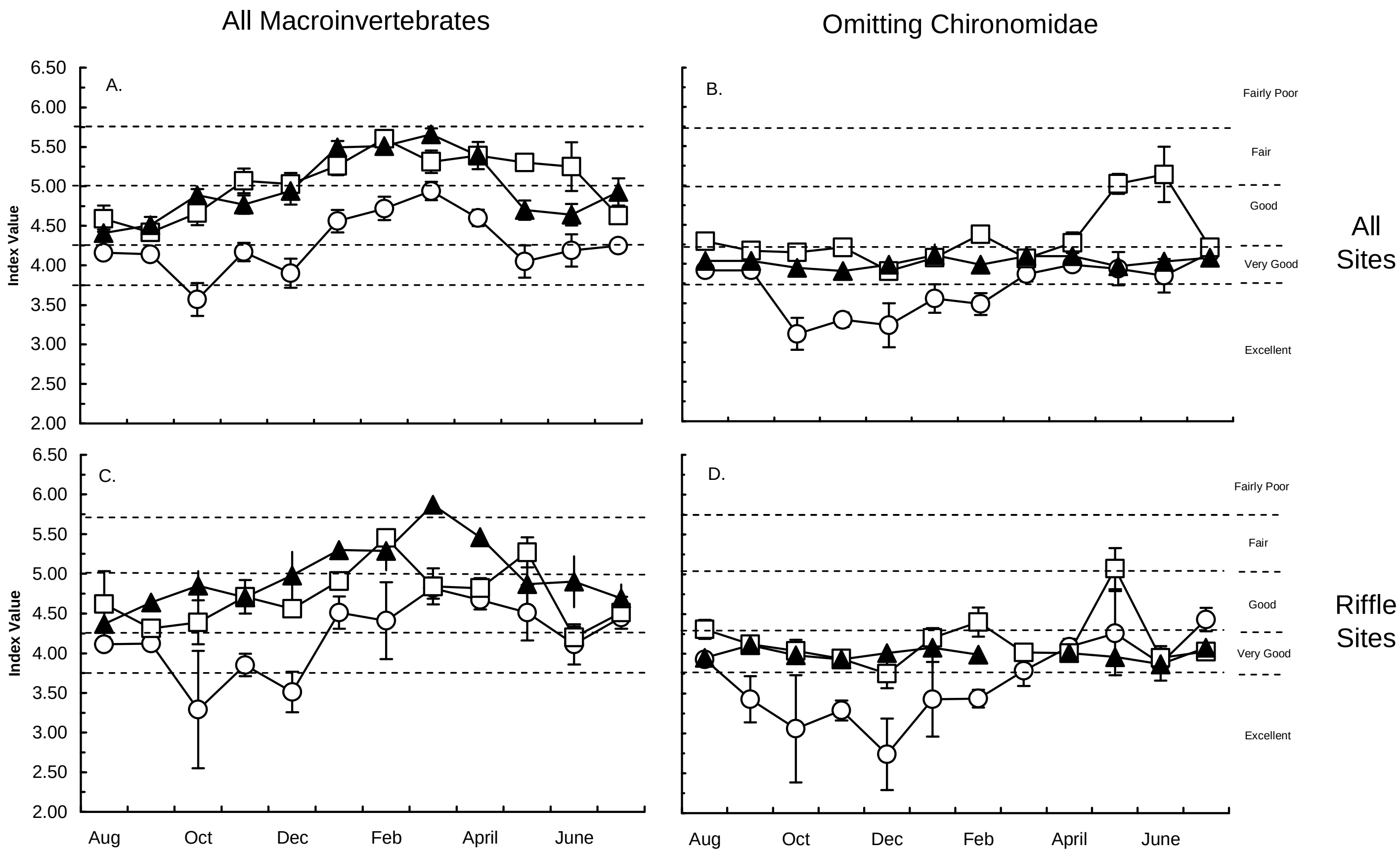
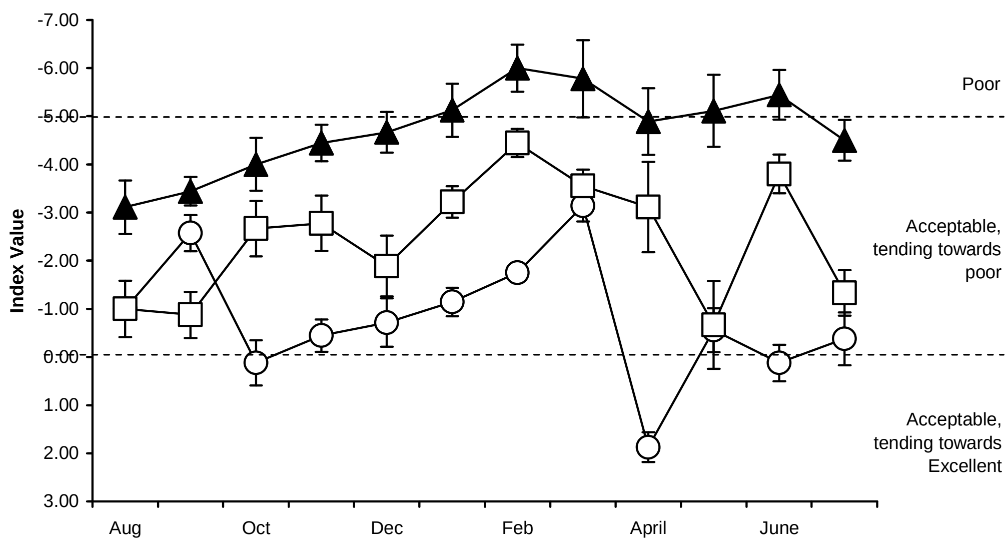
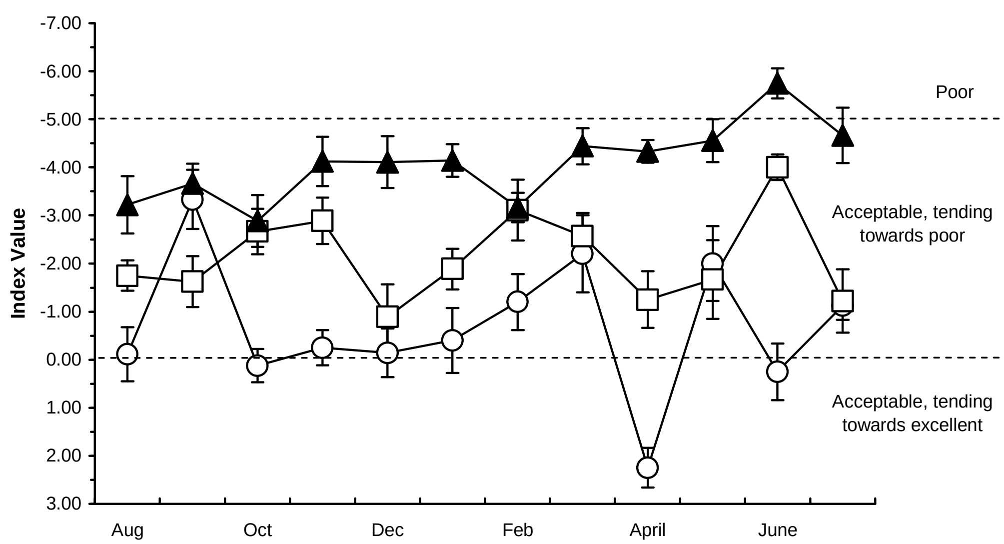


Figure 8. Mean monthly P51 values (+1 SE) calculated for (A) all macroinvertebrates and (B) omitting Chironomidae in Cowpie Creek(o), Nippersink Creek(□), and Lawrence Creek(▲).

A.



B.



water quality) throughout the year, although LC exhibited a poorer rating than the other streams on only one occasion (Figure 8A).

Temporal trends in biotic/multimetric assessments

Cowpie Creek. Similar temporal (monthly) patterns emerged among the biotic and multimetric indices in each stream. In CC, significant positive correlations ($P \leq 0.05$) were found between BI, MBI and FBI monthly index values (Table 9). The correlations were reflected in stream ratings, such that all biotic indices displayed poorest ratings in March (Figures 5A-8A). Patterns in BI and FBI water quality ratings were most similar, exhibiting poorest ratings from mid-winter through mid-spring and then again in mid-summer and late fall. Patterns in MBI values (Figure 6A) were similar to the BI and FBI, but showed less variation in ratings throughout the year, indicating poorest ratings on only two occasions, early spring and early summer. Despite the decline in water quality during those times, water quality ratings were still "very good".

Nippersink Creek. There was slightly more agreement between monthly biotic and multimetric indices in NC than in CC. P51 assessments, whose values increase with improved water quality, was negatively correlated ($p \leq 0.05$) with the biotic indices, whose values decrease with improved water quality, in NC, whereas, in CC, there was no correlation (Table 9). In NC, all monthly multimetric and biotic index values were significantly correlated ($P \leq 0.05$) except for the comparison between the MBI and FBI (Table 9). Significant correlations between biotic and multimetric indices were positive,

Table 9. Pearson Product-Moment correlation coefficients of monthly values including all macroinvertebrates and omitting Chironomidae for the BI, MBI, FBI, P51, EPT, %EPT, Taxa Richness (TR), Chironomid Taxa Richness (CTR) and Non-insect Taxa Richness (NITR) in Cowpie Creek (CC), Nippersink Creek (NC) and Lawrence Creek (LC). The * denotes p≤0.05. Correlations with P51 are negative due to the index's opposite numbering scale.

Stream		All Macroinvertebrates									Omitting Chironomidae					
		BI	**MBI**	**FBI**	**P51**	**EPT**	**%EPT**	**TR**	**CTR**	**NITR**	**BI**	**MBI**	**FBI**	**P51**	**%EPT**	**TR**
CC	**BI**	1.000									1.000					
	MBI	0.822*	1.000								0.840*	1.000				
	FBI	0.908*	0.583*	1.000							0.935*	0.760*	1.000			
	P51	-0.340	-0.392	-0.333	1.000						-0.169	-0.398	-0.200	1.000		
	EPT	-0.153	-0.227	-0.298	0.505	1.000					NA	NA	NA	NA	NA	
	%EPT	-0.218	-0.275	-0.341	0.353	0.949*	1.000				-0.403	-0.606*	-0.563*	0.554*	1.000	
	TR	0.172	0.093	0.102	0.524	0.304	-0.009	1.000			0.231	0.126	0.188	0.338	-0.131	1.000
	CTR	0.370	-0.023	0.345	-0.006	0.321	-0.073	0.625*	1.000		NA	NA	NA	NA	NA	NA
	NITR	-0.028	0.128	0.037	-0.081	-0.239	-0.553*	0.194	-0.329	1.000	NA	NA	NA	NA	NA	NA
NC	**BI**	1.000									1.000					
	MBI	0.717*	1.000								-0.066	1.000				
	FBI	0.920*	0.484	1.000							0.924*	-0.119	1.000			
	P51	-0.740*	-0.665*	-0.697*	1.000						-0.310	-0.396	-0.471	1.000		
	EPT	-0.515	-0.406	-0.522	*0.859	1.000					NA	NA	NA	NA	NA	
	%EPT	-0.477	-0.217	-0.635*	0.804*	0.799*	1.000				-0.125	-0.362	-0.309	0.777*	1.000	
	TR	0.059	-0.124	0.273	-0.117	0.130	-0.479	1.000			0.357	-0.303	0.581*	-0.302	-0.076	1.000
	CTR	0.233	0.196	0.374	-0.219	0.065	-0.430	0.869*	1.000		NA	NA	NA	NA	NA	NA
	NITR	0.280	-0.073	0.240	-0.358	-0.423	-0.384	-0.081	-0.329	1.000	NA	NA	NA	NA	NA	NA
LC	**BI**	1.000									1.000					
	MBI	0.822*	1.000								0.547*	1.000				
	FBI	0.963*	0.673*	1.000							-0.045	0.201	1.000			
	P51	-0.568*	-0.239	-0.727*	1.000						0.838*	0.372	-0.290	1.000		
	EPT	-0.468	-0.263	-0.583*	*0.847	1.000					NA	NA	NA	NA	NA	
	%EPT	-0.501	-0.337	-0.591*	0.853*	0.972*	1.000				0.437	-0.258	-0.191	0.518	1.000	
	TR	-0.139	0.128	-0.271	.0415	0.627*	0.429	1.000			0.643*	0.349	-0.326	0.697*	0.628*	1.000
	CTR	-0.085	0.314	-0.140	-0.450	0.143	0.385	0.746*	1.000		NA	NA	NA	NA	NA	NA
	NITR	0.348	0.152	0.485	-0.828*	-0.821*	-0.757*	-0.620*	-0.404	1.000	NA	NA	NA	NA	NA	NA

except for those correlations with P51, which were negative due to the opposite scale of the index.

General patterns emerged among the BI and FBI in NC showing high index values in the late winter and early spring, with a gradual decline in values by mid-summer. MBI and P51 values tended to decline by late spring, exhibiting better water quality than earlier in the year than than indicated by the BI and FBI. General patterns were also seen in the index ratings. The BI and FBI (Figures 5A and 7A) both displayed poorest ratings from late fall through early summer. The MBI's poorest rating, which was "Good" (Figure 6A), occurred less often throughout the year than the BI and FBI, displaying poorest water quality from mid- to late fall and mid-winter through mid-spring. Improved MBI ratings occurred much earlier in the spring compared to the BI and FBI. P51 ratings did not vary throughout the year, although in May, P51 displayed the best water quality scores in NC and the error associated with mean ratings was in the "Acceptable tending towards excellent" category (Figure 8A). This was in contrast to the BI and FBI ratings, which indicated some of the poorest ratings during that time (Figures 5 and 7). MBI ratings did show trends similar to P51 in May, showing a marked improvement in water quality at that time (very good) (Figure 6).

Lawrence Creek. All biotic indices in LC were positively correlated to each other ($P<0.05$) (Table 9). Because of the reverse scales of P51 and the biotic indices, it's not surprising that the former was negatively correlated ($p\leq 0.05$) with the latter, except for MBI (Table 9). MBI values tended to decline (improved water quality) slightly earlier in the spring after the peak in winter index values (reduced water quality) compared to P51 (Figures 6A and 8A). The MBI also showed a consistent improvement in ratings from late winter through late spring, while P51 showed a decline in water quality in late spring and early summer. Patterns in BI and FBI ratings were most similar of the indices throughout the year. Both indices indicated poorest ratings (rating of Fair) from late-winter through mid-spring, with a subsequent improvement until mid-summer (Figures 5A and 7A). In contrast, the MBI, despite significant correlations with BI and FBI values, indicated the poorest rating (rating of Good) to occur only in mid-winter (Figure 6A) and indicated that water quality improved in late winter, which was earlier in the year compared to the BI and FBI. P51 (Figure 8A) showed similar trends to the BI and FBI from mid-winter through early-spring, indicating poorest ratings (Poor) at that time. However, trends in P51 ratings differed from the other indices in May and June, showing some of the poorest ratings of the year (poor/ acceptable tending towards poor) when the other indices showed improved water quality ratings.

Biotic/Multimetric Assessment Comparisons. Overall, the biotic indices displayed the most similar seasonal patterns of all index types. Although the MBI was consistently correlated with the BI and FBI, patterns in BI and FBI ratings were most similar of the indices throughout the year. In all three streams the MBI showed a pre-summer trend of improved water quality, somewhat earlier than similar increases in the BI and FBI. In NC and LC, which were the two streams for which P51 was correlated with the BI and FBI, P51 tended to deviate from the other indices in late spring.

In addition to the ability of the indices to reflect variability in macroinvertebrate communities, the various rating scales of each index also dictated index sensitivity in determining water quality. Mean annual CVs for P51 values were higher than those of the biotic indices in CC and NC, but not in LC (Table 10), however P51 water quality ratings changed little due to the wide range of values within each rating category (Table 2). As a result, P51 values never varied more than 2 quality ratings per year. The BI also exhibited 2 ratings per year, likely due to low mean annual CVs in comparison to the other indices. In contrast, the FBI rated CC with four different ratings throughout the year, which probably resulted from the combination of high mean annual index CV values (Table 10) and narrower rating categories compared to the other indices (Table 2). The MBI, which had the highest mean annual index CV values of the biotic and multimetric indices in LC, rated LC with three different ratings throughout the year (Table 10).

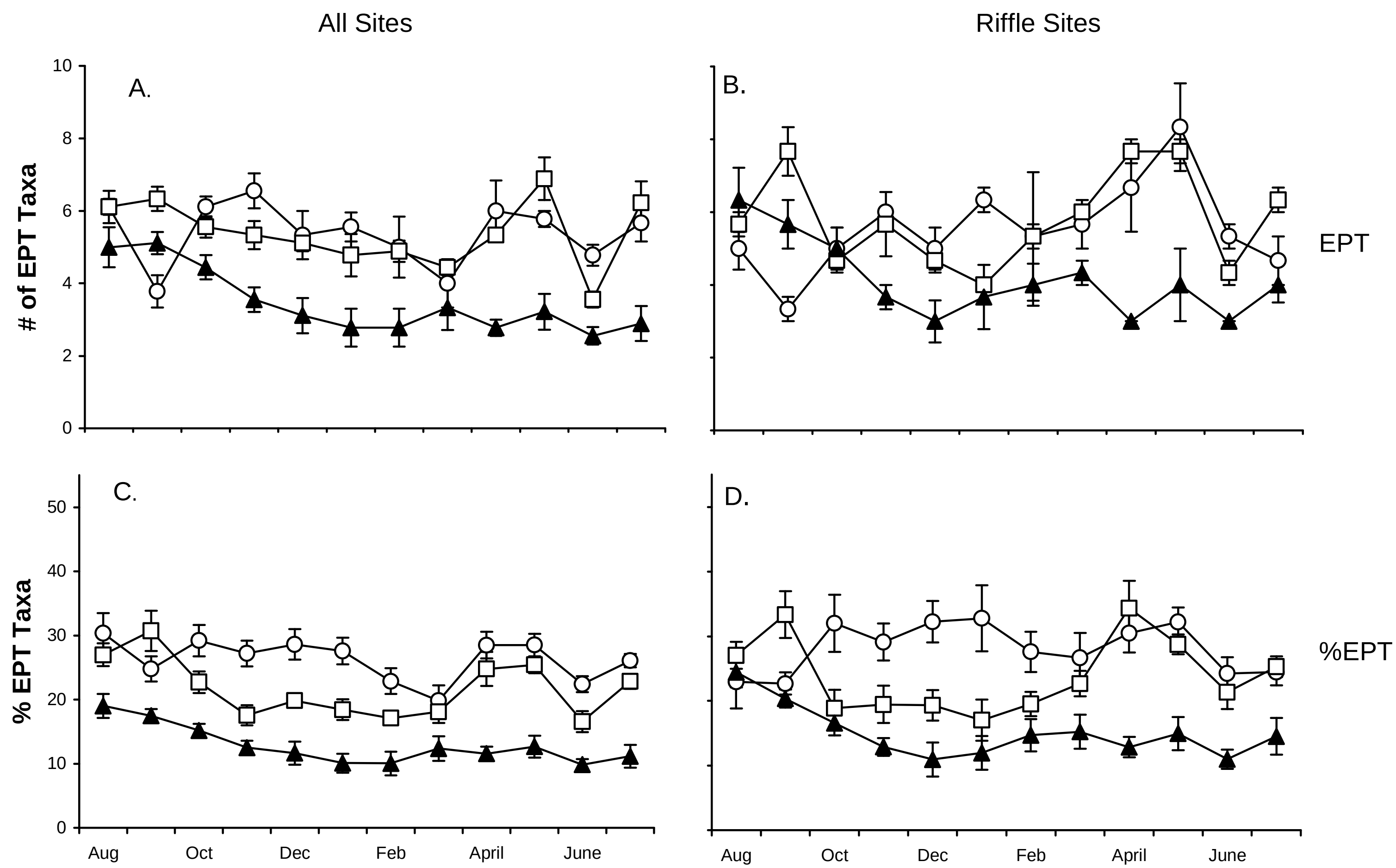
Temporal trends in EPT taxa richness and percent abundance

The degree of similarity of the EPT metrics to the other indices differed among streams. Although there were no significant correlations between either EPT taxa richness metric (EPT and %EPT) and the biotic and multimetric indices in CC, significant correlations were found between the EPT metrics and several other indices in NC and LC. In NC there was a significant positive correlation ($P \leq 0.05$) between the EPT metric and P51 (Table 9). Both the EPT (Figure 9A) and P51 (Figure 8A) metrics exhibited similar temporal patterns throughout the majority of the year, with the exception of differences in early winter when the decline in P51 values, but not EPT metrics, suggested improved water quality, but EPT did not. Differences in patterns among these two indices also occurred in mid-spring, however the variability of the P51

Table 10. Mean annual Coefficients of Variation (CV%) calculated for the BI, MBI, FBI, P51, EPT, %EPT, Taxa Richness (TR), Chironomid Taxa Richness (CTR) and Non-insect Taxa Richness (NITR) in Cowpie Creek (CC), Nippersink Creek (NC) and Lawrence Creek (LC). Values are calculated for all sites (margins and riffles) and riffle sites for assessments including all macroinvertebrates and those omitting Chironomidae. P51 requires that all sites be assessed and was therefore not used in riffle site assessments.

		CC		NC		LC	
		All Macroinvertebrates	Omitting chironomids	All Macroinvertebrates	Omitting chironomids	All Macroinvertebrates	Omitting chironomids
All Sites	BI	6.3	7.9	5.4	6	7.8	4.9
	MBI	6.2	9.4	6.3	5.9	9.0	12
	FBI	8.8	9.3	7.5	8.6	8.5	1.6
	P51	14.9	16.5	12.1	9.1	6.9	6.5
	EPT	15.9	NA	17.1	NA	25.8	NA
	%EPT	12.2	14.1	20.9	16.2	23.6	21.7
	TR	10.0	11.5	12.2	5.8	6.1	7.1
	CTR	25.6	NA	26.0	NA	8.6	NA
	NITR	22.9	NA	21.7	NA	14.6	NA
Riffle Sites	BI	10.0	13.5	5.3	5.9	6.4	7.6
	MBI	9.2	11.2	4.4	7.1	7.8	12.5
	FBI	11.0	13.5	7.9	8.1	8.3	1.8
	EPT	22.1	NA	22.6	NA	25.6	NA
	%EPT	13.7	NA	24.4	NA	26.3	NA
	TR	14.8	22.4	10.9	11.6	6.7	6.7
	CTR	18.9	NA	26.8	NA	11.9	NA
	NITR	45.1	NA	34.3	NA	19.0	NA

Figure 9. Mean monthly Ephemeroptera-Plecoptera-Trichoptera (EPT) (A,B) and %EPT (C,D) (+1 SE) calculated using all sites (A,C) and riffles sites (B,D) in the assessments of Cowpie Creek (o), Nippersink Creek (□) and Lawrence Creek (▲).



value was high. Regardless, P51 ratings did not change during those two occasions. The %EPT metric in NC (Figure 9C) was negatively correlated with the FBI ($P \leq 0.05$) and positively correlated with P51 ($P \leq 0.05$) (Table 9). Although FBI values or ratings did not reflect the high %EPT scores in late spring and low %EPT scores in early summer as did P51 values, the FBI had some of the lowest values and best ratings of the year in early fall (Figure 7A), a time when the percentage of EPT taxa was highest. The FBI also displayed lowered index values (improving water quality) beginning in late spring, a period when %EPT increased. %EPT abundance, which was negatively correlated with the FBI ($P \leq 0.05$), also showed a similar pattern to the FBI. Percent EPT abundance declined while FBI index values increased, both indicating poorest water quality in late winter/early spring (Figure 10). P51 exhibited patterns similar to %EPT taxa richness and FBI in late spring and early fall, however, the BI and MBI did not. The EPT taxa richness metrics were not significantly correlated with any other taxa richness measures in NC.

In LC both EPT taxa richness metrics (EPT and %EPT) were negatively correlated with the FBI ($p \leq 0.05$) and positively correlated with P51 ($p \leq 0.05$) (Table 9), indicating consistent trends among the indices. Percent EPT abundance was also positively correlated with P51 ($p \leq 0.05$) (Table 11). All of the EPT metrics showed a gradual decline from August - July, indicating a decline in water quality (Figures 9A and 10). Of the biotic and multimetric indices, P51 values most closely reflected the consistent decline in EPT taxa throughout the year (Figure 8A). In contrast to the EPT metrics, the biotic indices showed improved water quality in early summer, approaching values and ratings similar to those in late summer and early fall. As with the biotic and

Figure 10. Mean monthly percent abundance of *Gammarus*, non-insects, Chironomidae and EPT in all sites and riffle sites in Cowpie Creek (CC), Nippersink Creek (NC) and Lawrence Creek (LC). The * to the right of the group denotes significance differences between all sites and riffle sites for a given month. Differences were analyzed using a one-way ANOVA and Tukey's HSD p<0.05.

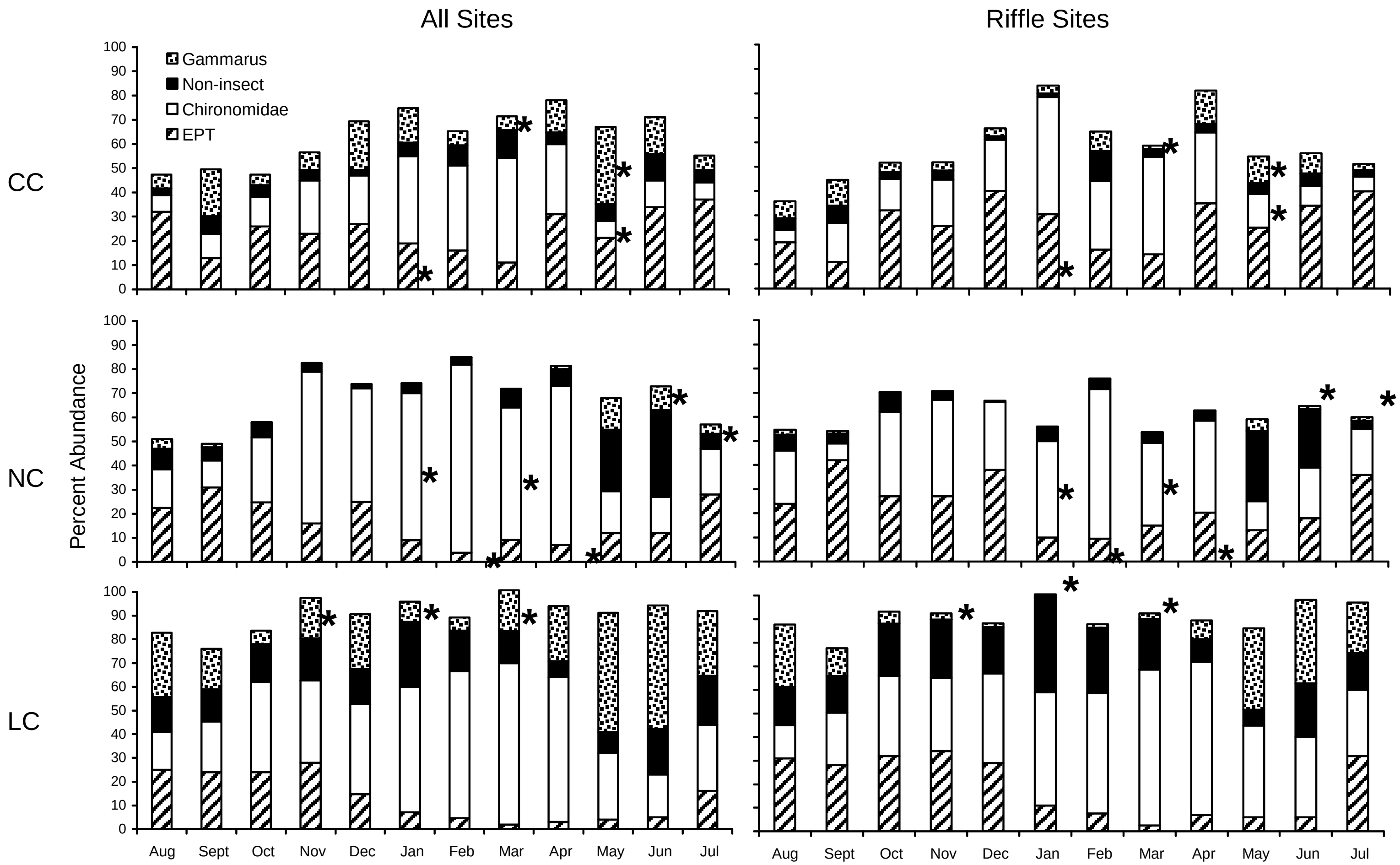


Table 11. Pearson Product-Moment correlation coefficients of monthly values including all sites and riffle sites for the BI, MBI, FBI, P51, percent EPT Abundance (% EPT-A), percent Chironomidae Abundance (% Chir-A), percent non-insect Abundance (% NI Abundance) and percent *Gammarus* Abundance (% Gam-A) in Cowpie Creek (CC), Nippersink Creek (NC) and Lawrence Creek (LC). The * denotes p≤0.05.

Stream		**All Sites**				**Riffle Sites**		
		BI	**MBI**	**FBI**	**P51**	**BI**	**MBI**	**FBI**
CC	**%EPT-A**	-0.040	0.313	-0.201	-0.290	-0.214	0.423	0.186
	%Chir-A	0.479	0.053	0.637*	0.060	0.295	0.086	0.442
	%NI-A	0.370	0.387	0.214	0.492	0.211	0.101	0.101
	%Gam-A	-0.316	-0.236	-0.169	-0.169	0.072	0.190	-0.062
NC	**%EPT-A**	-0.525	-0.113	-0.632*	0.246	-0.321	-0.042	-0.286
	%Chir-A	0.589*	0.237	0.749*	-0.283	0.561*	0.096	0.608*
	%NI-A	-0.325	-0.333	-0.276	0.150	-0.480	-0.079	-0.479
	%Gam-A	-0.307	-0.379	-0.314	0.116	-0.541*	-0.015	-0.660*
LC	**%EPT-A**	-0.218	0.104	-0.378	0.605*	-0.249	0.203	-0.473
	%Chir-A	0.415	-0.090	0.620*	-0.643*	0.333	-0.135	0.599*
	%NI-A	0.400	0.579*	0.327	0.025	0.550*	0.614*	0.468
	%Gam-A	-0.405	-0.177	-0.463	0.186	-0.609*	-0.460	-0.619*

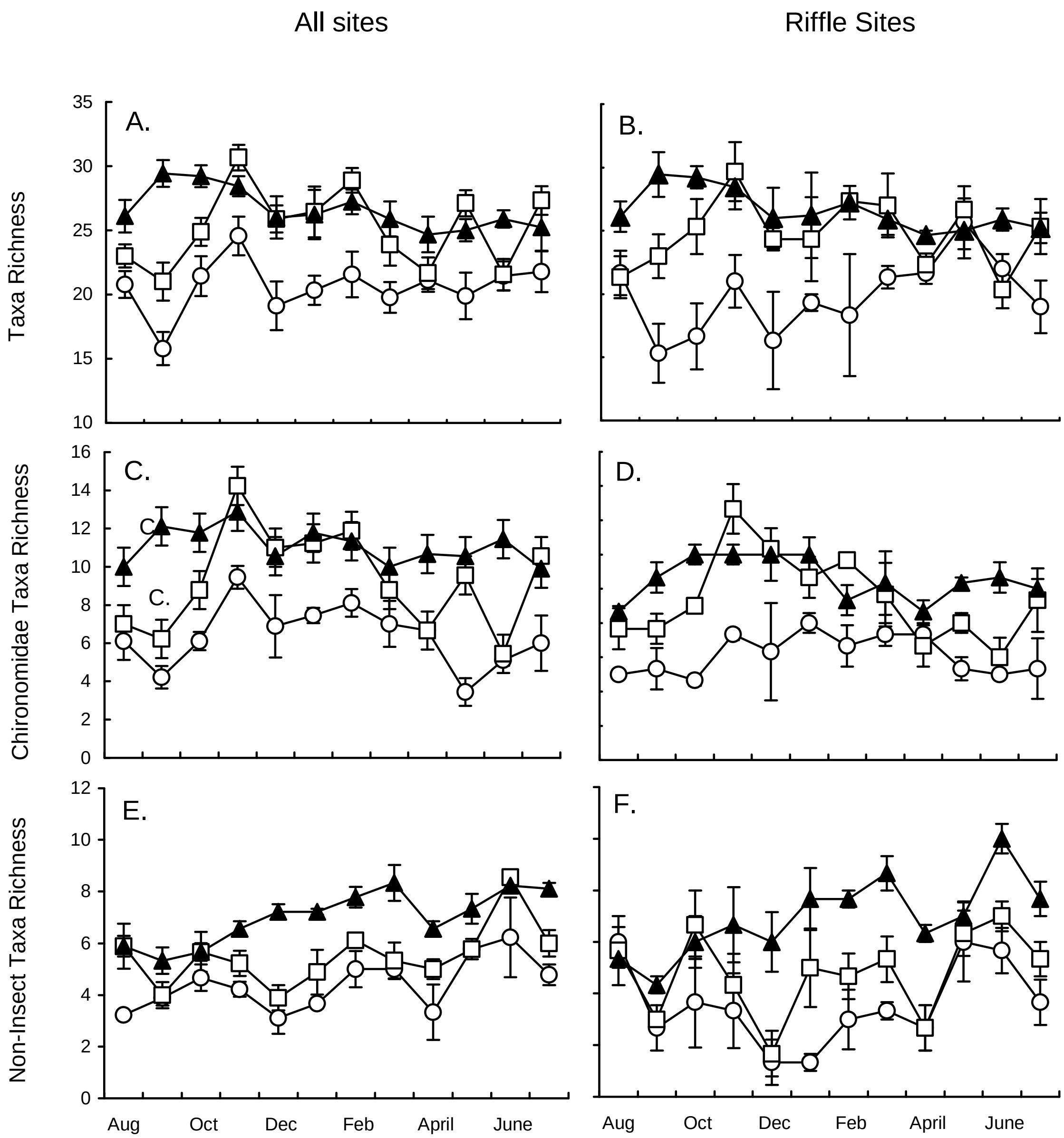
multimetric indices, the EPT index was positively correlated with taxa richness ($P \leq 0.05$). The number of EPT and total taxa declined starting in late summer and throughout the following year. Both EPT metrics also were negatively correlated with non-insect taxa richness ($p \leq 0.05$). As the number of EPT taxa gradually declined starting in late summer (Figure 9A), the number of non-insect taxa generally increased (Figure 11E).

Overall, patterns in P51 and the FBI among streams were more similar to the EPT than were the BI and MBI. The number of EPT taxa was significantly higher in CC and NC compared to LC throughout most of the year (1-way ANOVA, $F_{(2,11)}=38.5$, $p \leq 0.05$) (Figure 9A). Although P51 rated LC with “poor” water quality several months throughout the year, most P51 values were lower (decline in water quality) in LC than the other streams. The broad rating scale for P51 did not allow for distinction in water quality among streams.

Temporal trends in taxa richness and percent abundance

Taxa richness was positively correlated with chironomid taxa richness in all three streams ($p \leq 0.05$) (Table 9). Chironomid and total taxa richness were most strongly correlated in NC, suggesting midges greatly influenced the macroinvertebrate community in NC. In CC and NC patterns were similar among both metrics from late summer to early spring, with both metrics showing a general pattern of lower richness in the early fall and increasing until late fall, and a slight increase in late winter (Figure 11A,C). However, patterns in percent abundance of Chironomidae differed between CC and NC, increasing steadily in CC until early spring and peaking each season except summer in NC (Figure 10). In LC, both taxa richness metrics (chironomid taxa richess and total taxa

Figure 11. Mean monthly taxa richness (A,B), Chironomidae taxa richness (C,D) and non-insect taxa richness (E,F) (+1 SE) in all sites (margins and riffles) and riffle sites of Cowpie Creek (o), Nippersink Creek(□) and Lawrence Creek (▲).



richness) gradually declined from fall through early summer of the following year, during which time percent abundance of Chironomidae increased until declining in the spring. Temporal patterns in chironomid taxa richness and total taxa richness were similar, suggesting that midges contributed highly to temporal changes in macroinvertebrate communities of the streams.

Of the multimetric and biotic indices, the FBI showed the most similarity to percent abundance of Chironomidae, and the two indices were positively correlated in all three streams (Table 11) ($p \leq 0.05$). Temporal patterns of the BI most closely reflected the increase in abundance of chironomids throughout the year in NC, while temporal patterns of P51 most closely reflected the increase in abundance of chironomids throughout the year in LC (Figure 10). Percent abundance of Chironomidae was positively correlated with the BI in NC and negatively correlated with P51 in LC ($p \leq 0.05$) (Table 11).

Percent abundance of non-insects was highest in LC (Figure 10). However, only the MBI was significantly correlated with percent abundance of non-insects in LC. Percent abundance of *Gammarus* was highest in LC, but not significantly correlated with any of the indices.

Riffle Sites

Temporal trends in biotic/multimetric assessments

*Cowpie and Nippersink Creek***.** For the three biotic indices, there were no differences in index ratings between riffle sites and all sites in CC, however, in NC all indices showed better ratings in riffle sites compared to all sites on several occasions and at similar times of the year (Figures 5A,C – 7A,C) (Table 12A). For instance, all three biotic indices indicated differences in ratings between sites in mid-spring. In addition, the BI and FBI indicated differences in ratings between sites in mid-winter. Of the indices, the BI indicated the most differences between sites throughout the year, with better water quality in early summer in riffle sites compared to all sites, in addition to mid-spring and mid-winter site differences (Figure 5A,C).

Lawrence Creek. In Lawrence Creek, the FBI differed in quality ratings between all sites and riffle sites in early spring. However, unlike the trend in NC, ratings in riffle sites depicted a decline in water quality when compared to all sites.

Biotic/multimetric assessment comparisons

Limiting assessments to riffle sites altered index ratings on only a few occasions throughout the year. Because of high variability, mean index values in some months overlapped categories and, thus, were assigned two ratings. Where differences in ratings existed between all sites and riffle sites, the use of riffle sites generally resulted in better stream water quality ratings.

Temporal trends in EPT taxa richness and percent abundance

The EPT taxa richness metric differed significantly between all sites and riffle sites in early (1-way ANOVA, $F_{1,5}=48.9$, $p\leq0.05$) to mid-spring (1-way ANOVA, $F_{1,5=}36.7$, $p\leq0.05$) in NC and in early (1-way ANOVA, $F_{1,5}=15.9$, $p\leq0.05$) to mid-summer in LC (1-way ANOVA, $F_{1,5}=15.3$, $p\leq0.05$) (Figure 9). All biotic indices in NC in mid-spring indicated better water quality because of more EPT taxa (Figures 5A,C, 6A,C, and

Table 12. Months listed indicate the time of year when index ratings (A) and taxa richness metrics (B) differed between all sites (margin and riffle) and riffle sites for all macroinvertebrate (biotic indices and taxa richness metrics) and non-chironomid assessments (biotic indices only). Richness metrics were tested for significance with 1-way ANOVA, Tukey ($p \leq 0.05$).

A.

STREAM	INDEX	All Macroinvertebrates	Omitting Chironomidae
CC	BI	-	-
	MBI	-	-
	FBI	-	Sept
NC	BI	Jan, April, June	-
	MBI	April	-
	FBI	Jan, April	June
LC	BI	-	-
	MBI	-	March, July
	FBI	March	-

B.

STREAM	METRIC	All Macroinvertebrates
CC	EPT	-
	NITR	-
	CTR	Nov
NC	EPT	March - April
	NITR	April
	CTR	Dec
LC	EPT	June - July
	NITR	-
	CTR	-

7A,C), (Table 12B). None of the biotic or multimetric indices reflected differences in EPT between all sites and riffle sites in LC.

Although EPT taxa richness in CC did not differ between all sites and riffle sites, percent abundance of EPT did differ between sites in mid-winter (Figure 10) (1-way ANOVA, $F_{(1,5)}=10.9$, $p\leq0.05$). In NC, percent abundance of EPT differed between sites in late winter (1-way ANOVA, $F_{(1,5)}=10.4$, $p\leq0.05$) and mid-spring (1-way ANOVA, $F_{(1,5)}=23.5$, $p<0.05$). The BI and FBI showed improvements in ratings in late winter, but the MBI did not. There were no significant differences in percent abundance of EPT between sites in LC.

Temporal trends in taxa richness and percent abundance

Chironomid taxa richness did not differ between all sites and riffle sites except in CC in late fall (1-way ANOVA, $F_{1,5}=9.8$, $p\leq0.05$) and NC in early winter (1-way ANOVA, $F_{1,5}=16.0$, $p\leq0.05$) (Table 12B). On both occasions, the number of chironomid taxa was significantly lower in riffle sites compared to all sites (Figure 11C). The difference in chironomid taxa between riffle sites and all sites was not reflected in the biotic or multimetric index ratings during either time. In addition, rating differences between all sites and riffle sites ocurred when there were no significant differences between chironomid taxa richness at the two sites. Percent chironomid abundance in CC differed significantly between all sites and riffle sites in late spring (1-way ANOVA, $F_{1,5}=12.0$, $p\leq0.05$) and in NC in mid-winter (1-way ANOVA, $F_{1,5}=10.3$, $p\leq0.05$) and early spring (1-way ANOVA, $F_{1,5}=16.4$, $p\leq0.05$)(Figure 10). All indices had better water quality ratings in all sites compared to riffle sites in CC during late spring. In NC the FBI showed better ratings in riffle sites than all sites in mid-winter and early spring. The BI and MBI indicated better water quality in riffle sites than all sites in early spring in NC, but showed no difference in ratings in mid-winter.

Non-insect taxa richness differed significantly between all sites and riffle sites only in NC during mid-spring (1-way ANOVA, $F_{1,5}$=8.3, $p \leq 0.05$) (Figure 11E, Table 12B). Lower non-insect taxa richness in riffle sites compared to all sites during mid-spring was indicated by better biotic index ratings in riffles sites compared to all sites. Percent abundance of non-insects did not differ between sites.

Percent abundance of *Gammarus* differed between all sites and riffle sites during different times of the year in each stream (Figure 10). Percent *Gammarus* abundance in CC was significantly higher in all sites than in riffle sites in early (1-way ANOVA, $F_{1,5}$=10.8, $p \leq 0.05$) and late spring (1-way ANOVA, $F_{1,5}$=21.1, $p \leq 0.05$), in NC in early (1-way ANOVA, $F_{1,5}$=22.9, $p \leq 0.05$) to midsummer (1-way ANOVA, $F_{1,5}$=16.2 $p \leq 0.05$,) in NC and in LC in late fall (1-way ANOVA, $F_{1,5}$=30.8, $p \leq 0.05$), mid-winter (1-way ANOVA, $F_{1,5}$=29.4, $p \leq 0.05$) and early spring (1-way ANOVA, $F_{1,5}$=34.2, $p \leq 0.05$).

Omitting Chironomidae

All Sites

Differences in biotic index ratings between all macroinvertebrate and non-chironomid assessments occurred in all three streams (Table 13). For each index, most rating differences between assessments occurred in LC. The FBI differed the most in ratings between all macroinvertebrates and non-chironomid assessments, whereas P51 displayed the fewest differences. The MBI displayed slightly more differences than P51 in ratings between all macroinvertebrate assessments and those omitting midges, whereas the BI displayed slightly more differences than MBI in ratings between all macroinvertebrate and non-chironomid assessments. Omission of midges always resulted in improved ratings compared to those including all macroinvertebrates. In general, the omission of midges resulted in assessments showing greater water quality improvement in LC compared to the other two streams and greater similarity in water quality ratings between CC and LC than between NC and LC.

Temporal trends in biotic/multimetric assessments

Cowpie Creek. In Cowpie Creek, omitting chironomids from the assessments did not alter ratings from those made using all macroinvertebrates in P51 and MBI, however, chironomid omission in the BI and FBI did alter ratings for part of the year (Table 13). BI and FBI ratings differed between assessments including and omitting chironomids from late winter through mid-spring, with improved water quality ratings when midges were omitted (Figures 5A,B and 7A,B). FBI ratings also differed between assessments in late fall and mid-winter, improving in ratings with chironomid omission. The BI, MBI and FBI remained significantly correlated in CC ($P \leq 0.05$) regardless of changes in ratings resulting from non-chironomid assessments (Table 9). The %EPT richness measure for non-chironomid assessments was significantly correlated with the MBI, FBI and P51 ($p \leq 0.05$), whereas it was not correlated with these indices when all macroinvertebrates were used (Table 9). This suggested that midges heavily influenced the macroinvertebrate community in Cowpie Creek. Despite the importance of chironomids in CC, similar ratings between MBI and P51, regardless of whether midges were

Table 13. Months listed indicate the time of year when index ratings differed between all macroinvertebrate and chironomid omission assessments at all sites and riffle sites. NA denotes that P51 was not assessed in riffle sites. Because P51 assessments use all sites, riffle sites were not assessed.

	INDEX	All Sites	Riffle Sites
CC	BI	Feb - Apr	Mar - Apr
	MBI	-	-
	FBI	Nov, Jan - Mar	Sept, Jan - Apr
	P51	-	NA
NC	BI	Jan - Apr	Feb
	MBI	Jan - Feb	-
	FBI	Sept -Oct, Dec, Jan - Apr	Nov - Dec, Feb - April, Jul
	P51	-	NA
LC	BI	Jan - May, Jun	Apr - Jun
	MBI	Apr	Mar
	FBI	Jan - Dec	Sept-Feb, Mar-Jul
	P51	Feb	NA

included, indicates that it is not time efficient to include chironomids in MBI and P51 assessments of Cowpie Creek due to the lack of sensitivity of the two indices to community level changes.

Nippersink Creek. In NC, P51 was the only index in which ratings did not differ between all macroinvertebrate and non-chironomid assessments (Table 13). MBI ratings improved in mid- to late winter when chironomids were omitted (Figure 6A,B). BI ratings differed between assessments from mid-winter through mid-spring, also showing improvement when chironomids were omitted (Figure 5A,B). FBI ratings improved in early to mid-fall and early winter through mid-spring when chironomids were omitted (Figure 7A,B).

Although P51 and MBI were significantly correlated to each other and to the other biotic indices when all macroinvertebrates were included ($p \leq 0.05$), P51 and the MBI in NC were not significantly correlated to the other indices when midges were omitted from the assessments (Table 9). Despite the lack of correlation to the other biotic indices, P51 was the only index significantly correlated with %EPT when midges were omitted ($p \leq 0.05$), suggesting the index reflected the greater number of clean water taxa in relation to total taxa with the omission of chironomids. Regardless of the positive correlation with %EPT, P51 ratings did not differ between all macroinvertebrate and non-chironomid assessments, again suggesting that P51 ratings are not sensitive enough to detect community changes.

Lawrence Creek. In LC P51 ratings only differed between all macroinvertebrate and non-chironomid assessments in late winter (Table 13). The change in rating in February from “Poor” (all macroinvertebrates) to “Acceptable, tending towards poor” (midges omitted) changed the status of LC from having the poorest water quality of the three streams to having similar water quality (Figure 8A,B). MBI ratings also differed between assessments, but improved in April rather than February (Table 12A). BI ratings differed between assessments from mid-winter through early summer, improving in rating more often than MBI and P51 (Table 12A). FBI ratings differed between assessments in every month (Table 12A). The FBI was not significantly correlated with the BI, MBI, P51 and %EPT when midges were removed from the assessments. P51 also was not significantly correlated with %EPT (Table 9).

Comparisons of ratings among streams

Differences in ratings between all macroinvertebrate and non-chironomid assessments did not always result in changes in water-quality characterization among the streams. Except for the month of April, omitting midges from MBI assessments did not alter water quality relationships between the streams. In April, the omission of chironomids in LC assessments improved the MBI rating from “very good” to “excellent” and thus reflected similar ratings to CC and not to NC.

Omitting midges from BI assessments altered water quality relationships among streams more often throughout the year than the MBI. With midges omitted, NC was rated with the poorest water quality of the streams mid-winter through mid spring. Although ratings in NC improved with chironomid omission, ratings in LC improved by two rating categories rather than one, as in NC, resulting in LC ratings more similar to CC than to NC. The improved BI ratings from mid-spring through early summer in LC also resulted in similar ratings between CC and LC during that time (Figure 5 A,B).

Non-chironomid FBI assessments had a similar effect to those for the BI. The better FBI ratings in NC and LC in non-chironomids assessments compared to all macroinvertebrate assessments resulted in similar water quality ratings between all streams in early fall and early to mid-spring. Also, better ratings in non-chironomid versus all macroinvertebrate assessments in LC in late fall, late winter, and mid-spring through early summer resulted in similar ratings between CC and LC, rather than between NC and LC, with the exception of late fall, in which LC ratings were intermediate between CC and NC (Figure 7A, B).

Riffle Sites

Overall, when midges were omitted from assessments, biotic index ratings for riffle sites were similar to those from all sites, with a few exceptions (Table 12A). One exception was the FBI, which showed differences between riffle sites and all sites in CC (early fall). Low FBI values in CC riffle sites resulted in better water quality ratings in comparison to those in NC and LC. The FBI also showed differences between riffle sites and all sites in NC in early summer. Improved ratings in NC in riffle sites resulted in similar water quality among all three streams (Figure 7B,D). The MBI was the only index to show differences between all sites and riffle sites in LC, which occurred in early spring and mid-summer. In midsummer, MBI ratings using riffle sites improved from those of all sites (Figure 6B,D). Early spring ratings declined such that LC was more similar in rating to NC than to CC.

Annual Index Values and Water Quality Ratings

All Macroinvertebrates

All Sites

All indices rated CC with the best water quality (excellent to very good/good) of the three streams, with the exception of P51, which rated all streams similarly (acceptable tending towards poor) (Table 14). Both the BI and MBI assigned NC with the poorest ratings of the three streams. None of the indices suggested that LC, the stream with the lowest percentage of EPT taxa and significantly more non-insect taxa (Table 5), had the poorest water quality. The FBI and P51 indicated similar water quality ratings between NC and LC, however, P51 index values most closely reflected differences between streams. P51 showed similar patterns to chironomid and non-insect taxa richness measures among streams, which indicates significantly higher numbers of midge and non-insect taxa in LC compared to CC and NC in most months (1-way ANOVA, $F_{(2,11)}$=43.4, Tukey $p \leq 0.05$)(Figures 11C and 11E).

Riffle Sites

Nippersink Creek and LC riffle sites were rated similarly by the BI and MBI. However, the use of riffle sites in calculating the FBI resulted in three distinct ratings. The FBI was the only index to suggest that LC, the stream with the fewest percentage of EPT taxa and significantly higher number of non-insects (Table 5), had the poorest water quality. This differed from all site ratings, where the FBI indicated poorest water quality in NC. This was a result of better FBI water quality ratings in NC when riffle sites were used compared to all sites (Table 14). In fact, all indices showed better

Table 14. Mean annual index values (± SE) calculated for the BI, MBI, FBI and P51 in Cowpie Creek (CC), Nippersink Creek (NC) and Lawrence Creek (LC). Values are calculated for all sites (margins and riffles) and separately for riffle sites, including all macroinvertebrates and omitting chironomids. Ratings are: Excellent (E), Very Good (VG), Good (G), Fair (F) and Acceptable, tending towards poor (AP). Due to large error associated with mean values, streams may receive two ratings. Because P51 assessments use all sites, riffle sites were not assessed.

		CC				**NC**				**LC**			
		All Macroinvertebrates		**Omitting chironomids**		**All Macroinvertebrates**		**Omitting chironomids**		**All Macroinvertebrates**		**Omitting chironomids**	
		Index Value	**Rating**	**Index Value**	**Rating**	**Index Value**	**Rating**	**Index Value**	**Rating**	**Index Value**	**Rating**	**Index Value**	**Rating**
All Sites	**BI**	4.42 (±0.08)	VG	4.00 (±0.09)	VG	5.63 (±0.09)	F	5.01 (±0.09)	G	5.31 (±0.12)	G	4.40 (± 0.06)	VG
	MBI	4.49 (±0.08)	E	4.34 (±0.12)	E	5.91 (±0.12)	VG/G	5.69 (±0.10)	VG	5.40 (±0.14)	VG	5.22 (±0.18)	VG
	FBI	4.27 (±0.11)	VG/G	3.69 (±0.10)	E/VG	5.04 (±0.11)	G/F	4.33 (±0.11)	VG/G	4.99 (±0.12)	G/F	4.03 (±0.02)	VG
	P51	-0.80 (±0.38)	AP	-0.68 (±0.41)	AP	-2.45 (±0.36)	AP	-2.13 (±0.27)	AP	-4.71 (±0.25)	AP	-4.09 ± (0.23)	AP
Riffle Sites	**BI**	4.32 (±0.12)	VG	3.98 (±0.16)	VG	5.28 (±0.08)	G	4.84 (±0.08)	G	5.39 (±0.10)	G	4.59 (±0.11)	VG/G
	MBI	4.56 (±0.12)	E	4.40 (±0.14)	E	5.59 (±0.07)	VG	5.39 (±0.11)	VG	5.46 (±0.12)	VG	5.41 (± 0.20)	VG
	FBI	4.20 (±0.13)	VG/G	3.65 (±0.16)	E/VG	4.72 (±0.11)	G	4.15 (±0.10)	VG	4.99 (±0.12)	G/F	4.00 (±0.02)	VG

water quality ratings in NC when using only riffle sites. P51 assessments specifically include all sites, and, therefore, were not analyzed using only riffle sites.

Omitting Chironomidae

All Sites

Annual water quality assessments omitting midges generally improved stream ratings compared to assessments for which chironomid were included (Table 14). The FBI was the only index to show improved ratings in all three streams by omitting midges. All 3 biotic indices indicated better annual water quality ratings in NC in non-chironomid assessments compared to all macroinvertebrate assessments.   The BI also indicated better water quality in LC in non-chironomid assessments compared to all macroinvertebrate assessments, but not in CC in non-chironomid assessments. The MBI did not show better ratings in either CC or LC in non-chironomid assessments. Annual P51 water quality ratings did not differ between chironomid omission and all macroinvertebrate assessments in any stream, however index values indicated improvement in water quality when midges were omitted.

Improvement in some ratings by not including midges altered which streams exhibited the best and poorest water quality (Table 14). The MBI, which rated NC with the poorest water quality when midges were included, rated NC and LC similarly without midges, both equally poor in comparison to CC. The BI, which rated CC with the best water quality when midges were included, rated CC and LC similarly without midges, both exhibiting better water quality than NC. Although FBI ratings improved in each stream with the omission of midges, improvement was greater in LC than in NC. As a result, the FBI indicated that NC had the poorest water quality, rather than exhibiting similar ratings for NC and LC, both equally poor in comparison to CC, when midges were included. P51 rated the streams similar to one another regardless of chironomid inclusion or omission, but index values remained highest in CC (best water quality) and lowest in LC (poorest water quality) regardless of midge inclusion or omission. Although none of the index ratings indicated that LC, the stream with the lowest percentage of EPT taxa, had the poorest water quality, the MBI and P51 ratings both indicated similar water quality between NC (very good) and LC (acceptable, tending towards poor) regardless of midge inclusion or omission.

Riffle Sites

Index ratings for riffle sites were similar to those from all sites when chironomids were omitted, with the exception of FBI ratings in CC and NC and BI ratings in LC (Table 14). As a result, NC and LC were rated similarly by the FBI. The FBI no longer rated LC, the stream with the fewest percentage of EPT taxa, with the poorest water quality of the three streams as it did when riffle sites were used but midges were omitted.

Ability of Indices to Reflect Variation in Ordination Scores

Annual Patterns in Macroinvertebrate Communities

All Macroinvertebrates

Mean annual DCA axis scores were compared to mean annual index ratings of the biotic and multimetric indices (Table 15). The first ordination axis accounted for 42.5% of the variation in the macroinvertebrate community, whereas the second axis explained 31.6%

of the variation. Mean annual ordination scores on axis 1 were significantly lower in CC (1-way ANOVA, $F_{(2,11)}$=8.4, Tukey $p \leq 0.05$) than the other two streams and significantly higher in LC (1-way ANOVA, $F_{(2,11)}$=8.4, Tukey $p \leq 0.05$) compared to the other two streams. None of the indices annual ratings reflected differences in stream community structure depicted by axis 1. Although the biotic indices distinguished CC as the stream with the best ratings, FBI ratings did not distinguish between water quality in NC and LC and the BI and MBI rated NC with the poorest water quality of the three streams. P51 ratings did not distinguish between any of the streams. When only riffle sites were used in the assessments, FBI ratings accurately distinguished between axis annual scores. This was due to the change in annual rating in NC from “Good-Fair” to “Good” when only riffle sites were used.

Mean annual ordination scores on axis 2 were significantly higher in NC (1-way ANOVA, $F_{(2,11)}$=42.6, Tukey $p \leq 0.05$) compared to the other two streams. BI ratings best reflected differences in stream community structure depicted by axis 2, indicating best ratings in CC and poorest ratings in NC (Table 15). Although the MBI and FBI distinguished CC as the stream with the best water quality, ratings did not distinguish between water quality in NC and LC. When only riffle sites were used in the assessments, none of the indices accurately reflected community structure differences along axis 2.

Table 15. Annual DCA scores (± SE) and index ratings in Cowpie Creek (CC), Nippersink Creek (NC) and Lawrence Creek (LC) including all macroinvertebrates and omitting Chironomidae in the assessments.

				All Sites				Riffle Sites		
		Axis 1	**Axis 2**	**BI rating**	**MBI rating**	**FBI rating**	**P51 rating**	**BI rating**	**MBI rating**	**FBI rating**
All Macroinvertebrates	CC	107.07 (±7.29)	106.98 (±7.42)	Very good	Excellent	Very good-Good	Acceptable (tending towards poor)	Very good	Excellent	Very good-Good
	NC	153.87 (±9.40)	208.40 (±12.25)	Fair	Very good-Good	Good-Fair	Acceptable (tending towards poor)	Good	Very good	Good
	LC	253.57 (±7.75)	131.69 (±13.30)	Good	Very good	Good-Fair	Acceptable (tending towards poor)	Good	Very good	Good-Fair
Omitting chironomids	**CC**	153.30 (±7.40)	211.32 (±4.08)	Very good	Excellent	Excellent-Very good	Acceptable (tending towards poor)	Very good	Excellent	Excellent-Very good
	NC	131.49 (±9.92)	99.34 (±4.48)	Good	Very good	Very good-Good	Acceptable (tending towards poor)	Good	Very good	Very good
	LC	227.50 (±9.56)	154.80 (±5.12)	Very good	Very good	Very good	Acceptable (tending towards poor)	Very good-good	Very good	Very good

Omitting Chironomidae

When chironomids were omitted from the assessments, mean annual axis 1 scores were significantly higher in LC (1-way ANOVA, $F_{(2,11)}$=114.2,Tukey $p<0.05$) compared to the other two streams, with CC scores intermediate between NC and LC (Table14). None of the ratings accurately reflected differences in community structure depicted by the first axis.

Mean annual axis 2 scores were significantly higher in CC (1-way ANOVA, $F_{(2,11)}$=226.2, Tukey $p\leq0.05$) compared to the other two streams, with LC scores intermediate between NC and CC (Table 14). Although CC and NC alternated positions in ordinational space when midges were omitted, the MBI and FBI indicated CC to have the best water quality ratings. However, these two indices did not properly distinguish community structure changes between NC and LC. When only riffle sites were used in the assessments, none of the ratings accurately reflected community structure differences along axis 2.

Monthly Patterns in Macroinvertebrate Communities

All Macroinvertebrates

Although P51 was significantly correlated with DCA axis 1 ($p\leq0.01$, Table 6), it was difficult to determine if P51 ratings followed a water quality gradient on axis 1 due left side of the axis and several “Poor” ratings in LC on the right side of the axis (Figure 12). The FBI also appeared to correspond with axis 1, but with much more variance in ratings among similar axis values compared to axis 2 (Figure 13).

A water quality gradient was apparent along DCA axis 2 when monthly atings of the biotic indices were plotted with ordination scores. The best water quality ratings appeared lower on the axis while the poorest rating appeared higher on the axis. The BI and MBI better distinguished between the ratings along the axis than the FBI. For the MBI, most "Good" ratings, all of which were in NC except for one in LC, were higher on axis 2 than the "very good" ratings (Figure 14). However, "Very Good" ratings in early to mid-summer in LC overlapped "Excellent" ratings in CC. BI ratings differed between similar axis 2 values in CC and LC as well (Figure 15). "Good" ratings in late spring through mid-summer in LC shared similar axis 2 values as "Very Good" ratings in CC.

In contrast, the FBI showed less distinction between ratings along axis 2. As with the BI and MBI, there were months when FBI ratings differed between CC and LC and yet they shared similar axis 2 values. "Very Good" ratings in early fall and "Excellent" ratings in mid-fall in CC shared similar or higher axis 2 scores than ratings in LC in late summer through early fall, late fall, and late spring through mid-summer. FBI ratings in NC and LC differed among similar axis 2 values as well (Figure 13). "Fair" ratings mid-winter through mid-spring in LC shared similar axis 2 values to "Good" ratings late summer through mid-fall in NC. "Fair ratings in late spring (and early summer in LC) exhibited lower ordination values than most others throughout the year.

Omitting Chironomidae

As with assessments including all macroinvertebrates, it was difficult to determine patterns in P51 on DCA axis 1 when midges were omitted due to minimal variance in ratings. Although a water quality gradient appeared on axis 1, it was less apparent when midges were omitted (Figure 12). The axis 1 value for the "Acceptable…Excellent" rating in CC in mid-spring was higher than most axis 1 values with "Acceptable…poor" ratings in CC and NC. FBI ratings did not reflect axis 1 values of the three streams when midges were omitted from the assessments.

Similar to assessments including all macroinvertebrates, a water quality gradient was apparent along axis 2 when midges were omitted from the biotic indices. The biotic indices exhibited less rating overlap among similar axis values when midges were omitted from the assessment. The BI showed slightly better distinction between the ratings along the axis than the other biotic indices, exhibiting similar ratings among similar axis 2 scores among the streams (Figure 15). Patterns in MBI and FBI ratings were inconsistent with axis 2 values in several months. MBI ratings in NC in late spring (Very Good) were better than those in early spring (Good), however, axis 2 values in late spring were lower than those in early spring (Figure 14). FBI ratings in NC in mid-fall (Very Good) were better than ratings in early spring (Good), however axis 2 values in mid-fall were slightly lower than those in early spring (Figure 13).

Annual Variability Among Indices

All Macroinvertebrates

All Sites

Annual variability in this paper refers to the amount of variability in index values encountered among months throughout the year. Annual variability among the indices differed among streams, however, mean annual values indicated that the taxa richness

Figure 12. DCA analysis exhibiting monthly sample loadings coded by P51 ratings. Assessments included all macroinvertebrates (A) and omitted Chironomidae (B). Mean P51 water quality ratings are indicated by the following symbols: (●) Acceptable, tending towards excellent, (□) Acceptable, tending towards poor, and (▲) Poor. Mean monthly ratings having a standard error occuring in a rating category different from that of the mean are depicted as Mean P51 rating (+P51 rating error) and are indicated by the following symbols: (o) Acceptable, tending towards excellent (-Acceptable, tending towards poor), (■)Acceptable, tending towards poor (+Acceptable, tending towards excellent, (■) Acceptable, tending towards poor (-poor) and (Δ) Poor (+Acceptable, tending towards poor). Ordination values are connected by a dotted line for CC, solid line for NC and a dashed line for LC. Refer to Figure 2 for DCA values labeled by month.

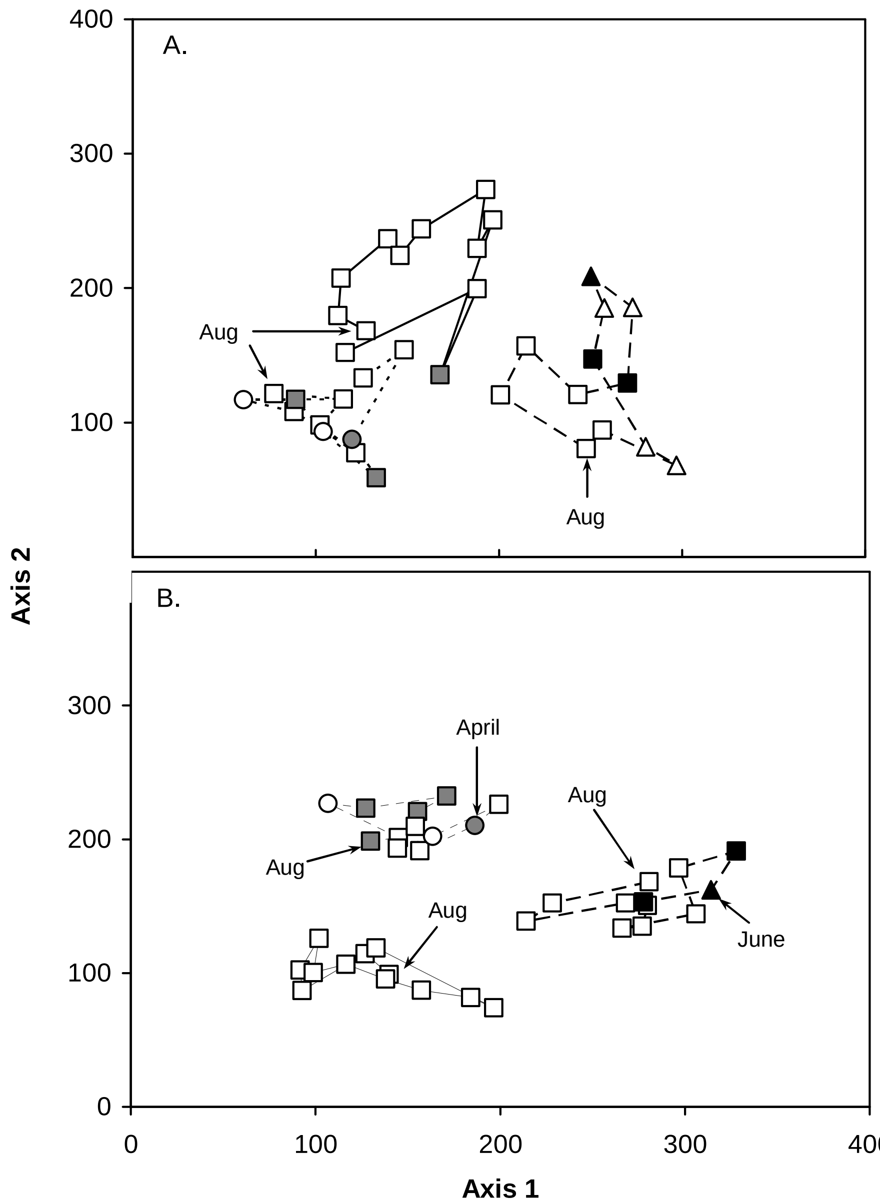


Figure 13. DCA analysis exhibiting monthly sample loadings coded by FBI ratings and showing mean FBI water quality ratings for each month. Mean monthly ratings having a standard error occuring in a rating category different from that of the mean are depicted as mean FBI rating (+FBI rating error). Ordination values are connected by a dotted line for CC, solid line for NC and a dashed line for LC. Assessments included all macroinvertebrates (A), all macroinvertebrates in riffle sites (B) and Chironomidae omitted (C). Refer to Figures 2 and 4 for DCA values labeled by month.

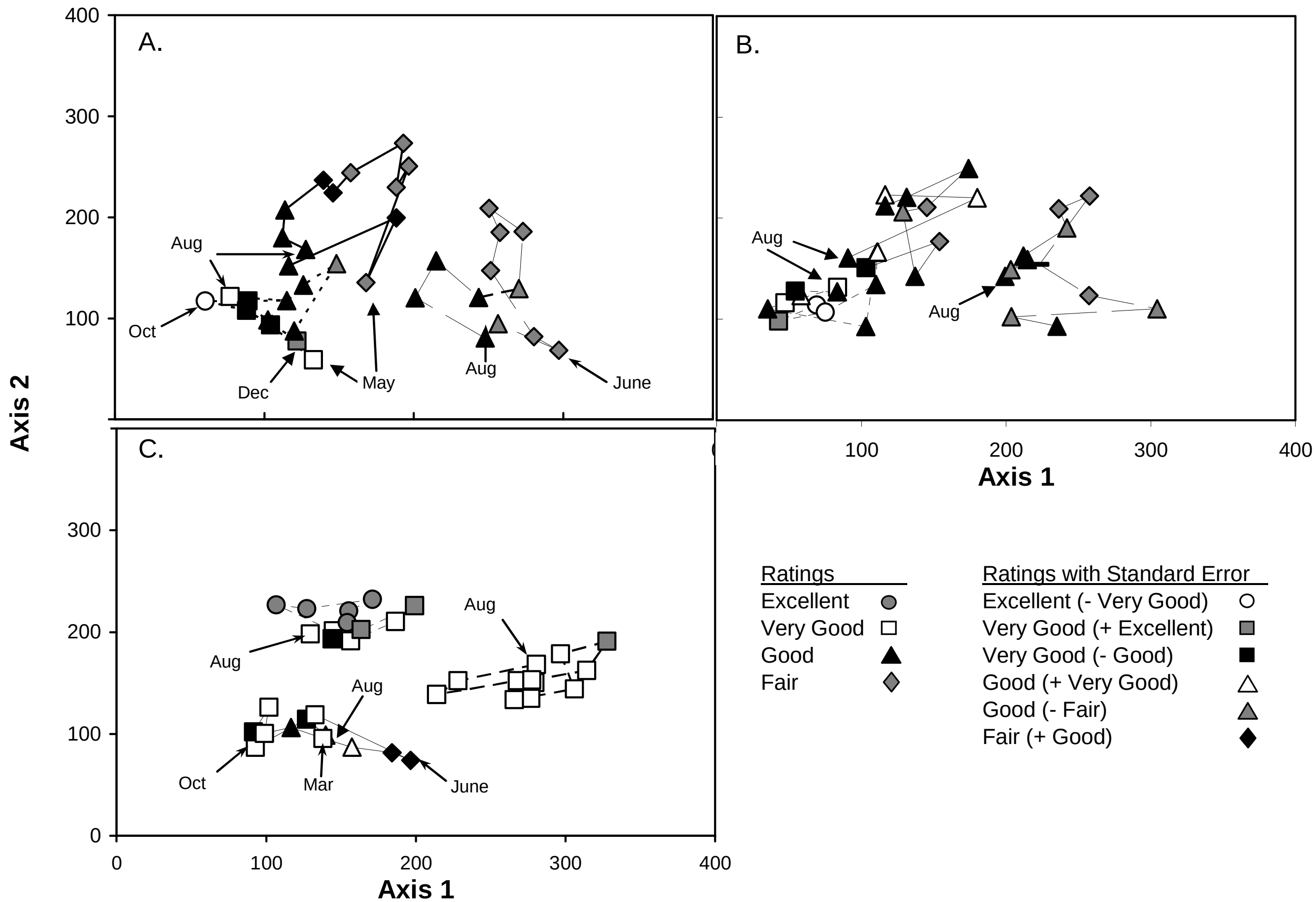


Figure 14. DCA analysis exhibiting monthly sample loadings coded by MBI ratings and showing mean MBI water quality ratings for each month. Mean monthly ratings having a standard error occuring in a rating category different from that of the mean are depicted as mean MBI rating (+MBI rating error). Ordination values are connected by a dotted line for CC, solid line for NC and a dashed line for LC. Assessments included all macroinvertebrates (A), all macroinvertebrates in riffle sites (B) and Chironomidae omitted (C). Refer to Figures 2 and 4 for DCA values labeled by month.

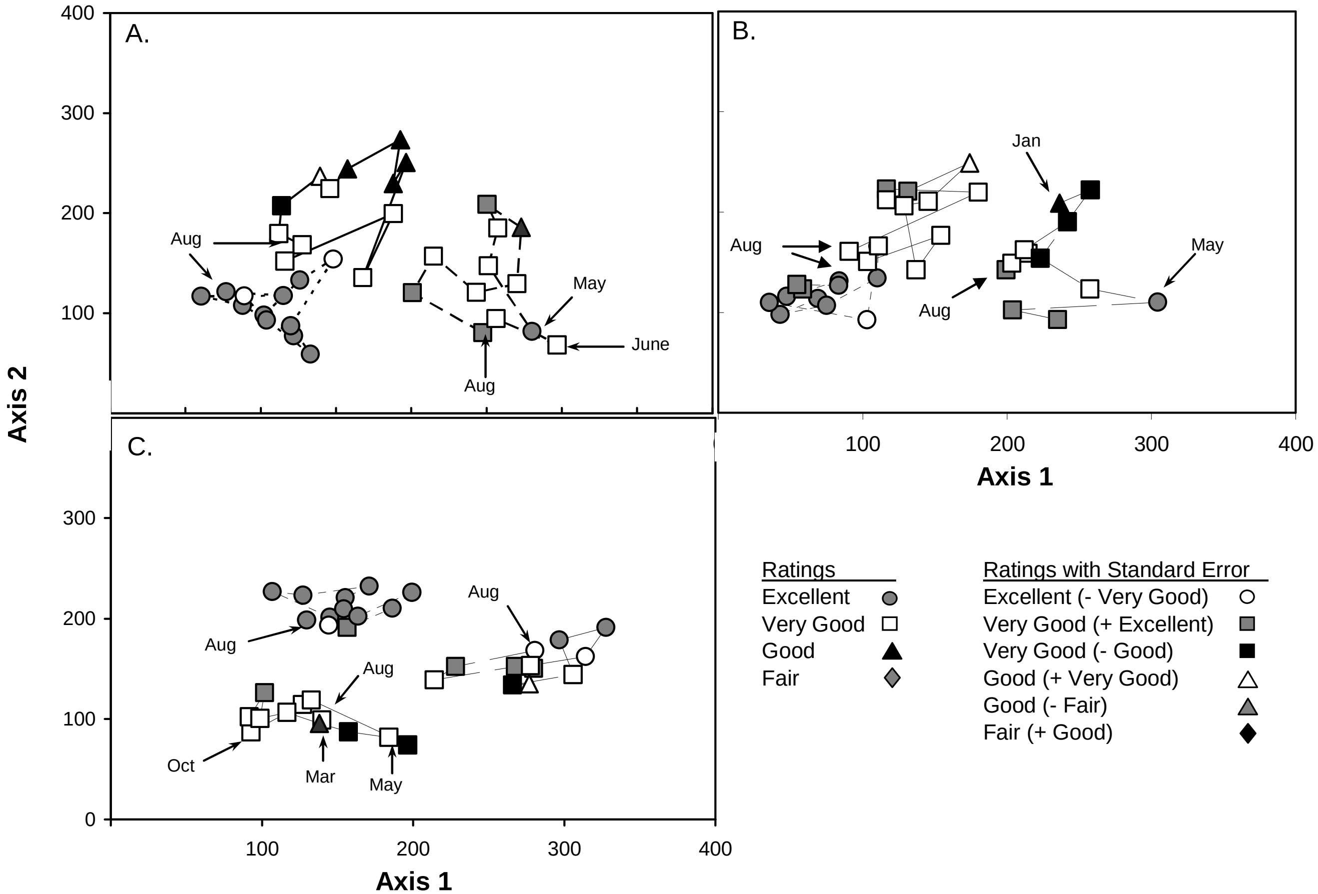
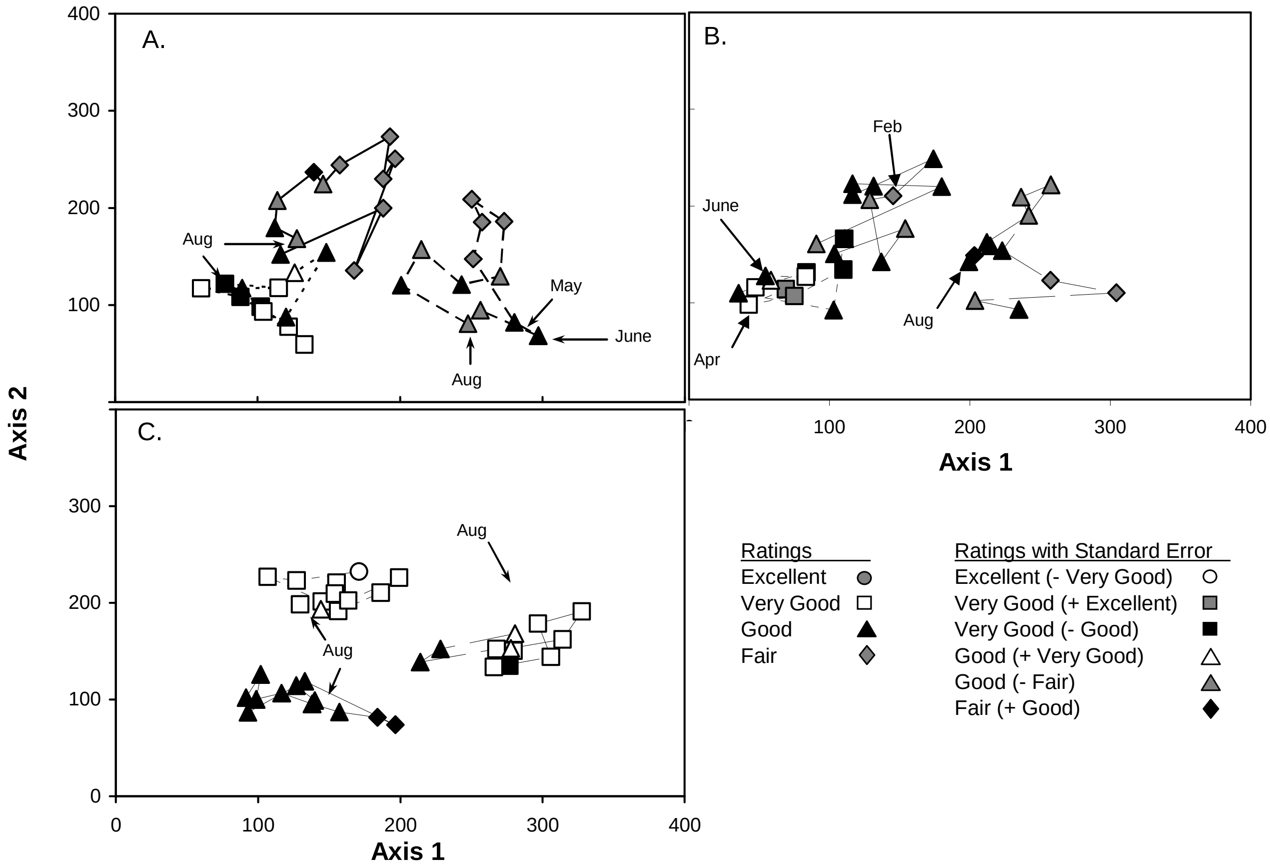


Figure 15. DCA analysis exhibiting monthly sample loadings coded by BI ratings and showing mean BI water quality ratings for each month. Mean monthly ratings having a standard error occuring in a rating category different from that of the mean are depicted as mean BI rating (+BI rating error). Ordination values are connected by a dotted line for CC, solid line for NC and a dashed line for LC. Assessments included all macroinvertebrates (A), all macroinvertebrates in riffle sites (B) and Chironomidae omitted (C). Refer to Figures 2 and 4 for DCA values labeled by month.



metrics generally were more variable throughout the year than the biotic and multimetric indices (Table 10). Chironomid taxa richness (CTR) and non-insect taxa richness (NITR) were more variable than the biotic and multimetric indices throughout the year in CC and NC. Although the EPT metrics displayed similar variability to P51 in CC and NC, EPT was more variable than the biotic indices. A different pattern in annual variability among the indices was evident in LC, where the EPT metrics were more variable than the other indices, including CTR and NITR. P51 was more variable than the biotic indices in CC and NC, but similar in variability in LC.

Riffle Sites

In CC, NITR was more variable than the other indices ($p \leq 0.05$) and the biotic indices were the least variable of the indices ($p \leq 0.05$). Variability among the indices in NC was similar to CC, except that the biotic indices were less variable in NC than CC. In contrast, %EPT and CTR were distinctly more variable in NC than in CC. Chironomid taxa richness and NITR were less variable in LC than in the other two streams.

Patterns in annual variability generally were similar between all site and riffle site assessments (Table 10). The most notable difference was that annual variability in NITR was much higher in the riffle sites than all sites in each stream. In CC, all indices showed only slight increases in variability except for CTR, which was less variable in riffle sites. In NC, only EPT and %EPT, in addition to NITR, had noticeably higher variability in riffle sites compared to all sites. Patterns in variability among indices in LC were similar among all site and riffle site assessments. As with all sites, biotic indices in riffle sites were the least variable of the indices. The MBI was slightly less variable in riffle sites than in all sites in NC and LC.

Omitting Chironomidae

All Sites

Annual variability of the indices in all sites was similar between all macroinvertebrate and non-chironomid assessments, with the exception of FBI assessments in LC (Table 10). The FBI was less variable in non-chironomid assessments compared to assessments including all macroinvertebrates in LC. Although P51 was more variable than the biotic indices in CC and NC when all macroinvertebrates were included in the assessments, it was more variable than the biotic indices only in CC when Chironomidae was omitted.

Riffle Sites

Most indices showed similar patterns to all sites when only riffle sites were assessed (Table 10). The exceptions were the BI and TR. The BI was slightly more variable in the riffle sites compared to all sites in CC. Taxa richness was more variable in the riffle sites compared to all sites in both CC and NC. There were no differences in variability between all sites and riffle sites in any of the indices in LC.

CHAPTER IV.

DISCUSSION

All Macroinvertebrates in Stream Assessments

Characterizing the Macroinvertebrate Community

Changes in macroinvertebrate community structure result from complex interaction of species composition, richness and abundance. One component alone can not fully describe community structure. Before a biotic or multimetric index can be deemed useful, its ability to reflect changes in macroinvertebrate community structure should be demonstrated. For instance, Cao *et al*. (1997) found that the Chandler score system was more effective than other indices tested to detect changes in community composition when taxa richness remained fairly stable along a pollution gradient. Another hurdle for indices is the influence of physical attributes on community structure. It could be debated that changes in the macroinvertebrate community influenced by natural physical attributes in the stream may alter an index rating, suggesting changes in water quality when they do not exist. One would expect the three streams in this study to differ in macroinvertebrate community structure due to physical differences alone. Ordination analyses are more commonly being used (Zamora –Muñoz *et al*. 1996, Calle-Martinez and Casa 2006) to assess macroinvertebrate community structure and to determine how physical and chemical parameters work together to shape tool in macroinvertebrate communities. Ordinations can be an effective evaluating an index's accuracy. Detrended Correspondence Analysis (DCA) used in this study confirmed that macroinvertebrate community structure of the three streams was distinctly different due to physical factors in the stream as well as other unknown factors, although likely due to enrichment. Community structure in CC and LC was least similar of the streams due to their physical attributes. Stream width and discharge contributed to the separation of the streams, increasing in width and discharge from CC (1^{st} order) to LC (2^{nd} order). Physical attributes also influenced macroinvertebrate community structure changes throughout the year in each stream. Cowpie Creek and Nippersink Creek displayed more annual variation in macroinvertebrate community structure than Lawrence Creek. These results were in agreement with Lenat and Crawford (1994), who indicated that changes in macroinvertebrate community structure were most likely to occur in smaller streams ranging from one to four meters in width. Cowpie and Nippersink Creeks are both less than or equal to four meters wide, whereas Lawrence Creek is greater than five meters wide. Although annual discharge was much lower in CC and NC than in LC, the banks of the streams, more so in CC than NC, exhibited scouring due to periodic flash flooding, which could be responsible for higher temporal variation in the community structure.

Unknown factors caused community structure in Nippersink Creek to differ from that of CC and LC. Macroinvertebrate community structure in LC was more variable that of CC or NC. Physical attributes of the streams failed to explain the variation along the second axis, however, it is possible that dissolved orthophosphate influenced species assemblages in NC due to extemely high phosphorus levels compared to the other two streams. All streams showed a decline in taxa richness and chironomid taxa richness with a simultaneous increase in chironomid abundance from winter to spring, highly influencing species placement on axis 2 and suggesting nutrient enrichment of the streams (Lenat, 1994). The increase in phosphorus would explain the correlation of the biotic indices to community structure, and hence, tolerance values of the organisms. Although corn crop fertilization can occur year round, Taylor (1991) showed that greatest fertilization of corn crops occurs in the spring prior to seeding, which would explain the changes in community structure and biotic index values during that time. Unfortunately nutrient data were collected only once during the study. The intent of nutrient sample collections was to determine approximate nutrient levels in each stream during summer. Therefore, it was not possible to use nutrient concentrations as factors in describing temporal patterns in community structure.

Annual Patterns in Macroinvertebrate Community Structure

All Sites

Indicator species

The presence of indicator species supported differences in stream communities found by the ordination analysis. It has been well documented that taxa richness and abundance of tolerant organisms, such as members of the family Chironomidae and non-insect groups, are higher in agricultural streams than in forested streams due to the influx of nutrients, pesticides and sediments (Lenat and Crawford 1994, Reice and Wohlenberg, 1993). In this study, non-insect groups most heavily influenced the macroinvertebrate community structure of LC. Mean annual Chironomidae taxa richness also was highest in LC, however, midges were the most numerically dominant organism in NC.

Macroinvertebrate community structure in CC was influenced mostly by typical clean water indicators not found in LC, such as stoneflies (*Allocapnia* and *Shipsa*) and caddisflies (*Glossosoma* and *Neophylax*), organisms that also prefer more turbulent water and suggest less environmental impact to the stream. Although Simuliidae, also present on the left side of axis 1 and influencing community structure in CC, are not clean water indicators, they are found in turbulent water with abundant substrate for attachment. Cowpie Creek also exhibited the lowest mean annual chironomid taxa richness of the three streams. Nippersink Creek was influenced by a combination of both clean water (*Helicopsyche*) and more pollution tolerant taxa (*Cricotopus*), suggesting water quality in NC to be intermediate of CC and LC. The combination of both turbulent and non-turbulent habitats increased habitat heterogeneity and thus the diversity of organisms in Nippersink Creek. Abundance of preferred habitat could explain the presence of *Helicopsyche* in NC. The dominant organism in LC, *Gammarus*, typically lives in cleaner water. However, they can thrive in areas in which substrate is impacted with sediments and microbial growth due to sewage, provided that water is fast flowing, shallow and well oxygenated (Hynes, 1966). Preferable physical habitat conditions in LC is a likely explanation for the proliferation of *Gammarus* in LC. The examples of habitat preference outweighing effects of pollution for *Simulium*, *Helicospyche* and *Gammarus* supported findings by Kerans *et al*. (1992) that habitat preference of macroinvertebrates can bias pollution assessments due to misinterpretation of taxa richness, abundance and tolerance values of indicator species. U.S. EPA (Yuan, 2006) recently published a guidance document for states to refine their specific tolerance values. Included in the document are goals to document sensitivities of macroinvertebrates to a variety of physical and chemical factors that will aid in index capability to reflect changes in macroinvertebrate community structure, and thus help determine sources of impact to a stream. Unfortunately more guidance from US EPA is needed to make sure that states have the tools necessary to derive tolerance values appropriate for their region and knowledge of conducting assessments so that results are comparable among different regions.

Taxa richness

Ephemeroptera-Plecoptera-Trichoptera (EPT), %EPT, percent EPT abundance, CTR, NITR and taxa richness each depicted changes in macroinvertebrate community structure related to physical parameters. The mean annual number of EPT taxa and %EPT taxa was highest in CC and lowest in LC. Lenat and Crawford (1994) found EPT taxa richness to be lower in agricultural streams than in forested streams. The forested riparian zone along much of CC from the headwaters to the study site and the small forested fragment in the sample reach of NC could explain the higher number of EPT taxa in the stream. Lamberti and Berg (1995) stressed that canopied woodland sections of streams can enhance stream health and biotic recovery from agricultural stresses. In addition, the large cobble substrate in conjunction with little sedimentation in CC could have provided adequate habitat for EPT taxa. Lawrence Creek had much less riparian canopy cover than the other streams and the substrate was small gravel layered with fine sediments. Due to the influence of physical factors, taxa richness was also lowest in CC and highest in LC. Lenat (1984) found total taxa richness to be lower in streams affected by agriculture impact than in forested streams. Although it is not possible to conclude that LC was least impacted by agriculture, it is likely that higher stream order of LC compared to the other streams partially influenced higher taxa richness in LC. Minshall *et al*. (1985) found taxa richness to increase with stream order in the lower orders, a phenomenon attributed to increased heterogeneity in moderately larger streams.

Biotic/multimetric indices

Although FBI and P51 values appeared to be strongly influenced by physical stream attributes, neither index rated the streams in a manner to reflect stream community structure differences attributed to those factors. Ratings by the FBI indicated greater distinction between the streams (CC less impacted than NC and LC) than P51 ratings, which rarely distinguished between streams throughout the year. Similar ratings were probably a result of the wide range of values in each water quality category. P51 also exhibited the fewest water quality categories of the biotic and multimetric indices, resulting in a lack of sensitivity to changes in water quality.

The BI and MBI were the only two indices that did not show a significant relationship to stream community structure related to physical attributes of the streams. However, the BI and MBI, as well as the FBI, were associated with differences in stream community structure influenced by undetermined factors, of which annual BI ratings best reflected. It is possible that the undetermined factors represent a gradient in dissolved oxygen among the streams due to excessive die-off of plant or algal material resulting from nutrient enrichment, which is the intended measurement of biotic indices (BI, MBI and FBI). As mentioned earlier, changes in community structure of the streams were indicative of the effects of heaviest fertilizer application in the spring. It is also possible that correlations between the biotic indices and the factors are related to discrepancies in biotic index tolerance values of certain organisms, for example *Gammarus* and *Cricotopus*. *Gammarus* in LC and *Cricotopus* in NC strongly influenced community structure in the two streams along the second axis. Hilsenhoff (1998) reported that intolerant species can inhabit larger, polluted streams during late autumn to early spring when water temperatures are cool. Although tolerance values are quite low for *Gammarus* (BI and FBI-4, MBI-3), Hilsenhoff (1988) considered *Gammarus pseudolimnaeus* to be a fairly tolerant organism that may inhabit more polluted systems in cooler seasons. Unfortunately, BI tolerance values for *Gammarus* are based on genus rather than species level. The strong influence of *Gammarus* in macroinvertebrate community structure in LC could explain the stream's similar or lower water quality rating to NC, which could be an inaccuracy if *Gammarus* is responding to preferable habitat more so than pollution levels in LC, as mentioned earlier. The P51 and richness indices, except taxa richness, are not dependent on *Gammarus* abundance and, therefore, are not influenced by the varied tolerance values. Another possible reason for poorer biotic index ratings in NC compared to LC, particularly by the MBI, could be the inability to distinguish *Cricotopus* from *Orthocladius* in LC during most of the year. Lower index values of the *Cricotopus/Orthocladius* group (MBI-6), and thus better water quality indicators, compared to *Cricotopus* (MBI-8), which highly influenced the macroinvertebrate community structure of NC, could be partially responsible for observed differences in water quality ratings between the two streams.

It is likely that the FBI more closely reflected changes in community structure because of physical and unknown factors than the other biotic indices due to the balancing effect of family level tolerance values. For example, FBI ratings for NC on many occasions were similar to LC, rather than higher, because the family level Chironomidae (FBI-6) has a lower tolerance value than does the generic level of *Cricotopus* (BI-7, MBI-8)). Similar to the FBI, P51 and the EPT metrics were not dependent on the identification of *Cricotopus* and *Orthocladius*, and therefore index values were not heavily influenced by these organisms. Although, in this study, the FBI seemed to indicate pollution of NC and LC well (both having similar impact) due to the index's capability to reflect both physical and other additional factors in the streams, it appeared to be by chance. Had the index overestimated water quality in NC by assigning the family level value to an organism that exhibits better genus and species level values than family level values, differences in water quality between the streams would have appeared greater than it should, assuming the genus/species level values are accurate. Because chironomids show species-specific responses to different levels and types of pollution, species level identifications can add valuable information to assessments, provided midge identifications are correct (Berg and Hellenthal, 1990).

Riffle Sites

Proposed sampling protocols for using different indices are not consistent, making comparing metrics difficult. Therefore, to facilitate comparisons among the indices, a single sampling method was used in this study for all indices. The aim of the sampling design was two-fold. The first was to sample macroinvertebrates from multiple areas in each stream to fully represent macroinvertebrate community structure. As mentioned earlier, Kerans *et al*. (1992) noted that limiting sampling to a particular habitat could introduce error into bioassessments by either overemphasizing or lessening the measure of human impact. They suggested a stratified sampling method due to the different levels of biological condition found in different habitats, specifically pool and riffle sites. In their study, Kerans *et al*. (1992) found that 8 of the 10 metrics examined differed significantly between pool and riffle sites. To examine differences between habitats, the present study focused on the margin and riffle areas of the streams. In instances where riffles flowed into margins, samples were relocated no more than a meter upstream or downstream of the transect to avoid high velocities in margin samples that were similar to that of the riffle. The second goal of the sampling design was to standardize the sampling method to accurately compare the ability of each index to reflect the entire macroinvertebrate community structure. Diamond (1996) warned that trends in benthic assemblages over time can be diminished if data from different bioassessment methods, including sampling protocols, are compared. As a result of both sampling goals, comparisons of index values were made between those obtained from particular habitats (riffles) and those obtained from a variety of habitats (riffles and margins). Index ratings for riffle site assessments were compared to DCA that ordinated riffle sites.

Biotic indices

Hilsenhoff (1988b) suggested limiting macroinvertebrate collections to riffle areas with current velocities greater than 0.3 m/s. In two of three streams studied by Hilsenhoff (1988b), no significant differences were found between BI values calculated for samples collected from different current velocities, however, mean BI values tended to be higher in slower currents. In the present study, BI, MBI and FBI index values were also calculated for riffles sites. Monthly index ratings from these sites were examined for their ability to reflect overall differences in stream macroinvertebrate community structure.

In general, using riffle sites to calculate indices rather than all sites resulted in stream ratings that more closely followed stream community differences influenced by physical factors throughout the year. This was particularly true for the BI and FBI. As a result, NC and LC were rated more similarly when only riffle sites were considered due to better mean annual index values for riffle sites than for all sites in NC. The difference in ratings was due to higher tolerance values of organisms in NC stream margins compared to those in NC riffles, which was not the case in LC. This study supported the findings of Kerans *et al*., (1992) that the bias in impact can occur in some streams but not others, depending on the distribution of habitat. Only the MBI reflected changes in macroinvertebrate community structure in riffle assessments due to other unknown factors determined by DCA axis 2, suggesting that the index is less sensitive than other indices to changes in physical habitat. Of the indices examined, annual MBI values did not distinguish between all sites and riffle sites, rating NC and LC similarly regardless of sites. It’s unclear why MBI ratings were similar between sites in NC. Perhaps the occurrence of non-insects, a group used in the MBI calculations but not in the BI or FBI, across margin and riffles maintained the similarity of ratings between sites

Taxa richness and percent abundance

Similar to all site assessments, all taxa richness and percent abundance measures examined in riffle sites reflected changes in macroinvertebrate community structure influenced by physical factors. On several occasions throughout the year, midges were less abundant and EPT was more abundant in riffles than in all sites in NC, whereas no differences occurred between sites in LC. This phenomenon could be due to differences in habitat between the streams, such as slower current velocities in the stream margins of NC compared to LC. As previously discussed, constraining the habitat sampled (all sites vs. riffle sites) can influence the taxonomic groups and abundances sampled due to their distributions in the streams. Kerans *et al*. (1992) suggested that limiting the habitat sampled can result in the loss of important information regarding water quality due to differences in the spatial structure of macroinvertebrate communities. Lenat (1990) found that in lower order streams, mid-channel communities will have the same assemblages as those near the margins and thus there is no need for multihabitat sampling. However, the present study showed that macroinvertebrate communities differed between all sites and riffle sites in both first and second order streams, suggesting a need for multi-habitat sampling.

Seasonal Patterns in Macroinvertebrate Communities

All Sites

Biotic/multimetric indices

Of the biotic and multimetric indices, P51 best reflected macroinvertebrate community structure influenced by physical factors throughout the year. Although P51's best water quality ratings were assigned to CC, the ratings did not cluster as closely compared to ratings of the biotic indices, possibly indicating difficulty of the index to assess stream systems subject to either low levels of anthropogenic stress or highly variable physical factors, such as those in Cowpie Creek. Lenat (1990) reported that unimpacted streams are most influenced by seasonal variation in community structure due to physical attributes. Temperature can affect variability due to differences in hatching and emergence of insect taxa and high ccurrent velocities can decrease population densities due to scouring. In agriculturally impacted streams, variability in macroinvertebrate communities can be reduced and seasonal changes are more likely associated with sediment, nutrients or pesticide inputs. Seasonal P51 ratings tended to misrepresent changes in the community attributed to water quality when natural seasonal variability was a stronger influence than agricultural impacts. In particular, ratings by P51 did not reflect changes in community structure in CC (April) and ratings were different (better) than in months with similar community structure. This appeared to be influenced by the presence of *Gammarus*. Ratings in April were mostly influenced by high EPT taxa richness and percent abundance, but most likely due to a change in physical parameters of the stream, *Gammarus* was also abundant in April. As a result, CC community structure appeared to show more similiarity to LC, but had better ratings than in months with similar community structure due to overall lower tolerance values. Although EPT and non-insect taxa are incorporated into the P51 metrics, amphipods are not included in the assessment unless they are numerically dominant in community structure. Therefore, information about community structure is lost when amphipods are highly abundant, yet another taxonomic group is dominant. P51 was the only index with ratings that reflected physical differences between NC and LC. This was partly due to lower percent abundances of *Gammarus* in NC than in LC. There is likely an imbalance in the multiple metrics of P51. A metric accounting for the presence of organisms responding to organic enrichment, such as abundance of the midges *Chironomus* or *Cricotopus* (Barbour *et al*., 1992) , may help to balance the metrics, especially when these midges are present but not dominant in the community. It is important to include metrics that indicate a range in both the type and level of degradation (Fore *et al*. 1996). In addition, caution must taken when selecting metrics so that they represent entire communities, yet do not include metrics that mask changes in others. The State of Illinois is currently working on developing a multimetric index that includes both taxa richness metrics and a biotic index (IEPA, 2002). Provided that tolerance values are re-evaluated, using both types of indices could provide additional information regarding changes in water quality due to physical and chemical attributes of streams.

The biotic indices showed a greater disagreement between community structure and ratings than P51, including April in CC. As with P51, *Gammarus* was the source of some discrepancy in index ratings due to its numerical dominance in the stream. The BI showed the effects of high *Gammarus* abundance in each season, whereas the FBI was less affected, showing differences only in CC in early winter and late spring. The BI and FBI also showed a disagreement between NC and LC ratings during periods when community structures were similar due to physical conditions in the streams. The differences were due to high percent abundance of chironomids in NC (Jan-Jun) and *Gammarus* abundance in LC (Aug-Nov). As water quality indicators, midges indicate greater impact in NC than LC during that time, however, the indices did not account for physical factors that allowed *Gammarus* to dominate in LC.

The MBI showed the least agreement between ratings and changes in macroinvertebrate community structure, demonstrating agreement in ratings between CC and NC related to physical attributes only half of the year and only in mid-winter between NC and LC. The MBI was the most taxonomically resolute index of those examined in this study, however the index uses a lower tolerance value for *Gammarus* than the BI and FBI, thus increasing the difference between the tolerance value and the organism's species loading along axis 1. Lower tolerance values of *Gammarus* can severely underestimate pollution in shallow, well-oxygenated streams impacted by sewage, where *Gammarus* can thrive.

As expected, biotic indices reflected a better agreement between index ratings and undetermined factors (axis 2) than with physical parameters, suggesting that the indices reflected changes in community structure due only to organic enrichment. Despite the overall better agreement between biotic index ratings and the axis, inconsistencies still existed. The MBI showed the fewest inconsistencies between ratings and changes in macroinvertebrate community structure in all streams. However, the MBI showed no changes in ratings in CC throughout the year. This is due in part to the large range of index values in the "Excellent" rating. The "excellent" rating includes organisms with tolerance values as high as 5, which includes many species of *Hydropsyche*. Hydropsychids are somewhat more tolerant than cased-caddisflies because they can avoid the surface of the substrata where effects of pollution can be greater (Barbour *et al*. 1992, Hynes 1966). Due to the pooling of *Hydropsyche* into the "excellent" category, the MBI is likely showing a lack of sensitivity to small changes in environmental stress in least impacted streams.

Taxa richness and percent abundance

The discrepancy between axis 2 values and index ratings in summer was likely due to a peak in discharge during that time. The BI and FBI represented the resulting physically-influenced change in community structure as a decline in water quality. All three streams showed a peak in discharge in June, however the disturbance affected each stream differently. Although Wallace *et al*. (1996) suggested that EPT taxa are relatively insensitive to natural disturbance, Lenat (1990) found more EPT taxa to be present during times of decreased flow due to less nonpoint source pollution. This is a possible explanation for the low EPT taxa richness NC in June in the present study. Hilsenhoff (1988b) suggested avoiding stream assessments during the summer months due to poor water quality conditions caused by reduced dissolved oxygen levels and increased temperature. This study indicated that water quality in summer did not decline in all streams as evidenced by water quality improvements in CC due to high EPT percent abundance regardless of the decline in EPT taxa richness. Water quality ratings in LC also improved in June, primarily due to high percent abundance of the amphipod *Gammarus*, as indicated by the BI and FBI. The increase in *Gammarus* may have been due to dislodgement of upstream communities during high flow. *Gammarus* populations also may have increased due to desirable physical conditions such as high current velocity, creating optimal oxygen levels as a result of turbulence and nutrient dilution (Lenat, 1988). In contrast, the June peak in discharge in NC resulted in an increased percent abundance of the isopod *Caecidotea*, driving the ratings toward a decline in water quality, which would coincide with increased agricultural run-off. The changes in community structure in each stream depicted by the biotic indices in June are characterized more by percent abundance and less by taxa richness.

Total taxa richness did not provide much information regarding water quality. Taxa richness in CC remained fairly stable from winter through summer, indicating the metric's lack of sensitivity to changes in chemical and physical attributes of the stream, and thus a lack of sensitivity towards changes in the macroinvertebrate community. Taxa richness in LC also appeared robust to changing stream dynamics and did not vary much throughout the year, although there was a decline in taxa over time. The similarity in temporal patterns of taxa richness in CC and LC made it difficult to distinguish possible differences in water quality between the two streams. Minshall *et al*. (1981) reported taxa richness to be stable in clean water habitats, which could explain the fairly stable values in CC over time. However, stable taxa richness can also be indicative of constant environmental stress. The taxa richness metric, if used alone, would not reveal the difference between the two types of environments. Taxa richness was variable in NC, indicating different levels of environmental stress over time. This was likely due to rain events increasing nutrient loading into the stream. Without nutrient data, however, this could not be verified.

Riffle Sites

Biotic indices

Seasonal patterns in index ratings reflected changes in the macroinvertebrate community in riffle sites differently from all sites, depending on the stream assessed and the index used for the assessment. The BI and FBI ratings better reflected community structure throughout the year in CC in riffle sites compared to all sites, whereas MBI ratings better reflected community structure in LC. Limiting sites to riffles only eliminated a majority of *Gammarus* from assessments, and thus balanced the effect of low MBI tolerance values in LC. However, the indices ratings also showed a general inability to reflect community structure in riffle sites throughout the year (MBI in CC, FBI in NC and BI in LC). Bonada *et al*. (2006) showed that the relative importance of different habitat can vary seasonally and that certain habitats may indicate a greater level of impairment than others. A complex set of factors that influence community structure annually, ranging from life histories, functional feeding, predation, natural disturbance to various types and levels of anthropogenic disturbance, is difficult to eliminate from assessments simply by limiting habitats sampled. To add to the complexity, the patterns in variability of macroinvertebrate communities differ from year to year (McElravy *et al*., 1989). Multivariate methods are emerging as a way to describe seasonal variation in macroinvertebrate communities. These methods help to identify how natural and anthropogenic factors are influencing the spatial and temporal dynamics of macroinvertebrate communities (Zamora-Muñoz and Alba-Tercedor, 1996).

Taxa richness and percent abundance

The percent abundance of *Gammarus* in CC and NC in riffles site assessments was significantly less in spring and summer, respectively, than in all site assessments, thus reducing discrepancies between BI/FBI ratings and community structure depicted by physical factors. Chironomids greatly influenced the orientation of streams along axis 2 when all sites were assessed, but significantly fewer chironomids in NC during January and March contributed to the better agreement between the BI, FBI and axis 1 in riffle site assessments. Fewer chironomids in the riffle site assessments compared to all sites gives the appearance that organic enrichment is less and that changes in community structure are more indicative of physical influences in the streams. This study shows clearly that discrepancies in water quality can occur when using biotic indices without included taxa richness and abundance metrics in assessments, ultimately resulting in the failure to observe anthropogenic impacts to streams.

Temporal Variability Among Indices

This study has demonstrated that indices rate stream water quality and track temporal changes differently in stream macroinvertebrate communities. Both aspects are important in determining the applicability of the indices. Knowledge of temporal variation in the indices is necessary to help avoid making inaccurate water quality assessments.

All Sites

Bioassessment indices have been reported to exhibit large amounts of variation throughout the year (Hannaford and Resh 1995, Hilsenhoff 1998, Szczytko 1989). The indices examined in this study were sensitive to temporal changes in insect assemblage structure. Some indices were more sensitive than others, exhibiting mean annual coefficients of variations (CV) ranging from 6.5% (BI) to 22% (EPT taxa). The variability of a particular index also differed among streams. High temporal variability in bioassessment indices has typically been regarded as undesirable, reflecting an index's inability to discriminate between water quality ratings. However, current research has suggested that closer examination of structural changes in the macroinvertebrate community over time might offer some insight as to whether or not a stream site is truly impacted (Linke *et al*., 1999). Therefore, similarities as to how indices reflect macroinvertebrate community structure over time is important and determines the utility of an index in inter-agency comparisons.

One goal of this study was to determine if indices reflected changes in macroinvertebrate community structure during similar times of the year so as to suggest an optimal season for sampling. Collectively, index ratings did not reflect changes in macroinvertebrate community structure in September, January, April, May or July due to influences of both physical attributes of the streams and other unknown factors.

Trends in water quality determination, i.e., similar changes in ratings, detected by the biotic and multimetric indices differed during half of the year (Sept, Nov, and Mar-Jun). Of the remaining months, all index ratings showed agreement in water quality changes and reflected changes in community structure. The most likely reason for the differences in water quality determination is that indices reflect different aspects of the macroinvertebrate community. For many biotic indices, tolerance values are derived on the basis of a particular environmental stress. If the stress is something other than what the index measures, it can be overlooked. For multimetric indices, the lack of key metrics emphasizing certain taxa groups can also result in the underestimation or overestimation of anthropogenic stresses. Using metrics that supply the same community information may also have the same impact by weighting one aspect of the community more heavily than others, resulting in a skewed representation of community structure.

(Norris, 1995). Not only are there difficulties associated with index development, but with the natural variability in macroinvertebrate communities themselves. As this study showed, the problem with comparing water quality among streams in a specific month is that each macroinvertebrate assemblage is stream-specific and responds differently to the physical environment. Sampling during a season that community structure most reflected anthropogenic impact would be ideal, however, that season could vary from stream to stream depending on the nature of the impact.

Generally in this study, taxa richness metrics displayed higher annual variability than biotic and multimetric indices. Szczytko (1989) found EPT to be the most variable index among the biotic and richness indices examined. Hilsenhoff (1988b) compared variability of the EPT index to a modified version of the BI and also found the EPT to be highly variable. He suggested that EPT is temporally sensitive to changes in physical attributes of the stream as well as functional measures of the macroinvertebrate community. Although the present study supported the views that EPT metrics are more variable than biotic and multimetric indices, it also was found that Chironomid Taxa Richness (CTR) and Non-insect Taxa Richness (NITR) were generally more variable than the EPT metrics. The higher variability of the EPT metrics compared to the biotic indices in this study could possibly be associated with differences in subsampling. The biotic indices were calculated using the required fixed-count subsampling method, whereas the EPT metrics were calculated using the fixed-fraction method. The fixed-count method establishes a particular number of organisms to subsample, which was 100 organisms for the biotic and multimetric indices used in this study. The fixed-fraction method establishes a fractional portion to subsample. Courtemanch (1996) suggested that fixed-fraction subsampling is the only reliable method for calculating richness because of the natural increase in taxa with sample size. He concluded that a fixed number of organisms can limit the number of taxa found. It is likely that a greater number of taxa sampled from the macroinvertebrate community will result in higher variability among samples and over time. In this study, the biotic indices, which used the fixed-count subsampling method, displayed distinctly lower annual variability than EPT metrics. The multimetric index, which also used the fixed-count method, revealed similar annual variability to EPT in CC and variability intermediate to the biotic indices and EPT in NC. However, annual variability of the multimetric index in LC was much lower compared to the other streams and was similar to the biotic indices, making an overall conclusion about the inherent level of temporal variability in the index difficult. The CTR and NITR metrics were added to the study at a later date, and therefore were calculated using fixed-counts. Like P51, CTR and NITR were similar in variability to EPT in CC and NC, but not in LC. These findings suggest that although taxa richness metrics were generally the most variable throughout the year, the degree of variability of all indices was stream-dependent.

The level of variability associated with the FBI in this study was inconsistent with the results of Szczytko (1989). He found the FBI to exhibit the lowest variability of the indices tested, which included the BI, EPT, and taxa richness. However, the results of the present study showed the FBI to be slightly more variable throughout the year than the BI and MBI in CC and NC and more variable than the BI and taxa richness in LC. In contrast to temporal variability in ratings, annual FBI ratings exhibited a lower sensitivity to stream differences in macroinvertebrate community structure and, therefore, ratings were more similar among streams compared to the other biotic indices. Although CV's of ordination axes showed that annual variation among streams differed, the FBI did not reflect differences in variation, demonstrating a lack of sensitivity to detect differences in impact among streams. These results support a study by Hilsenhoff (1990), which found that the FBI lacked ability to discriminate between different levels of water quality. In this study, the lower variation of annual FBI ratings between streams was a result of averaged monthly values. Reduced variability, theoretically due to greater numbers of organisms with the same tolerance values, appeared to be counter-balanced somewhat by the smaller range of values in FBI rating categories compared to the other indices.

Riffle Sites

The only obvious change in annual variability from all sites to riffle sites was a decrease in variability of CTR in CC. Thus, chironomid populations are likely more variable in the margins of CC during certain times of the year than in the margins of NC and LC. This was seen as an increase in variability of the second ordination axis for CC when riffle sites were used in the DCA. Biotic index ratings in CC were similar between all sites and riffle sites throughout the year, indicating a lack of response to changes in community structure that are likely due to physical influences. Lack of response to physical stream characteristics is a particular goal of biotic indices, however, it seems likely that the indices will be less effective at attaining this goal as physical and anthropogenic disturbances become more complex.

Omitting Chironomidae from Stream Assessments

In some respects, omitting chironomids from assessments would be beneficial to bioassessment programs. The expertise and time involved in sampling, sorting, and identifying Chironomidae can decrease the efficiency of rapid bioassessment programs. Midge abundances are typically reduced in high flow, especially in streams with sand or gravel sediments (Lenat 1983). Due to the strong influence of physical factors on chironomid distribution, their use in water quality determination can be obscured. Midge taxa metrics also can result in conflicting conclusions concerning water quality. Chironomid taxa can increase in moderately enriched streams, but decrease in highly impacted streams. The CTR metric if used alone may not always indicate differences between healthy and highly polluted streams (Lenat 1983). However, this study has shown that the omission of Chironomidae in bioassessments also can influence water quality assessments. The ability for indices to portray macroinvertebrate community structure improved in some streams but declined in others when Chironomidae was omitted from the assemblage structure.

Characterizing the Macroinvertebrate Community

Midge communities are often inadequately sampled, resulting in high variability in sampled densities and thus high variability in assessments. Ordination of stream sites indicated that macroinvertebrate community structure in the three streams was distinctly different along the first two ordination axes when chironomids were omitted. The omission of midges indicated an overall greater similarity in community structures of CC and NC along the first axis. Community structure in LC showed fewer similarities to CC and NC on the second axis.

Annual variability in community structure of CC along the first axis was slightly lower when Chironomidae was omitted from the analysis than when included, however variability in NC and LC was slightly greater when chironomids were omitted. There was less variability of macroinvertebrate communities along the second ordination axis when midges were omitted. Lenat (1983) showed that midges are highly sensitive to changes in stream discharge. In this study, the periods of high discharge in CC did not appear to have an effect on annual variability of non-chironomid assessments, as shown by the similarity of community structure in June to other months regardless of all macroinvertebrate and non-chironomid assessments. However, community structure in June in NC and LC was different than in other months, suggesting that discharge could have been an important factor influencing macroinvertebrates other than midges in community structure in June.

Annual Patterns in Macroinvertebrate Community Structure

All Sites

Indicator species

Removing Chironomidae from the assessments resulted in different species influencing streams along both ordination axes. On the first axis, *Helicopsyche* was more influential as a clean water taxon when midges were omitted and *Shipsa*, *Neophylax* and *Allocapnia*, all clean water taxa, were less influential. *Gammarus*, an amphipod that is tolerant to impacts of sedimentation, was most influential in LC with the omission of midges, whereas *Microtendipes* and *Tanytarsus*, also tolerant to impacts of sedimentation, were midges most influential in all macroinvertebrate assessments. Nonetheless, as with assessments that include chironomids, the gradient from clean water insect taxa to non-insects was likely associated with a relationship between macroinvertebrates and their physical habitat requirements related to current velocity. On the second axis, the most influential clean water indicator did not change (*Shipsa*), however *Gammarus*, which had axis values similar to clean water taxa when Chironomidae was included was less influential in the absence of Chironomidae. *Caecidotea* was located near clean water taxa on the axis when Chironomidae was included, but was closer to the opposite end of the axis in the absence of Chironomidae. As with chironomid inclusion assessments, it is unclear what is influencing the arrangement of macroinvertebrates along the second axis when chironomids were omitted, although tolerance values seem to reflect a gradient in enrichment along the axis. If macroinvertebrate community structure is related to enrichment on axis 2 in this study, the presence of *Helicopsyche* in CC is a reflection of physical attributes of the stream and not enrichment.

Biotic/multimetric indices

The ability of the indices to rate annual stream water quality according to overall differences in stream macroinvertebrate community structure along axis 1 (physical attributes) did not change when Chironomidae were omitted from the assessments. P51 values best reflected changes in macroinvertebrate community structure, but still lacked discriminatory power and ability to distinguish differences in water quality among the three streams. Regardless, it appeared that the omission of Chironomidae from biotic index assessments reduced an index's ability to distinguish between macroinvertebrate communities depicted by axis 1.

In contrast, the ability of the FBI to rate annual stream water quality according to differences between macroinvertebrate community structure along axis 2 improved when Chironomidae was omitted from the stream assessments. Macroinvertebrate communities in NC and LC were less similar in riffle sites than all sites, which resulted in the FBI, the index which has been shown to have lower sensitivity to changes in water quality (Hilsenhoff, 1990), to detect the differences in community structure. The BI and MBI did not show improvements in detecting water quality differences along the second ordination axis and P51 ratings were the same for each stream, regardless of the presence or absence of midges.

Taxa richness

Taxa richness was the only richness metric that did not explain changes in macroinvertebrate community structure based on axis 1 (physical factors) when Chironomidae was omitted from assessments. The lack of correlation between axis 1 and taxa richness indicates that chironomids made up a large component of total taxa richness and strongly influenced community structure on the first ordination axis.

Riffle Sites

Biotic indices

As with all macroinvertebrate assessments, annual index ratings for riffle sites in non-chironomid assessments did not show improvement over that of all sites in describing variation in community structure in the streams along either axis. The FBI better reflected community structure in riffle sites compared to all sites when all macroinvertebrates were used in assessments. The omission of midges from assessments removed information about community structure that had enabled the FBI to depict stream differences when riffle sites were used.

Taxa richness

Omitting chironomids from assessments in combination with limiting analyses to riffle sites produced only one significant correlation with richness measures (NITR and DCA axis 1). Therefore, it can be concluded that all other taxa richness measures did not explain differences in community structure between streams under these limitations. Chironomids are an important group in assessments due to their sensitivity to moderate levels of pollution. Certain species of midges can be indicators of the onset of mild to moderate organic pollution in a healthy stream (Hynes, 1966). There are few other taxonomic groups that can serve the same purpose. A typical macroinvertebrate assemblage is usually dominated by facultative organisms that are neither tolerant nor intolerant (Fore *et al*., 1996). Non-insect groups were not abundant enough in the three study streams so that biotic indices portrayed the same degree of information without the presence of midges in assessments.

Seasonal Patterns in Macroinvertebrate Community Structure

All Sites

Biotic/multimetric indices

P51 was the only index in which monthly ratings reflected community structure associated with axis 1 (physical and unknown parameters) with the same consistency between all macroinvertebrate and non-chironomid assessments in all three streams. In particular, P51 ratings in April and June in all three streams, which were periods of peak discharge, did not change between assessments when biotic index ratings improved. Lawrence Creek community structure had the highest percentage of midges of all the streams and is likely the reason that the omission of midges improved LC P51 index values. In general, P51 appeared very robust, and changed little with omission of midges, which was likely due to the relative unimportance of the group in P51 assessments. A multimetric index incorporating metrics that describe all aspects of the macroinvertebrate community should be sensitive to changes in the community. A chironomid metric would most likely aid the P51 index in determining anthropogenic impacts in streams. Some midge taxa can be indicative of specific types of impairments and several metrics may aid P51 in distinguishing different types of impact. Calle-Martinez and Casa (2006) found 6 species of chironomids that responded directly to water quality impairment along a wide gradient of impairments, thereby allowing potential improvement in index sensitivity.

With the omission of midges, the biotic indices showed less similarity between NC and LC water quality throughout the year and more similarities between CC and LC.

With midges omitted from assessments, *Gammarus*, which has much lower tolerance values than the midge community in LC, was most influential in shaping the macroinvertebrate community in LC, resulting in improved index ratings similar to CC. Although monthly ratings of the BI, MBI and FBI generally improved in non-chironomid assessments, the previous example demonstrates that the rating changes did not properly reflect changes in the macroinvertebrate community. Although community structure between streams was more distinct when midges were omitted from assessments, the indices reflected seasonal changes in community structure differently. For instance, BI and FBI ratings better reflected macroinvertebrate community changes in CC throughout the year, whereas the MBI and FBI showed less capability to reflect the macroinvertebrate community in NC, showing that index ability was dependent on the stream assessed. Again, P51 exhibited fewer rating differences between the three streams over time when midges were omitted, however, the ability to reflect changes in macroinvertebrate community structure did not improve or decline, indicating a lack in sensitivity to community structure changes resulting from the omission of midges. Although there is potential error involved in sampling and processing midges, this study suggests that the error associated with omitting them from assessments is likely greater. The variability midges add to assessments due to different life histories (Berg and Hellenthal 1990) can actually benefit assessments simply by their persistent presence. Many indicator species are present at only certain times of the year. If bioassessments do not encompass that time frame, potential information about water quality is excluded from evaluation.

Taxa richness

Chironomidae taxa richness appeared to be a driving force in seasonal changes in the ratings of the BI, MBI and FBI. Seasonal patterns in macroinvertebrate community structure without midges were slightly more similar among streams than when midges were included. Declines in water quality seemed to parallel high CTR in winter and spring, although the MBI was the only index to indicate improved water quality in the summer when CTR declined. The omission of chironomids from assessments resulted in improved index ratings in winter and spring.

Riffle Sites

Although a DCA ordination was not used to assess riffle sites when midges were omitted from assessments, it appears that restricting sites to riffles while omitting chironomids does not have an effect on assessments.

Temporal Variability among Indices

All Sites

Both non-chironomid and all macroinvertebrate index assessments displayed similarly ranges in annual variability. Both types of bioassessments showed taxa richness metrics to be the most variable. However, the variability of the indices subject to the omission of Chironomidae did not change as predicted. For example, although community structure in CC showed lower temporal variability when Chironomidae was omitted in comparison to chironomid inclusion, the indices did not reflect the lowered variability, and instead showed slightly higher temporal variability. Although temporal variability in community structure in NC and LC increased slightly with the absence of Chironomidae, not all indices increased in temporal variability. The BI and FBI were less variable in the absence of midges in LC, with the FBI being impacted the greatest. Using family level tolerance values rather than generic level values likely reduced variability among monthly index values. Most family level tolerance values for organisms collected in this study were very similar. The removal of one of the more extreme values, such as Chironomidae, likely reduced variability of FBI values in LC, although the effect was not seen in CC or NC. Regardless, the BI, FBI and P51 indicated that LC community structure was the least variable of the three streams. It is unclear why none of the indices depicted NC as the stream with the most temporally variable community structure.

Riffle Sites

Temporal variability in riffle sites was similar to that of all sites when chironomids were omitted from biotic and multimetric assessments. Regardless of whether variability increases or decreases, sampling macroinvertebrates from particular habitats and omitting taxa groups from the analyses misrepresents macroinvertebrate community structure. Nijboer *et a*l. (2005) found that, although Chironomidae were one of the most important taxonomic groups in defining overall macroinvertebrate community structure, subsets of indicator taxa or single taxonomic groups alone did not adequately characterize the overall macroinvertebrate community structure and they concluded that all macroinvertebrates should be used in assessments. Although limiting taxonomic components of rapid assessment may be useful in quickly determining sites that are at potential risk and in need of further study, they are not recommended methods for long-term water quality monitoring programs.

CHAPTER V.

CONCLUSION

Argument for Using Community Structure in Assessments

This study has shown that bioassessment indices are sensitive to temporal changes in macroinvertebrate community structure and that biotic indices reveal slightly different patterns in temporal variability of presumed water quality than multimetric and taxa richness metrics. Even among biotic indices, temporal patterns in water quality evaluated using the MBI differed slightly from the BI and FBI. This study shows that it is difficult to find a single time period during the year to compare water quality results among different indices.

Although it is generally thought that seasonal variability in community structure makes it difficult for bioassessment indices to reflect organic enrichment, variability in macroinvertebrate community structure can actually be helpful in determining impact to a stream. This study indicated that physical factors in streams were strongly influencing macroinvertebrate community structure. To fully determine how organic enrichment or pollutant loading is affecting a community, it is important to know how the community naturally changes throughout the year due to the physical environment. A change in the macroinvertebrate community related to physical factors can easily be misinterpreted as an impact to the stream. This study showed that it is necessary to include all parameters, values. physical and chemical, in bioassessments to aid in interpreting bioassessment index values.

Suggestions for Improving Indices

Many studies have shown that biotic indices reflect organic enrichment independent of physical factors in streams. However, the effects of physical stream attributes on the community must be taken into consideration to fully understand shifts in community structure and to avoid erroneous changes in water quality. The underlying reason for water quality misrepresentation lies in tolerance values used in biotic indices, which can bias the taxa richness component of the indices. In this study, several macroinvertebrate tolerance values were noted as outliers on the DCA. For future use, especially when biotic indices are used independent of taxa richness metrics, tolerance values of outliers need to be re-evaluated so that biotic indices can better detect community changes attributed to physical attributes and thus more reliably reflect information about macroinvertebrate community structure.

This study found less variability among FBI tolerance values due to the grouping of macroinvertebrates into family categories, thereby reducing the influence of outliers, i.e., genera with questionable tolerance values. Although this can ultimately mask small changes in the macroinvertebrate community structure, the FBI remains a good tool for assessing areas upstream and downstream of industrial or wastewater treatment plants where changes in the macroinvertebrate community are greater and therefore more detectible by the FBI. The benefit to using the FBI is that macroinvertebrates can be identified in the field, making assessments much more rapid.

The P51 index reflected differences in community structure among streams as defined by the physical attributes, yet the rating scale was too indiscriminant to show changes in water quality between the streams. The range of values in the rating scale should be adjusted so that changes in water quality can be detected

Restricting Habitat Sampled

Restricting the habitat sampled during assessments reduces information on variability in community structure and produces an inaccurate picture of the macroinvertebrate community. In this study, limiting sites to riffles did not decrease temporal variability due to physical factors within the streams, but did remove similarities among the streams, giving the appearance of three more distinctly different streams. Rather than removing information about community structure, it is more appropriate to re-evaluate tolerance values.

Omitting Chironomidae from Assessments

As with restricting the habitats sampled, limiting macroinvertebrates included in assessments to only certain taxonomic groups also reduces information depicting the composition and variability in community structure. In this study, omission of chironomids from assessments affected the seasonal variability stream evaluations differently and ultimately altered seasonal water quality assessments.

Implications of Applying Bioassessment Indices

Information obtained from existing stream assessments is important in helping states develop water quality standards, such as those for nutrient and dissolved oxygen limits. Tolerance values play a key role in determining existing water quality assessments. Indices that are not clearly depicting changes in the macroinvertebrate community because of bias in macroinvertebrate tolerances can have costly consequences for state programs. By removing the error associated with tolerance values and examining the variability in community structure more closely through taxa richness and abundance metrics, biologists will have a better understanding of how to compare water quality assessments between streams and to determine which streams are truly impacted. Multimetric indices are a good example of such an approach. These qualitative indices retain information about the macroinvertebrate community, yet enable a much more rapid assessment.

Table 16. Recommendations for future bioassessments.

Development of an index	Strict evaluation of tolerance values for biotic indices Selection of metrics, including biotic index, that represent all aspects of macroinvertebrate community structure Adjust sensitivity of rating scale to detect both low and high levels of pollution
Use of multivariate analysis to test the index	Ability to relay proper information about the impact to the macroinvertebrate community Ability to provide information about impact to the community on a temporal basis
Standardization of field and laboratory protocols	Sampling all macroinvertebrate habitat Inclusion of all macroinvertebrates in assessments Lowest taxonomic identification possible
Consensus on when to sample during the year	Assess particular level of impact rather than particular time of year Level dependent on the specific type of impact

REFERENCES

Ballogh, D., C.E. Davies, and F.H. Jones. 1976. Biological assessment of water quality in three British rivers: The North Esk (Scotland), the Ivel (England) and the Taff (Wales). Water Pollution Control 75: 92-110.

Barbour, M.T., J.L. Plafkin, B.P. Bradley, C.G. Graves, and R.W. Wisseman. 1992. Evaluation of EPA's rapid bioassessment benthic metrics: metric redundancy and variability among reference stream sites. Environmental Toxicology and Chemistry 11: 437-449.

Berg, M.B. and R.A. Hellenthal. 1990. Data variability and the use of chironomids in environmental studies: the standard error of the midge. Pages 1-8 In: W.S. Davis [ed]. Proceedings of the 1990 Midwest Pollution Control Biologists Meeting. U.S. Environmental Protection Agency Region V, Environmental Sciences Division, Chicago, IL. EPA-905-9-90/005.

Bergman, E.A. and W.L. Hilsenhoff. 1978. Baetis (Ephemeroptera: Baetidae) of Wisconsin. The Great Lakes Entomologist 11: 125-135.

Bonada, N., H. Dallas, M. Rieradevall, N. Prat and J. Day. 2006. A comparison of rapid bioassessment protocols used in 2 regions with Mediterranean climates, the Iberian Peninsula and South Africa. Journal of the North American Benthological Society 25: 487-500.

Calle-Martinez, D. and J.J. Casas. 2006. Chironomid species, stream classification and water-quality assessment: the case of 2 Iberian Mediterranean mountain regions. Journal of the North American Benthological Society 25: 465-476.

Camargo, J.A. 1992. Temporal and spatial variations in dominance, diversity, and biotic indices along a limestone stream receiving a trout farm effluent. Water, Air, and Soil Pollution 63: 343-359.

Cao, Y., A.W. Bark and W.P. Williams. 1997. Analysing benthic macroinvertebrate community changes along a pollution gradient: a framework for the development of biotic indices. Water Research 31: 884-892.

Chessman, B.C. and P.K. McEvoy. 1998. Towards diagnostic biotic indices for river macroinvertebrates. Hydrobiologia 364: 169-182.

Chutter, F.M. 1972. An empirical biotic index of the quality of water in South African streams and rivers. Water Research 6: 19-30.

Courtemanch, D.L. 1996. Commentary on the subsampling procedures used for rapid bioassessments. Journal of the North American Benthological Society 15: 381-385.

Diamond, J.M., M.T. Barbour, J.B. Stribling. 1996. Characterizing and comparing bioassessment methods and their results: a perspective. Journal of the North American Benthological Society 15: 713-727.

Fore, L.S., J.R. Karr and R.W. Wisseman. 1996. Assessing invertebrate responses to human activities: evaluating alternative approaches. Journal of the North American Benthological Society 15: 212-231.

Gauch, H.G. 1982. Multivariate Analysis in Community Ecology. Cambridge University Press, Cambridge.

Gerritsen, J. 1995. Additive biological indices for resource management. Journal of the North American Benthological Society 14: 451-457.

Hannaford, J.H. and V.H. Resh. 1995. Variability in macroinvertebrate rapid-bioassessment surveys and habitat assessments in a northern California stream. Journal of the North American Benthological Society 14: 430-439.

Hill and Gauch, 1980. Detrended correspondence analysis, an improved ordination technique. Vegetatio 42: 47-58.

Hilsenhoff, W.L. 1977. Use of arthropods to evaluate water quality of streams. Technical Bulletin No. 100. Department of Natural Resources, Madison, WI.

Hilsenhoff, W.L. 1982. Using a biotic index to evaluate water quality in streams. Technical Bulletin No 132. Department of Natural Resources, Madison, WI.

Hilsenhoff, W.L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomologist 20: 31-39.

Hilsenhoff, W.L. 1988a. Rapid field assessment of organic pollution with a family-level biotic index. Journal of the North American Benthological Society 7: 65-68.

Hilsenhoff, W.L. 1988b. Seasonal correction factors for the biotic index. The Great Lakes Entomologist 21: 9-13.

Hilsenhoff, W.L. 1990. Data variability in arthropod samples used for the biotic index. Pages 47-52 In: W.S. Davis [ed]. Proceedings of the 1990 Midwest Pollution

Control Biologists Meeting. U.S. Environmental Protection Agency Region V, Environmental Sciences Division, Chicago, IL. EPA-905-9-90/005.

Hilsenhoff, W.L. 1998. A modification of the biotic index of organic stream pollution to remedy problems and permit its use throughout the year. The Great Lakes Entomologist 31: 1-12.

Hutchens, J.J., Jr., K. Chung and J.B. Wallace. 1998. Temporal variability of stream macroinvertebrate abundance and biomass following pesticide disturbance. Journal of the North American Benthological Society 17: 518-534.

Hynes, H.B.N. 1966. The biology of Polluted Waters. Hazell, Watson and Viney LTD. Aylesbury, England.

Illinois Environmental Protection Agency (IEPA). 1987. Pages 1-51 In: Quality assurance and field methods manual, Division of Water Pollution Control, Section C: Macroinvertebrate monitoring. Springfield, Illinois.

Illinois Environmental Protection Agency, Bureau of Water. 2002. Water Monitoring Strategy 2002-2006. IEPA/BOW/02-005.

Kerans, B.L., J.R. Karr, S.A. Ahlstedt. 1992. Aquatic invertebrate assemblages: spatial and temporal differences among sampling protocols. Journal of the North American Benthological Society 11: 377-390.

Kolkwitz, R. and M Marsson. 1909. Okologie der tierischen Saprobien. Breitage zur Lehre von der biologischen Gewasserbeurteilung. Int. Revue Ges. Hydrobiol. Hydrogr. 2, 126-152.

Lamberti, G.A. and M.B. Berg. 1995. Invertebrates and other benthic features as indicators of environmental change in Juday Creek, Indiana. Natural Areas Journal 15: 249-258.

Lenat, D.R. 1983. Chironomid taxa richness: natural variation and use in pollution assessment. Freshwater Invertebrate Biology 2: 192-198.

Lenat, D.R. 1984. Agriculture and stream water quality: a biological evaluation of erosion control practices. Environmental Management 8: 333-344.

Lenat, D.R. 1988. Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates. Journal of the North American Benthological Society 7: 222-233.

Lenat, D.R. 1990. Reducing variability in freshwater macroinvertebrate data. Pages 19-32 In: W.S. Davis [ed]. Proceedings of the 1990 Midwest Pollution Control

Biologists Meeting. U.S. Environmental Protection Agency Region V, Environmental Sciences Division, Chicago, IL EPA-905-9-90/005.

Lenat, D.R. and J.K. Crawford. 1994. Effects of land use on water quality and aquatic biota of three North Carolina Piedmont streams. Hydrobiologia 294: 185-199.

Lind, O.T. 1979. Handbook of Common Methods in Limnology. C.V. Mosby, St. Louis, MO. Pp. 163.

Linke, S., R.C. Bailey and J. Schwindt. 1999. Temporal variability of stream bioassessments using benthic macroinvertebrates. Freshwater Biology 42: 575-584.

Mackay, R.J. 1978. Larval identification and instar association in some species of Hydropsyche and Cheumatospyche (Trichoptera: Hydropsychidae). Entomological Society of America 71: 499-509.

Margalef, D.R. 1957. Information theory in ecology. General Systems 3: 36-71

Marsh, P.C. and T.F. Waters. 1980. Effects of agricultural drainage development and benthic invertebrates in undisturbed downstream reaches. Transactions of the American Fisheries Society 109: 213-223.

Matthews, R.A., A.L. Buikema, Jr., J. Cairns, Jr. and J.H. Rodgers, Jr. 1982. Biological monitoring. Part IIA. Receiving system functional methods, relationships and indices. Water Research 16: 129-139.

McElravy, E.P., G.A. Lamberti and V.H. Resh. 1989. Year to year variation in the aquatic macroinvertebrate fauna of a northern California stream. Journal of the North American Benthological Society 8:51-63.

Merritt, R.W. and K.W. Cummins (eds). 1996. An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing Co., Dubuque, IA.

Michigan Department of Environmental Quality. 1996. Pages 1-43 In: Update of Great Lakes and environment assessment section (GLEAS) Procedure 51 metric scoring and interpretation. Surface Water Quality Division staff report.

Minshall, G.W., R.C. Petersen, Jr. and C.F. Nimz. 1985. Species richness in streams of different size from the same drainage basin. The American Naturalist 125: 16-38.

Minshall, G.W. 1981. Structure and temporal variations of the benthic macroinvertebrate community inhabiting Mink Creek, Idaho, U.S.A., a 3rd order rocky mountain stream. Journal of Freshwater Ecology 1:13-26.

Morihara, D.K. and W.P. McCafferty. 1979. The Baetis larvae of North America (Ephemeroptera: Baetidae). Transactions of the American Entomological Society 105: 139-221

Murphy, P.M. 1978. The temporal variability in biotic indices. Environmental Pollution 17: 227-236.

Nijboer, R.C., P.F.M. Verdonschot, D.C. Van Der Werf. 2005. The use of indicator taxa as representatives of communities in bioassessment. Freshwater Biology 50: 1427-1440.

Norris, R.H. 1995. Biological monitoring: the dilemma of data analysis. Journal of the North American Benthological Society 14: 440-450.

Norris, R.H. and A. Georges. 1993. Analysis and interpretation of benthic macroinvertebrate surveys. Pages 195-223 In: D.M. Rosenberg and V.H. Resh [eds.] Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, Inc., New York.

Omernik, J.M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77: 118-125.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. US Environmental Protection Agency, Washington, DC. EPA 444/4-89-001.

Polls, I. 1994. How people in the regulated community view biological integrity. Journal of the North American Benthological Society 13: 598-604.

Reice, S.R. and M. Wohlenberg. 1993. Monitoring freshwater benthic macroinvertebrates and benthic processes: measures for assessment of ecosystem health. Pages 287-305 In: D.M. Rosenberg and V.H. Resh [eds.] Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, Inc., New York.

Resh, V.H. and E.P. McElravy. 1993. Contemporary quantitative approaches to biomonitoring using benthic macroinvertebrates. Pages 159-194 In: D.M. Rosenberg and V.H. Resh [eds.] Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, Inc., New York.

Resh, V.H. and J.K. Jackson. 1993. Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. Pages195-223 In: D.M. Rosenberg and V.H. Resh [eds.] Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, Inc., New York.

Resh, V.H., R.H. Norris and M.T. Barbour. 1995. Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. Australian Journal of Ecology 20: 108-121.

Resh, V.H. and J.D. Unzicker. 1975. Water quality monitoring and aquatic organisms: the importance of species identification. Journal of the Water Pollution Control Federation 47: 9-19.

Rosenberg, D.M., H.V. Danks and D.M. Lehmkuhl. 1986. Importance of insects in environmental impact assessment. Environmental Management 10: 773-783.

Rosenberg, D.M. and V.H. Resh. 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates. Pages 1-9 In: D.M. Rosenberg and V.H. Resh [eds.] Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, Inc., New York.

Schuster, G.A. and D.A. Etnier. 1978. A manual for the identification of the larvae of the caddisfly genera <u>Hydropsyche</u> <u>pictet</u> and <u>Symphitopsyche</u> <u>ulmer</u> in eastern and central North America (Trichoptera: Hydropsychidae). U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, Ohio. EPA-600/4-78-060.

Schwenneker, B.W. and R.A. Hellenthal. 1984. Sampling considerations in using stream insects for monitoring water quality. Environmental Entomology 12: 741-750.

Spindler, P. 1996. Using ecoregions for explaining macroinvertebrate community distributions among reference sites in Arizona. Arizona Department of Environmental Quality, Flagstaff, AZ.

Stark, J.D. 1993. Performance of the macroinvertebrate community index: effects of sampling method, sample replication, water depth, current velocity and substratum on index values. New Zealand Journal of Marine and Freshwater Research 27: 463-478.

Stone, M.K. and J.B. Wallace. 1998. Long-term recovery of a mountain stream from clear-cut logging: the effects of forest succession on benthic invertebrate community structure. Freshwater Biology 39: 151-169.

Szczytko, S.W. 1989. Variability of commonly used macroinvertebrate community metrics for assessing biomonitoring data and water quality in Wisconsin streams. Pp. 12-22. In W.S. Davis and T.P. Simon [eds.] Proceedings of the 1989 Pollution Control Biologists Meeting. U.S. Environmental Protection Agency Region V, Environmental Sciences Division, Chicago, IL EPA-905-9-89/007.

Taylor, H.H. 1991. Fertilizer application timing. Situation and Outlook Report: agricultural resources.

United States Environmental Protection Agency. 1996. Summary of state biological assessment programs for streams and rivers. Washington, DC.

Vinson, M.R. and C.P. Hawkins. 1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. Journal of the North American Benthological Society 15: 392-399.

Wallace, J.B., J.W. Grubaugh and M.R. Whiles. 1996. Biotic indices and stream ecosystem processes: results from an experimental study. Ecological Applications 6: 140-151.

Washington, H.G. 1984. Diversity, biotic and similarity indices. A review with special relevance to aquatic ecosystems. Water Research 18: 653-694.

Wright, I.A., B.C. Chessman, P.G. Fairweather and L.J. Benson. 1995. Measuring the impact of sewage effluent on the macroinvertebrate community of an upland stream: the effect of different levels of taxonomic resolution and quantification. Australian Journal of Ecology 20: 142-149.

Yuan, L.L. 2006. Estimation and Application of Macroinvertebrate Tolerance Values. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC. EPA/600/P-04/116F.

Zamora-Muñoz, C. and J. Alba-Tercedor. 1996. Bioassessment of organically polluted Spanish rivers, using a biotic index and multivariate methods. Journal of the North American Benthological Society 15: 332-352.

Zamora-Muñoz, C., C.E. Sainz-Cantero, A. Sanchez-Ortega, and J. Alba-Tercedor. 1995. Are biological indices BMPW’ and ASPT’ and their significance regarding water quality seasonally dependent? Factors explaining their variations. Water Research 29: 285-290.

Zar, J.H. 1984. Biostatistical Analysis. Second edition. Prentice-Hall, Inc, New Jersey.

VITA

Nia M. Haller began attending the College of Arts and Sciences at Northeastern State University in the summer of 1990. In 1991, she transferred to the University of West Florida, where she received a B.S. in Marine Biology in 1993. In the summer of 1993, she started a two-year cooperative agreement between Western Illinois University and the USEPA at Sabine Island, FL, studying the effects of molluscicidal agents on nontarget organisms. Nia entered the Department of Biology at Loyola University Chicago in 1995 to pursue her M.S. in aquatic ecology. Her research centered on the temporal comparisons of bioassessment indices in three Midwestern streams. After the completion of field work and data analysis in 2000, Nia began working at the Illinois Natural History Survey (Lake Michigan Biological Station) in conjunction with Illinois-Indiana Sea Grant as a research and outreach assistant. In 2001, she accepted a permanent position with the Illinois Environmental Protection Agency (IEPA) in the Bureau of Water, Surface Water Section, as a stream biologist. Since 2001, she has continued to write her master's thesis while furthering her career at IEPA and enjoying motherhood.

THESIS APPROVAL SHEET

The thesis submitted by Nia M. Haller has been read and approved by the following committee:

Martin B. Berg, Ph.D.
Professor of Biology
Loyola University Chicago

Christopher Peterson, Ph.D.
Professor of Natural Science
Loyola University Chicago

Nancy C. Tuchman, Ph.D.
Professor of Biology
Loyola University Chicago

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

____________________ ______________________________________

Date Director’s Signature