

NOVEL APPROACHES IN MACROINVERTEBRATE BIOMONITORING

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Doctor of Philosophy**

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TABLE OF CONTENTS

Acknowledgements.....	ii
List of Tables.....	vi
List of Figures.....	viii
Abstract.....	x
Chapter 1 Dissertation introduction.....	1
Chapter 2 Literature review.....	4
Chapter 3 A Least Desired Index for Assessing the Effectiveness of Grass Riparian Filter Strips in Improving Water Quality in an Agricultural Region.....	9
3.1 Abstract.....	9
3.2 Introduction.....	10
3.3 Methods and Materials.....	12
3.3.1 Study Approach.....	12
3.3.2 Study Sites.....	13
3.3.3 Sampling and Identification.....	14
3.3.4 Analysis.....	14
3.3.5 Metric Selection, Scoring and Index Development.....	15
3.3.6 Assessment of Test Sites and Index Comparisons.....	16
3.4 Results.....	17
3.4.1 Study Sites.....	17
3.4.2 Metric Selection, Scoring, and Index Development.....	17
3.4.3 Assessment of Test Sites and Index Comparisons.....	18
3.5 Discussion.....	19
3.5.1 Study Sites.....	19
3.5.2 Metric Selection, Scoring, and Index Development.....	19

3.5.3	Assessment of Test Sites and Index Comparisons.....	22
3.6	Conclusions.....	24
Chapter 4 Seasonal Predictability of a Benthic Macroinvertebrate Community in an		
	Ozark Stream in Central Missouri, USA.....	37
4.1	Abstract.....	37
4.2	Introduction.....	38
4.3	Methods and Materials.....	43
4.3.1	Sampling and Processing.....	43
4.3.2	Rare and Transient Taxa.....	46
4.3.3	Metric Assessment.....	46
4.3.4	Maturity Weighted vs. Standard Abundance.....	46
4.3.5	Multivariate Assessment.....	47
4.4	Results.....	49
4.4.1	Rare and Transient Taxa.....	49
4.4.2	Metric Assessment.....	49
4.4.3	Maturity Weighted vs. Standard Abundance.....	50
4.4.4	Multivariate Assessment.....	50
4.5	Discussion.....	52
	References cited.....	82
Appendix A. Mean number of individuals for taxa of reference and anti-reference		
	streams for sample periods I, II, and III for Chapter 3. Only taxa with mean	
	numbers ≥ 1 are included.....	93
Appendix B. Raw data excluding rare and transient taxa for Chapter 4.....		
		96
Appendix C. Raw maturity weighted data for Chapter 4.....		
		100
Appendix D. SAS program for calculating metrics used in Chapter 4.....		
		104
Vita.....		
		114

LIST OF TABLES

Table	Page
1. Metrics tested for differences between reference and anti-reference sites.....	26
2. Redundancy, excessive variation, zero values, and ANOVA results of the nine metrics that significantly differed between reference and anti-reference streams.....	27
3. Correlation matrices for B-IBI (A) and LDI (B) metric selection.....	28
4. Test site scores for B-IBI and LDI.....	29
5. Suggested GRFS test site condition classification for B-IBI and LDI scores.....	30
6. Mean metric values, standard deviation (SD), sample size (n), and metric scores with B-IBI and LDI at two test sites, and metric response to impact.....	31
7. Physical attributes per taxonomic group used for determining individual maturity classifications.....	63
8. Physico-chemico-temporal factors considered for metric multiple regressions and discriminant function analysis of RIVPACS models.....	64
9. Operational taxonomic units (OTUs) considered rare or transient from samples taken from the Burris Fork in central Missouri. Rare and transient as defined in the text are given as tentative assignments. Some taxa are given more than one designation to account for uncertainty or their versatility.....	65
10. Metrics representing each metric type with a coefficient of variation (CV) less than 20 calculated over the Model Development Period.....	67
11. Multiple regression models used for predicting test date metric values.....	68
12. Metrics used for multiple regression. Model F-values, <i>p</i> -values, error degrees of freedom, and mean square error (MSE) are included. The mean predicted square error (MSPE) of the test dates are also given.....	69

13. Metrics calculated from standard data and their maturity-weighted analogues.
Means and standard deviations (SD) were based on sampling dates for which the target groups of the metric were present (*n*). Paired comparisons were used for t-test and signed rank test. The number of *n* sampling dates that were positive as a result of the standard metric subtracted from its maturity weighted analogue and the number of sampling dates where the maturity analogue represented a $\pm 5\%$, 10%, and 20% change is given.....70

14. Maturity weighted abundances of macroinvertebrate taxa (listed by operational taxonomic unit) used for cluster analysis in the classification of seasons for the Burris Fork in Missouri. All dates of the Model Development Period (20 December 2002 – 20 December 2003) are shown.....71

15. Group membership probabilities, similarity, and O/E model results for communities sampled from the Burris Fork in central Missouri. Discriminant function analysis was used to find the probability of group membership to seasons (winter, summer, autumn) determined from temporal variables from all dates covering the Model Development Period (MDP) (20 December 2002 – 20 December 2003). Probabilities of season membership for test dates (*t*₁, *t*₂, and *t*₃) were determined from the MDP discriminant functions.....72

16. Physico-chemico-temporal variable measurements from Burris Fork in central Missouri used for metric multiple regressions and discriminant function analysis of RIVPACS models. Dates covering the Model Development Period (20 December 2002 – 20 December 2003) for test dates (*t*₁, *t*₂, and *t*₃) are given.....73

LIST OF FIGURES

Figure	Page
1. Locations of study sites.....	32
2. Scoring criteria for a metric that decreases in response to increasing impact. The end of the top and bottom whiskers represent the 95 th and 5 th percentiles, respectively. The top and bottom of the box represent the 75 th and 25 th percentiles, respectively. The dot in the middle of the box represents the median. For reference streams, the 95 th percentile determines the maximum limit for this metric. For anti-reference streams, the 5 th percentile determines the minimum limit.....	33
3. Scatter plots of reference and anti-reference sites for all seasons in 2-dimensional DFA space derived from environmental parameters. Open and filled symbols represent reference and anti-reference sites, respectively. Triangles, circles, and squares represent spring, summer, and fall, respectively.....	34
4. Scatter plots of reference (●), anti-reference (□), and test sites (Δ) in 2-dimensional DFA space derived from environmental parameters for all seasons.....	35
5. Reference and anti-reference scoring criteria determined for nine metrics. Square root transformed metrics are indicated by 'a'.....	36
6. Flow chart showing the steps to construct a RIVPACS model showing different pathways for constructing expected communities based on occurrence frequency and relative abundances. Taken after Flotemersch et al. (2006).....	74
7. Observed and expected multiple regression trends for selected richness metrics. Vertical lines indicate the end of the Model Development Period (20 December 2002 – 20 December 2003) and the beginning of the test dates.....	75

8. Observed and expected multiple regression trends for selected diversity metrics. Vertical lines indicate the end of the Model Development Period (20 December 2002 – 20 December 2003) and the beginning of the test dates.....	76
9. Observed and expected multiple regression trends for selected tolerance metrics. Vertical lines indicate the end of the Model Development Period (20 December 2002 – 20 December 2003) and the beginning of the test dates.....	77
10. Observed and expected multiple regression trends for selected functional feeding and composition metrics. Vertical lines indicate the end of the Model Development Period (20 December 2002 – 20 December 2003) and the beginning of the test dates.....	78
11. Cluster dendrogram showing sampling date classifications and biologically derived seasons from select Operational Taxonomic Units, based on semi-partial R ² dissimilarity distance.....	79
12. Discriminant function analysis scatter plot of Model Development Period sampling dates (20 December 2002 – 20 December 2003) and test dates (t1, t2, and t3).....	80
13. Abundance in thousands per sampling date. Striped bars indicate total abundance, solid black bars indicate total abundance minus taxa without maturity weightings (e.g. Oligochaeta), and white bars indicate maturity weighted abundance.....	81

ABSTRACT

The increasing popularity of benthic macroinvertebrate use for biological monitoring and assessment of lotic systems calls for new applications for regions with a lesser known fauna and an overall better understanding of how community measures are influenced by seasonal variability. Two separate studies are presented to address aspects of biological monitoring that are seldom studied. The first study focused on evaluation of Grass Riparian Filter Strip effectiveness in north central Missouri. Streams within conservation areas and unprotected reaches were sampled to identify metrics that could distinguish between undesired and best attainable conditions. These metrics were used to construct two assessment tools, a Benthic Index of Biotic Integrity (B-IBI) and a novel technique we call a Least Desired Index (LDI). LDI works in a reciprocal fashion to B-IBI in that the lowest scoring criteria of the metrics are set by anti-reference stream conditions, or conditions that represent an undesirable quality. B-IBIs were developed from the conservation stream samples and the LDI was developed from the unprotected stream reaches. Two test sites with established GRFS were sampled and assessed with the B-IBI and the LDI. B-IBI determined that the GRFS sites showed moderate improvements where the LDI showed that the GRFS sites made no improvements. The LDI was considered to be a more realistic assessment considering that the GRFS at the test sites had only been established for 2 years and the reference streams used to construct the B-IBI were most likely impaired. The second study utilized a state designated reference stream as a case study for temporally profiling the community structure. Samples of benthic macroinvertebrates and water quality were taken approximately monthly to fulfill four objectives. The first objective was to determine if a temporal profile of the community might better reveal members of the community that are rare and transient. The second objective was to consider the maturity of each individual in weighting abundances and how these might affect the interpretation of the

community structure. The third objective examined annual variability of 120 metrics. Multiple regressions with physico-chemico-temporal variables were created to see if these metrics could be predicted for samples taken during the following year. The last objective was to construct a River Invertebrate Prediction and Classification System (RIVPACS) model that classified seasons based on the biota and to use this model to predict and compare the community composition of samples taken during the following year. Rare and two types of transient taxa were recognized. Rare taxa were considered to be residents of the community, but infrequently encountered. Transient type I taxa were considered adventitious, and transient type II were short lived. Maturity weighted abundances showed significant changes in community measures and were found to be useful in aspects of other analyses. Few metrics exhibited low variation over the annual period, and fewer were predictable, indicating that seasonal variation can have a significant impact on measurements used in biological monitoring. The RIVPACS model biologically classified three seasons. Overall, the model moderately predicted community structure, also highlighting that macroinvertebrate communities are difficult to consistently quantify. Lotic habitats are important resources but are dynamic systems exhibiting challenging perspectives with regard to monitoring and quality assessment.

CHAPTER 1

Dissertation Introduction

Water is an important resource that is necessary for the existence of life. Anthropogenic activities have degraded stream habitat quality, and consequently have negatively affected the quality of human life. Recently, there has been an interest in preserving balanced communities that resemble natural conditions (Karr and Dudley 1981). There has been an increased effort in biological monitoring as a means of assessing biological integrity (Karr 1991). Biological monitoring of running waters is an essential application for assessing ecological risk, which enables human kind to mandate better management practices for sustaining healthy stream environments (Karr and Chu 1999). Streams are important sources of freshwater and although major conservation efforts have been implemented, methods for determining stream health is still poorly defined (Karr 1999).

Biological assessments have been developed for freshwater bodies as a means of assessing water and habitat quality (Barbour et al. 1999). Aquatic macroinvertebrates have become more commonly used in freshwater biological assessment and monitoring. Macroinvertebrates represent a diverse group of organisms that occupy a variety of niches within freshwater ecosystems. Moreover, there are many species, each differing in tolerance or sensitivity to disturbances, thereby collectively providing information about the quality of their habitat. Biological monitoring is an effective approach for assessing stream habitat quality because the sample community reflects disturbances that can be chronic or acute, whereas, physical or chemical monitoring often reflects only short time periods that may miss specific disturbance events (Chandler 1970). Furthermore, analyses from water samples can only target specific compounds that must be decided upon before analysis. Consequently, as more compounds are analyzed the more expensive the analysis becomes. Biological sampling requires fewer samples and

reflects the conditions that have affected the community over a period of time. Further, community trends over several years can be used to identify a point in time the quality of the habitat began to change.

The aim of this dissertation was to develop novel techniques and methodologies in benthic macroinvertebrate bioassessment studies towards the improvement or refinement of habitat quality measurements and assessments. Two projects were conducted, each addressing a separate set of objectives.

The first project (Chapter 3) involved the construction of a benthic macroinvertebrate Index of Biotic Integrity (B-IBI) in the north-central region of Missouri. Macroinvertebrate assemblages were poorly known from this region and reference condition streams (controls) were of compromised quality. The first objective of this project was to develop metrics (ecological measures that respond in a predictable fashion to disturbances) suitable for habitat evaluation in this region. The second objective was to construct a B-IBI for evaluating the effectiveness of Conservation Reserve Program (CRP) practices in restoring stream habitat quality. Grass filter strips had been shown to reduce sediments and potential nutrient loading (National Research Council 1993), but macroinvertebrate recovery had never been evaluated from these best management practices.

The second project (Chapter 4) was a case study of a single reference stream in central Missouri. The sampling regime was designed to capture the structure of the benthic macroinvertebrate community over seasons within a single year. The first objective of this study was to identify rare and transient taxa based on the belief that repeated sampling of a stream reach throughout a year would indicate which organisms are part of the sample community. Decisions based on whether rare taxa should be retained in bioassessments have been considered in both extremes (Faith and Norris 1989, Hawkins et al. 2000, Robinson et al. 2000, Carter and Resh 2001, Turak and Koop

2003), but these decision have not been based on whether the subjects are actually part of the community. The second objective was to identify metrics that express low variability over the annual continuum and to determine if they were predictable from a second season of sampling. The perfect metric would show the same value every time a sample was taken, regardless of the time of year and would only change in response to an alteration in the habitat quality. Because metrics do change with season, identifying environmental variables that can be associated with natural changes in the environment is important for calibrating metrics with regard to seasonal variability. The third objective was to examine whether weighting abundances by the maturity of each individual would have an influence on community measurements. The premise is that a less mature individual contributes less to the community compared to more mature individuals in terms of consumption and potential energy for other organisms. The last objective was to construct a predictive model based on biologically derived seasons. The model was used to construct community assemblages for test dates, and comparisons were made to the observed assemblages.

Benthic macroinvertebrate bioassessments are a common means of evaluating habitat quality of freshwater. However, progress and advancements of techniques in this science have been slow (Rosenberg and Resh 1993). Refinements of ecological measurements based on biology, development of new approaches in habitat evaluation, and a better understanding of the functional relationships between benthic macroinvertebrates and their environment will help future bioassessment studies to be more effective in identifying habitat quality.

CHAPTER 2

Literature Review

Macroinvertebrate biological assessments have existed for nearly a century (Kolkwitz and Marsson 1909). The earliest biological assessment techniques in the United States were in the form of surveys (Cairns and Pratt 1993), but have recently gained widespread national attention as an effective means for monitoring water quality (Plafkin et al. 1989). Towards the fulfillment of the Clean Water Act, the United States Environmental Protection Agency mandated that states monitor and provide biological information on their surface waters. All states have adopted some type of biological monitoring program, utilizing a variety of organisms. Benthic macroinvertebrates are the most commonly used organisms in lotic biomonitoring programs (Rosenberg and Resh 1993, Barbour et al. 1999) and there is a number of reasons why they are excellent for biological monitoring (Rosenberg and Resh 1993, Barbour et al. 1999, Flotemersch et al. 2006): First, they are ubiquitous, abundant, and diverse. Changes in water and habitat quality follow a replacement of one species with another and theoretically such a relationship can be plotted across a gradient, thus acting as a measure of overall quality. Macroinvertebrates are relatively easy to sample and there is an acceptable working knowledge of their taxonomy in developed countries. Benthic macroinvertebrates are generally sedentary in nature and are directly exposed to the conditions of a given area. Lastly, a variety of analytical applications have been developed for assessing habitat quality based on benthic macroinvertebrate community structure.

Although many bioassessment approaches exist, there are two main types of benthic macroinvertebrate biological assessments: multimetric and multivariate. Multimetric bioassessments evaluate benthic macroinvertebrate community structure with a series of ecological measures called metrics. Each metric is selected to represent

an attribute of the community (Barbour et al. 1995) that shows a value change in response to increased anthropogenic activities (Barbour et al. 1999). The metrics are aggregated into an index with scoring criteria based on a set of reference site metric values in what is known as a benthic macroinvertebrate Index of Biotic Integrity (B-IBI) (Karr 1991). With this assessment tool, test sites are scored by comparison of their metric values to the reference metric thresholds. Biocriteria thresholds are usually established for specified areas based on biogeographical classifications (Barbour et al. 1999). Such classifications may include ecoregions (Jessup and Gerritsen 2002, Hargett and Zumberge 2006, Montana Department of Environmental Quality 2006, North Carolina Department of Environment and Natural Resources 2006), drainage units (Sarver et al. 2002), elevation (Lawson 2005), or even sampling methods (Bode et al. 2002). These types of classifications are often referred to as *a priori* approaches because the regional assignments for stream classification are determined before data are analyzed.

Multivariate approaches to benthic macroinvertebrate bioassessment are based on reference sites sampled over a range of biogeographical regions (Wright et al. 1984). Ordination or cluster analysis is performed on the macroinvertebrate assemblages and in this way sample sites are biologically classified. Each site class is characterized by the occurrence frequencies of each taxonomic component of that class. Discriminant functions are created for each class from a set of environmental variables that are considered to have little influence from anthropogenic activities, such as longitude, latitude, and altitude (Flotemersch et al. 2006). Observed assemblages at test sites are compared to expected community assemblages that are predicted from the discriminant functions and the frequency distributions of those classes. In the case of the River Invertebrate Prediction and Classification System (RIVPACS), the discriminant functions are used to find the probability of a test site belonging to each of the classes.

The probabilities are multiplied by the taxonomic frequencies expected from each class and the observed taxonomic frequency is divided by the expected and the further the value is from 1 the more dissimilar, and thus degraded, the test site is considered to be. A model developed in Canada, known as the Benthic Assessment of Sediment (BEAST), is similar to RIVPACS except that the test site is associated with the most probable reference class and the comparison of the test site taxa is conducted in ordination space using probability ellipses based on the reference class taxa (Reynoldson et al. 1995). These types of approaches to bioassessment are referred to as *a posteriori* because the reference classifications for which test sites are compared are determined by the biota after they are enumerated.

One of the fundamental differences between the multimetric and multivariate approach is that the multimetric classifications are based on regional, usually geographic information, while the multivariate approach bases its classifications on biological information directly obtained from sampling. There are advantages and disadvantages to both methods (Reynoldson et al. 2001, Bonda et al. 2006), and trends in the bioassessment industry point towards the utilization of both methods for assessment. One of the fundamental similarities between these two methods is that they both rely on reference streams to determine biological criteria for test sites. However, a lack of good quality reference streams will hinder both of these approaches and represents a major problem for bioassessments in general (Hughes 1995).

In the absence of quality reference sites, researchers have devised ways to assess test site conditions by other means. Some studies have used the best score for each metric attained from all sample sites as the reference score (Fausch et al. 1984, Bozzetti and Schulz 2004). Others have used historical data as a means of reconstructing reference conditions that no longer exist (Hughes et al. 1998). Recently there has been a need to define reference condition as it relates to “naturalness”, separate from other

states of condition used for test site evaluation (Stoddard et al. 2006). Although the use of non-traditional reference conditions has been necessary for enabling test site assessment, best attainable conditions may not exhibit the ultimately desirable state of habitat quality. Thus, bioassessment models constructed with these types of “reference conditions” might be liberal in assessment of test sites; small improvements towards the actual desired condition at a test site will be assessed as large improvements if compared to references that were not themselves desirable.

Metrics require rigorous testing in order to provide the most meaningful understanding of their relationships with environmental factors (Norris, 1995). Many metrics are representations of community characteristics such as abundance, richness, and diversity. More precise metrics have been developed to focus on specific target groups within the community, such as functional feeding groups, and habitat preferences (Merritt and Cummins 1996). Other metrics are determined by incorporating each organism’s tolerance to impacts (Woodiwiss 1964, Chandler 1970, Cutter 1972, Hilsenhoff 1977, Zweig and Rabeni 2001). However, there are metric types and data modifications that have not been considered.

Multivariate approaches have only recently been applied to benthic macroinvertebrate bioassessments (Wright et al. 1984). As such, there are many opportunities to explore refinements and applications. Researchers have considered many approaches to classification (Clarke et al. 2003), strategies for selecting environmental variables for the discriminant function analysis (Van Sickle et al. 2006), expected test site allocation among reference sites (Reynoldson et al. 2001), and comparative levels for test site assessment (Hawkins et al. 2000). It has been suggested that the classification step could be removed entirely (Linke et al. 2005). Many research questions are available for exploration with this new technique in bioassessments.

Furthermore, the predicted community assemblage can be used in ways other than calculating overall richness.

Natural lotic systems in temperate regions are dynamic environments that contain benthic macroinvertebrate communities representing a wide variety of life histories. Many aquatic insect life histories and development rates are influenced by temperature (Ward and Stanford 1982, Ward 1992, Williams and Feltmate 1992) and thus are cued to different life stages as temperatures change with season (Vannote and Sweeney 1980). Resource partitioning may exist temporally due to thermal conditioning (Merritt and Cummins 1996), thus some species may replace other species leaving the system as terrestrial adults. Furthermore, measurements taken at one point in time may miss important events (weather related or otherwise) that could have had an affect on the community structure prior to sampling (Cooper and Barmuta 1993). Therefore, samples taken at different points in time may not be comparable. It is of interest to identify metrics that are least affected by seasonal changes because these measures will show more consistent assessment, regardless of season.

Biological assessment of benthic macroinvertebrates is still a developing science. Many opportunities exist for evaluating habitat quality in relation to community structure (Rosenberg and Resh 1993, Carter and Resh 2001). Exploration of new techniques, measurements, and approaches to bioassessment, coupled with improved understanding of macroinvertebrate life histories and how they are affected by the natural environmental conditions will contribute to the advancement of interpreting bioassessment results.

CHAPTER 3

A Least Desired Index for Assessing the Effectiveness of Grass Riparian Filter Strips in Improving Water Quality in an Agricultural Region

(This chapter has been published in *Environmental Entomology* 36: 713 – 724)

3.1 Abstract

Unprotected streams within the agricultural Midwest region of the United States are subject to sedimentation, nutrification, and agricultural chemicals. Grass Riparian Filter Strips (GRFS) have been implemented as a best management practice to minimize sedimentation and associated materials that are harmful to aquatic ecosystems, however, few studies have examined the benthic community response to GRFS installation. This study introduces a Least Desired Index (LDI) a multimetric approach of evaluating benthic communities in response to GRFS installation. LDI was determined in a reciprocal fashion to that of a benthic macroinvertebrate Index of Biotic Integrity (B-IBI). When pristine conditions are not available for the use of B-IBI, anti-reference sites, representing least desired conditions, can be used as a means of constructing an LDI. A B-IBI and LDI were constructed in the Claypan Till Plains Subsection of Missouri and comparatively used to evaluate two test sites where tall fescue GRFS were installed. Five metrics were used to develop the B-IBI and six for the LDI. The LDI tended to be more conservative at evaluation in comparison to the B-IBI. Paired t-tests showed that LDI and B-IBI were significantly different at scoring test sites. The LDI assessed both test sites as showing no response to GRFS installation, whereas the B-IBI suggested moderate improvement. The LDI was considered to be a better index for evaluation because reference sites used to develop the B-IBI were more variable compared to LDI and were considered to be impacted. The chironomid community produced some of the most useful metrics.

3.2 Introduction

Streams are an essential component of the environment, providing fresh water for much of the terrestrial biota. However, the quality of water may be compromised by anthropogenic activities which often degrade the ecological integrity of both aquatic and terrestrial systems. Some studies have shown that aquatic invertebrate community structures are altered by agricultural practices (Courtemanch and Gibbs 1980, Lenat 1984). Barton (1996) found that adjacent land use effects were most influential early in the growing season, and Schulz and Liess (1999) indicated that aquatic invertebrate numbers significantly decreased immediately after insecticide application on adjacent crops.

Small streams of the central Great Plains region historically have been regarded as poor fish and wildlife habitats, and therefore conservation measures have been neglected (Zale et al. 1989). Specifically, canalization, impoundment construction, riparian vegetation loss, and increased agricultural influence have resulted in the degradation of these systems (Zale et al. 1989). Effects on streams due to riparian vegetation loss include an increase in temperature, bank erosion, sedimentation, nutrient and pesticide inputs, and losses of stream woody debris and allochthonous materials (Roth et al. 1996).

Grass riparian filter strips (GRFS) have been promoted and implemented as a means of reducing sedimentation and associated nutrients and pesticides in streams (National Research Council 1993). Established buffer strips have been shown to successfully restore riparian wildlife habitat (Schultz et al. 1995), while acting as temporary sinks for excess phosphorus (Osborne and Kovacic 1993). Riparian buffer strips have been shown to influence water temperatures in Ontario trout streams as far as 2.5 km downstream, thus influencing fish communities (Barton et al. 1985). However,

few studies have investigated the response of benthic macroinvertebrates to these conservation techniques.

Biological monitoring of running waters is essential for assessing ecological risk, which enables better management for sustaining a healthy environment (Karr and Chu 1999), and is an effective means towards defining biotic integrity (Karr 1991). A benthic macroinvertebrate Index of Biotic Integrity (B-IBI) is a technique commonly used for monitoring running waters and relies on the inclusion of reference streams which are essential for the development of these programs (Barbour et al. 2000). However, in many regions of the world, good quality reference conditions do not exist, which presents a major obstacle in conducting biological assessments (Hughes 1995). Thus, researchers have devised ways to assess test site conditions by means other than traditional reference conditions. Some studies have used the best score for each metric attained from all sample sites as the reference score (Fausch et al. 1984, Bozzetti and Schulz 2004). Although this provides a suitable comparison to the least disturbed condition, there is no certainty that this is a condition that is ultimately desirable. Others have used historical data as a means of reconstructing reference conditions that no longer exist (Hughes et al. 1998). Unfortunately, historical data are not always available. Recently, “Dirty Water Models” have been constructed as a multivariate model for predicting how test sites should positively respond to remediation measures (Linke et al. 2002). Although these approaches provide a means for evaluating test sites, they do not offer direct comparison to streams within the region, or they represent a condition that may not be desirable. However, if test site comparison is based on streams that are of a ‘least desired condition’, then test site departure should be viewed as a positive response to an implemented conservation measure. Thus, a multimetric Least Desired Index (LDI) can be used as a means of evaluating test sites in regions that do not have appropriate reference conditions for comparison.

The objectives of this study were to: (1) determine if a LDI based on metric scores of 'least desired conditions' is an effective means of evaluating test stream habitat condition, and (2) evaluate the effectiveness of GRFS installation in agricultural settings.

3.3 Methods and Materials

3.3.1 Study Approach

When good quality reference conditions for developing B-IBI multimetric criteria are unavailable, streams used as reference conditions may not represent the 'best attainable condition' (Stoddard et al. 2006). Theoretically, if a set of streams within a region are known to be highly impacted, then establishing criteria based on metric scores of a 'least desired condition' may be used for direct test site comparison when assessing the effectiveness of a conservation measure or best management practice. In the same fashion that percentiles of reference streams are used to establish the upper limit of metric score criteria in B-IBI (for metrics that decrease with increased impact), a LDI uses 'anti-reference' streams to establish the lower limit of metric score criteria. Thus, test site metric scores that deviate from the criteria designated by anti-reference streams are indications of improvement.

For the presented study, traditional reference sites used for developing B-IBI were identified as stream reaches within Missouri state conservation areas and were considered 'least disturbed conditions' (Stoddard et al. 2006), based on the presence of continuous riparian vegetation and canopy cover. Agricultural sites without vegetative buffers were considered 'least desired conditions,' and were used as anti-reference sites for the development of an LDI. Comparison of metric scores was made between B-IBI and LDI in order to select suitable metrics for test site evaluation. Metrics were then screened for excessive variation and redundancy, separately for each index. Scoring

criteria were then determined for metrics using B-IBI and LDI, separately, and test sites with established GRFS were individually evaluated with each index.

3.3.2 Study Sites

This study was conducted in the north-central Missouri counties Audrain, Monroe, and Randolph (Fig. 1). This is a low gradient region and flow is generally not recordable except during times of heavy precipitation. Land use in these counties is dominated by agricultural practices, primarily of cattle pastures and row crops of corn or soybean.

The reference sites were at Perche Creek, 39° 15' 20" N by 92° 25' 18" W; Skull Lick, 39° 13' 9" N by 92° 1' 4" W; and Young's Creek, 39° 20' 2" N by 91° 52' 11" W, in the Rudolf Bennitt, Clarence L. Northcutt Memorial, and Robert M. White II conservation areas, respectively. Each conservation area is managed by the Missouri Department of Conservation. Agricultural sites without a riparian buffer were located at Crooked Creek, 39° 36' 25" N by 92° 2' 16" W; Long Branch, 39° 20' 47" N by 92° 2' 37" W; and Otter Creek, 39° 36' 22" N by 92° 10' 10" W.

Two sites with previously established GRFS were used as test sites. Goodwater Creek, 39° 17' 49" N by 92° 7' 9" W, was seeded in the spring of 2000 with tall fescue totaling 5.58 ha. The GRFS was distributed unevenly on both sides of the stream due to meanders and existing trees. Thus, the width ranged from 2 to 33 m. The GRFS at Long Branch, 39° 18' 3" N by 92° 11' 42" W, also was seeded in the spring of 2000 with tall fescue, and the distribution was even on both sides of the stream, totaling 9.3 ha along the main trunk. The width of the GRFS was approximately 36 m. Both test sites occurred in the headwaters of watersheds with streams that were used as reference or anti-reference. However, Long Branch and Goodwater test sites were considered independent of the anti-reference and reference sites because they were 23.5 and 25.0 km, respectively, upstream. Thus, because of the imperceptible flow of streams in this region, biotic and abiotic influences between sites were considered negligible.

Each sample site was fed by numerous intermittent streams, thus sample sites were considered to be second order. All sites, except Perche Creek and Young's Creek, were within the Claypan Till Plains Subsection of the Central Dissected Till Plains ecoregion of Missouri (Omernik 1987, Nigh and Schroeder 2002). However, these two outliers were very close: Perche Creek was located 3.4 km into the Outer Ozark Boarder Subsection, part of the Ozark Highlands, and Young's Creek was located 1.3 km into the Mississippi River Hills Subsection of the Central Dissected Till Plains.

3.3.3 Sampling and Identification

Sampling of invertebrates was conducted at three different seasons. Specifically, samples were taken during two day intervals in spring on 4 and 5 May, summer on 22 and 23 June, and fall on 31 August and 1 September, 2002. Because new monitoring programs should be based on only one habitat type (Resh 1995), invertebrates were sampled only from stream margins with a water pump sampling device after Brown et al. (1987) modified by Wood (1999). The sampler isolated a 0.05 m² area and was run for 3 minutes while the substrate within was agitated with a potato rake. The water was filtered through a bag with 24 x 20 per inch mesh. Seven samples were taken at each site, preserved in the field with 95% ethanol, and taken to the laboratory for sorting and identification to the lowest taxonomic resolution possible. Three measures each of dissolved oxygen (mg/l and percent), conductivity, specific conductance, water temperature, and pH were taken at random locations at each site with field meters. Whole counts of macroinvertebrates were taken for all samples. Chironomid larvae were cleared with 10% KOH for ca. 12 hr, then transferred to a bath of 100% ethanol, and slide mounted with Euparal.

3.3.4 Analysis

The 7 samples from each site were numerically pooled by season. Functional feeding group and tolerance values were assigned to each taxon with data obtained from the

Missouri Department of Natural Resources (Sarver 2001) and other sources (Bode et al. 1991, Merritt and Cummins 1996, Barbour et al. 1999). A total of 24 macroinvertebrate metrics was calculated from the data (Table 1). Metrics that were not normally distributed over all seasons were square root transformed to enable parametric testing. Two-way ANOVA was used for each metric to test for differences between reference and anti-reference sites and among seasons. Interaction would indicate that seasonal variability is not the same for both land uses and thus not a suitable metric. Metrics that still did not meet criteria for parametric testing were rank transformed and analyzed with the Friedman test.

Discriminant function analysis (DFA) was used to find group classification of conservation and agricultural sites simultaneously for each season using environmental data as predictors. A separate DFA was used to find group classification of conservation, agricultural, and test sites with all seasons combined.

3.3.5 Metric Selection, Scoring and Index Development

Metric values were assessed with data pooled from all seasons. Metrics that were significantly different between reference and anti-reference sites were further scrutinized for redundancy and excessive variation in order to select metrics that were most appropriate for test site evaluation. Specifically, separate correlation matrices for metrics of reference and anti-reference sites were used to eliminate redundant measures at $P < 0.05$. Metrics with a coefficient of variation (CV) of 50 or higher were considered too variable and eliminated, and metrics containing a zero value within their range were eliminated.

After metrics were screened, two indices were developed; a B-IBI using reference streams and a LDI using anti-reference streams. For B-IBI, the 5th and 95th percentiles of reference stream metrics were used to establish the maximum score limits for metrics that increase and decrease, respectively, with impact. Conversely for LDI, the 95th and

5th percentiles of anti-reference stream metrics were used to establish the minimum score limits for metrics that increase and decrease, respectively, with impairment. After limits were set, the remaining area was equally divided by bisection and the new areas were given appropriate scores. Thus, for the hypothetical metric that decreases with impact (Fig. 2), the maximum limit of the highest score (5) for B-IBI is set by the 95th percentile of the reference sites (shown at 90) and the remaining scores (3 and 1) are established by divisions at 60 and 30. For the same metric, the 5th percentile of the anti-reference is used to set the minimum limit of the lowest score (1, shown at 10), and the remaining scores (3 and 5) are established by divisions at 40 and 70. Test site metrics that occur beyond these limits would score only the maximum or minimum, respectively for B-IBI or LDI.

Theoretically, of the metrics that are statistically different between reference and anti-reference, and increase with impact, the upper limit of the reference and the lower limit of the anti-reference should approximate, except in cases of extreme disturbance. The inverse should be true for those metrics that decrease with perturbation. Thus, there is assurance that there will be little overlap between reference and anti-reference given that metrics with overlapping variances will not be significantly different as analyzed with ANOVA.

3.3.6 Assessment of Test Sites and Index Comparisons

After test site scores were tallied for B-IBI and LDI separately, test sites were evaluated using a composite condition score criterion for GRFS effectiveness with each index. Resulting categorical test site conditions were then compared. Paired t-tests were used to test for differences between reference and anti-reference coefficients of variation. Additionally, paired t-tests were used to test for departure from zero between reference and anti-reference test site scores, for each test site, separately. All calculations and statistical tests were performed using SAS (SAS Institute 2001).

3.4 Results

3.4.1 Study Sites

During the sampling periods, a considerable amount of filamentous algae with dense populations of Cladocera was evident in the agricultural sites, and very little in the conservation sites. DFA classification showed a clustering of reference sites for summer and fall, anti-reference sites for summer and fall, and both land uses together for spring samples (Fig. 3). Functions 1 and 2 accounted for 68.0 and 21.5% of the variation, respectively.

Plots of the land use sites in 2-dimensional DFA space showed distinct groupings of reference, anti-reference, and test sites (Fig. 4). DFA correctly classified 100% of the anti-reference sites, 88.9% of the reference sites, and 100% of the test sites for the combined seasonal groupings. Functions 1 and 2 accounted for 60.3% and 39.7% of the variation, respectively.

3.4.2 Metric Selection, Scoring, and Index Development

Of 24 metrics tested (Table 1), nine were significantly different between conservation and agricultural sites (Tables 1, 2). A 9 x 9 correlation matrix was then constructed for conservation and agricultural sites, separately (Table 3).

For LDI metric selection, Tanytarsini/Chironomidae and percentage Tanytarsini were eliminated due to high variation and zero scores (Table 2). The correlation matrix (Table 3) showed that percentage Chironomini was significantly correlated with percentage Chironomidae and Tanypodinae/Chironomidae, thus percentage Chironomini was eliminated. All other metrics were retained for inclusion in the LDI.

Metric selection for the B-IBI was not as simple. The metrics Tanypodinae/Chironomidae, percentage Tanytarsini, percentage Oligochaeta, and *Procladius*/Chironomidae exhibited CV values greater than 50 (Table 2). The

correlation matrix (Table 3) revealed strong relationships between percentage Chironomina with percentage Chironomidae, and Chironomidae biotic index with biotic index. The two remaining metrics were not considered sufficient to constitute a suitable index, therefore, in cases in which two metrics were correlated with each other and no others, the metric with the lower CV was retained for inclusion in the B-IBI. Thus, Tanyptodinae/Chironomidae, percentage Tanytarsini, and percentage Oligochaeta were additionally used to develop the B-IBI, disregarding excessive variability or zero values.

As described above, the scoring range for each metric was assigned equal divisions by bisection from the maximum and minimum established values for B-IBI and LDI, respectively (Fig. 5). Some metrics, such as percentage Chironomidae, exhibited similar score ranges resulting from reference and anti-reference streams, whereas others, such as percentage Tanytarsini, widely differed in score ranges between reference and anti-reference sites.

3.4.3 Assessment of Test Sites and Index Comparisons

B-IBI comprised 5 metrics for test site comparison, thus 5 and 25 were the lowest and highest possible scores, respectively. Goodwater scored 11 and Long Branch scored 13 (Table 4). Suggested habitat conditions indicates that Goodwater has not shown any improvement after GRFS installation, whereas Long Branch has shown moderate improvement (Table 5).

LDI comprised 6 metrics for test site comparison with 6 and 30 as the lowest and highest possible scores, respectively. Goodwater and Long Branch each scored 8 (Table 4), indicating that neither site has shown any positive improvement to GRFS installation (Table 5).

Paired t-tests did not show that reference and anti-reference metric CVs were significantly different from zero ($t = 1.38$; $df = 8$; $P = 0.204$). However, paired t-tests of metric scores for Goodwater ($t = 3.16$; $df = 8$; $P = 0.013$) and Long Branch ($t = 2.53$; $df =$

8; $P = 0.035$) (Table 6) indicated a significant departure from zero when comparing score differences between references and anti-references for all nine metrics, thus suggesting that LDI was more conservative at scoring test sites. By the process of metric selection described above, it is clear that more correlations were significant between metrics within the reference group than within the anti-reference group (Table 2).

3.5 Discussion

3.5.1 Study Sites

Because of the non-flowing nature of these streams, the community inhabiting these systems tends to be represented by species characteristic of lentic habitats. Lack of current and limited invertebrate mobility tend to negate the physical connectivity between stream reaches. DFA reinforced the notion that test sites were not similar to either reference or anti-reference streams (Fig. 4), and therefore are unlikely pseudo-replicates of downstream sites.

The greater amounts of algae at agricultural sites can be explained by two environmental attributes: First, the reference sites tended to be well shaded whereas anti-reference sites were not. Second, anti-reference sites were open to nutrient inputs from pastures and row crops, whereas the reference sites had a buffer of trees and other vegetation.

3.5.2 Metric Selection, Scoring, and Index Development

The protocol for this study required three separate seasons of sampling for index development and test site evaluation. It is important to partition data by season to avoid temporal pseudo-replication (Hurlbert 1984). By partitioning the data as such, variation due to seasonal effects is accounted for and the power of the test is increased.

Seven of the nine metrics that differed between reference and anti-reference sites were based on aspects of richness or abundance of Chironomidae. Rabeni and Wang

(2001) suggested that removing this family altogether from biological assessment does not decrease sensitivity of the evaluation, at least for the four metrics they tested. However, Wymer and Cook (2003) highlighted the importance of including genus-level resolution of Chironomidae when studying the structure of aquatic insect communities. Results reported herein clearly show that midges are an important part of streams in agricultural landscapes and should not be disregarded, which agrees with the findings of other studies (e.g., Barton 1996). Additionally, the aquatic midge community may be a direct indicator of riparian vegetation; Delettre and Morvan (2000) have associated high adult midge richness and abundance with riparian vegetation.

Most of the metrics used for index development here have been commonly used in other bioassessments (Resh and Jackson 1993, Barbour et al. 1999). However, Tanypodinae/Chironomidae and *Procladius*/Chironomidae were developed for this study from direct observation of their abundance within the samples. Further consideration of their use and ecological significance is warranted.

The chironomid subfamily Tanypodinae is considered a pollution tolerant group (Lenat 1993), useful for identifying grades of eutrophication (Saether 1979), and has been used as a metric in other biological monitoring programs (Llansó 2002). The chironomid genus *Procladius* is apparently adapted to eutrophic conditions (Wiederholm 1980), but has been shown to be limited by extremely low oxygen concentrations (Berg et al. 1962). Paleolimnological studies have also associated *Procladius* with disturbances caused by anthropogenic activities (Porinchu and McDonald 2003). A more in-depth ecological explanation of the success of metrics based on these groups may be elucidated by understanding chironomid feeding preferences. *Procladius* is known to scour the surface of the substrate, preying on microcrustaceans, oligochaetes and other fauna (Thut 1969, Berg 1995), and has been reported as the main invertebrate predator in Oligochaeta dominated habitats (Edmonds

and Ward 1978). Other studies have shown *Procladius* to be distributed with microcrustaceans (Cardinale et al. 1998) and decrease in abundance with a decrease in abundance of oligochaetes (Lang 1998).

During all sampling seasons, the mean percentage of oligochaetes (primarily tubificids) was significantly greater at anti-reference than at reference sites. In addition, although numbers of microcrustaceans were not considered in this study, Cladocera were noticeably more prevalent at anti-reference than at reference sites. Oligochaetes have been associated with nutrient richness (Edmonds and Ward 1979, Johnson et al. 1993) and sedimentation (Robbins et al. 1989) and some Cladocera taxa are noted to favor algal habits (Whiteside et al. 1978), which may explain why these taxa are in large numbers at non-buffered agricultural sites. Thus, it is likely that *Procladius* and other Tanypodinae take advantage of an increased prey population and may be 'top-down' indicators of nutrification. That is, these taxa are present in large numbers due to increased abundance of tubificids and microcrustaceans, which are in large numbers in part because of a great abundance of algae and sedimentation, which is in great abundance due to nutrification, direct sunlight, and poor buffering capacity of the riparian zone.

A distinct partitioning of certain chironomid taxa was observed between the reference and anti-reference streams. Specifically, Tanypodinae, especially *Procladius*, tended to favor agricultural streams, whereas members of the tribe Tanytarsini favored conservation sites. When a metric of one of these types was selected as an acceptable measure for index development for one land use, it was eliminated from consideration for the other land use because it exhibits high variation accompanied by zero values. However, these metrics can still be considered because a zero value of Tanypodinae/Chironomidae at a test site is viewed as a positive departure from an

agriculturally perturbed habitat, as are zero values of percentage Tanytarsini as an indication of no improvement toward reference condition.

3.5.3 Assessment of Test Sites and Index Comparisons

Both test sites were similar to each other with regard to overall evaluation with LDI in that neither exhibited evidence of recovery. In contrast, B-IBI assessed Long Branch as showing moderate recovery, whereas Goodwater Creek was considered somewhere between moderate recovery and no recovery. Both streams scored higher with B-IBI than with LDI. Although paired t-tests did not detect differences in overall metric variability between reference and anti-reference CVs, individual reference metrics had high CVs, and thus were not appropriate for index development (Barbour et al. 1999). Considering the liberties that had to be taken with the development of the B-IBI (e.g., inclusion of highly variable metrics), the GRFS in this study do not appear to have been effective at restoring habitat quality in the 2 years since establishment. Schmitt et al. (1999) showed that 25 year-old filter strips were more effective at preventing sedimentation than were 2 year-old filter strips.

Test site scores generated by B-IBI were more generous than those by LDI. The performance of these metrics in relation to the given study sites can be viewed in two contrasting ways: First, the use of anti-reference sites and the development of LDI is very conservative, and perhaps more liberal criteria should be used for designating metric scores. Second, the LDI is sufficient and the B-IBI is not adequately adapted for assessment in this region. ‘Least disturbed conditions’ used as reference streams in this study may not have been appreciably different in quality from unprotected habitats within the region. Thus, only slight improvements in test sites might be misevaluated with B-IBI as strong improvements. Although statistical differences were detected between metrics of reference and anti-reference streams, a desired habitat condition may

have much greater (or lower) metric values than those that were obtained for reference sites in this study.

Our view follows the second line of thinking in that the LDI is not excessively conservative. The conservation areas used for reference sites were only small fragments in an agriculturally dominated landscape. Even though the streams were located within a buffer of trees and other vegetation, they are influenced by anthropogenic practices and are probably themselves impacted to a degree. Therefore, the test sites are being evaluated with impacted reference sites, obscuring the assessment. In contrast, the LDI is making comparisons only to a worst case scenario and, therefore, it acts as a more realistic model. Because, there is no maximum limit for the LDI, this index may become conservative for metrics that approach zero. Furthermore, some metrics may be bimodal. For instance, percentage Chironomidae is typically thought of as a metric that increases with disturbance, but in this region, it decreased with increased disturbance. It is likely that as physical improvements are made at a site with least desired conditions, percentage Chironomidae will increase, but at some upper threshold, the community structure will shift so that percentage Chironomidae decreases with improvements.

In addition to the problems associated with reference stream quality, the degree of impact experienced by each reference site is unique and partially explains the high variability among reference metrics. Even more, each reference site occurred in a different Ecoregion Subsection, thus contributing to variability. Granted that Perche Creek and Young's Creek are on the cusp of the Claypan Till Plains Subection, these Subsections are derived from physical characters, potential natural vegetation and soils (Nigh and Schroeder 2002), and reasonably have an influence on the assembly of the biota found there. Resh et al. (2000) have shown that local conditions can have extreme effects on variability between sites. All of the anti-reference sites were within the Claypan Till Plains Subection (although Crooked Creek is 3.2 km from the Mississippi

River Hills Subsection) and thus exhibited less metric variability than did the reference sites. Considering that there were no other natural areas close to the study region, this investigation demonstrated that the application of using anti-reference streams for assessing test site responses to remediation might be the only adequate means of evaluation.

3.6 Conclusions

A LDI is a suitable means of evaluating GRFS in the Claypan Till Plains Subsection of the Central Dissected Till Plains ecoregion within Missouri, even though results of this study indicated that GRFS did not result in improvement to test sites.

Although LDI does not identify a desired condition for test sites to achieve, it enables direct comparison with an undesired condition which test sites should not emulate. It is important to know the spectrum of metric scores that can be attained in an area. Knowing the ranges of both best attainable conditions and least desired conditions will give managers the most power when trying to define environmental standards. Index development using reference condition streams alone is the next best choice. However, if reference streams are not available or are of undesirable quality, then the use of anti-reference sites to establish metrics for LDI is sufficient, and sometimes better, for monitoring and assessing test sites. In areas in which there is a lack of reference streams, there probably is no shortage of candidate anti-reference streams.

Finally, Chironomidae appear to be a key taxonomic group for identifying habitat conditions within this region, as these sites tend to resemble ponds more than streams. Future studies might consider using midge pupal exuviae as an easier alternative, because they require less sorting time and are relatively easy to identify (Ferrington et al. 1991). Finer taxonomic resolution and enumeration of Oligochaeta and

microcrustaceans, respectively, might also result in more effective metrics and indices for use in these ecosystems.

Table 1. Metrics tested for differences between reference and anti-reference sites.

Biotic index *
 Chironomidae richness
 Chironomidae biotic index *
 Diptera richness
 EPT richness ^b
 EPT/Chironomidae
 Pentaneurini/Chironomidae
 Percentage *Caenis*
 Percentage Chironomidae *
 Percentage Chironomini ^a *
 Percentage Diptera and non-insects
 Percentage Ephemeroptera
 Percentage filterers ^a
 Percentage Oligochaeta *
 Percentage predators
 Percentage scrapers ^a
 Percentage shredders ^a
 Percentage Tanytarsini *
Procladius/Chironomidae ^a *
 Shannon diversity index
 Tanypodinae/Chironomidae *
 Tanytarsini/Chironomidae ^a *
 Total richness
Total richness without Chironomidae
 * significant difference (P < 0.05)
^a square root transformed
^b Friedman test

Table 2. Redundancy, excessive variation, zero values, and ANOVA results of the nine metrics that significantly differed between reference and anti-reference streams.

	No. of Correlations		Coefficient of Variation				Zero Values		ANOVA^a		
	reference	anti-reference	reference	anti-reference	reference	anti-reference	reference	anti-reference	F	df	P
Biotic index	2	0	8.40	3.55	no	no	no	no	18.67	1	0.0010
Tanypodinae/Chironomidae	1	1	98.03	36.80	yes	no	no	no	15.77	1	0.0019
Chironomidae biotic index	3	0	10.40	5.53	no	no	no	no	20.83	1	0.0007
Percentage Oligochaeta	1	0	80.92	35.03	no	no	no	no	9.08	1	0.0108
<i>Procladius</i> /Chironomidae ^b	2	0	101.57	21.10	yes	yes	no	no	16.23	1	0.0017
Percentage Chironomidae	1	1	19.90	21.33	no	no	no	no	20.88	1	0.0006
Percentage Tanytarsini	1	1	70.15	96.41	no	no	yes	yes	15.77	1	0.0019
Tanytarsini/Chironomidae ^b	2	1	42.24	63.01	no	no	no	yes	5.35	1	0.0392
Percentage Chironomini ^b	1	2	35.74	31.37	no	no	no	no	12.73	1	0.0039

^a (P < 0.05)

^b square root transformed

Table 3. Correlation matrices for B-IBI (A) and LDI (B) metric selection.

A									
	Tanypodinae/ Chironomidae	Chironomidae biotic index	Percentage Oligochaeta	<i>Procladius</i> / Chironomidae ^a	Percentage Tanytarsini	Percentage Chironomidae	Percentage Tanytarsini	Tanytarsini/ Chironomidae ^a	Percentage Chironomini ^a
Biotic index	- 0.164	0.827**	0.597	- 0.014	0.130	0.865**	0.093	0.568	
Tanypodinae/ Chironomidae	---	- 0.344	- 0.349	0.846*	- 0.106	0.011	0.059	- 0.221	
Chironomidae biotic index	---	---	0.829**	0.037	- 0.215	0.699*	- 0.275	0.141	
Percentage Oligochaeta	---	---	---	- 0.101	- 0.261	0.665	- 0.314	0.009	
<i>Procladius</i> / Chironomidae ^a	---	---	---	---	- 0.448	0.042	- 0.303	- 0.466	
Percentage Chironomidae	---	---	---	---	---	0.092	0.982**	0.681*	
Percentage Tanytarsini	---	---	---	---	---	---	0.056	0.489	
Tanytarsini/ Chironomidae ^a	---	---	---	---	---	---	---	0.624	

B									
	Tanypodinae/ Chironomidae	Chironomidae biotic index	Percentage Oligochaeta	<i>Procladius</i> / Chironomidae ^a	Percentage Tanytarsini	Percentage Chironomidae	Percentage Tanytarsini	Tanytarsini/ Chironomidae ^a	Percentage Chironomini ^a
Biotic index	0.353	0.238	0.281	0.532	- 0.391	- 0.423	- 0.341	- 0.371	
Tanypodinae/ Chironomidae	---	0.399	- 0.019	0.796*	0.143	- 0.079	0.224	- 0.359	
Chironomidae biotic index	---	---	0.089	0.343	- 0.270	- 0.285	- 0.202	- 0.138	
Percentage Oligochaeta	---	---	---	- 0.013	0.514	0.064	0.274	- 0.310	
<i>Procladius</i> / Chironomidae ^a	---	---	---	---	- 0.239	- 0.233	- 0.303	- 0.793*	
Percentage Chironomidae	---	---	---	---	---	0.347	0.899**	0.135	
Percentage Tanytarsini	---	---	---	---	---	---	0.127	0.183	
Tanytarsini/ Chironomidae ^a	---	---	---	---	---	---	---	0.406	

* , P < 0.05; ** , P < 0.01; ^a square root transformed

Table 4. Test site scores for B-IBI and LDI.

B-IBI		
Metric	Goodwater	Long Branch
Biotic index	3	3
Tanypodinae/Chironomidae	3	3
Percentage Chironomidae	1	1
Percentage Oligochaeta	3	5
Percentage Tanytarsini	1	1
Total	11	13

LDI		
Metric	Goodwater	Long Branch
Biotic index	1	1
Tanypodinae/Chironomidae	1	1
Chironomidae biotic index	1	1
Percentage Chironomidae	1	1
Percentage Oligochaeta	3	3
<i>Procladius</i> /Chironomidae	1	1
Total	8	8

Table 5. Suggested GRFS test site condition classification for B-IBI and LDI scores.

B-IBI Score	LDI Score	Description
5 – 9	6 – 12	Benthic habitat quality poor; no recovery apparent in response to GRFS.
13 – 17	15 – 21	Some quality benthic habitat; GRFS has promoted moderate recovery.
21 – 25	24 – 30	GRFS is effective; benthic community has recovered to least impaired habitat quality.

Table 6. Mean metric values, standard deviation (SD), sample size (n), and metric scores with B-IBI and LDI at two test sites, and metric response to impact.

Metric	Goodwater				Long Branch				metric response
	mean	SD	n	B-IBI LDI	mean	SD	n	B-IBI LDI	
Biotic index	8.44	0.35	3	3 1	8.41	0.36	3	3 1	increase
Tanypodinae/Chironomidae	42.05	14.00	3	3 1	56.19	14.69	3	3 1	increase
Chironomidae biotic index	8.46	0.33	3	3 1	8.51	0.30	3	3 1	increase
Percentage Oligochaeta	38.05	20.32	3	3 3	31.97	5.99	3	5 3	increase
<i>Procladius</i> /Chironomidae ^a	6.29	1.19	3	3 1	6.78	2.18	3	1 1	increase
Percentage Chironomidae	19.90	12.56	3	1 1	4.83	2.18	3	1 1	decrease
Percentage Tanytarsini	4.39	3.29	3	1 1	0.53	0.17	3	1 1	decrease
Tanytarsini/Chironomidae ^a	4.40	1.31	3	5 3	1.64	2.84	3	1 1	decrease
Percentage Chironomini ^a	2.29	0.58	3	1 1	1.23	0.40	3	1 1	decrease

^a square root transformed

Figure 1. Locations of study sites.

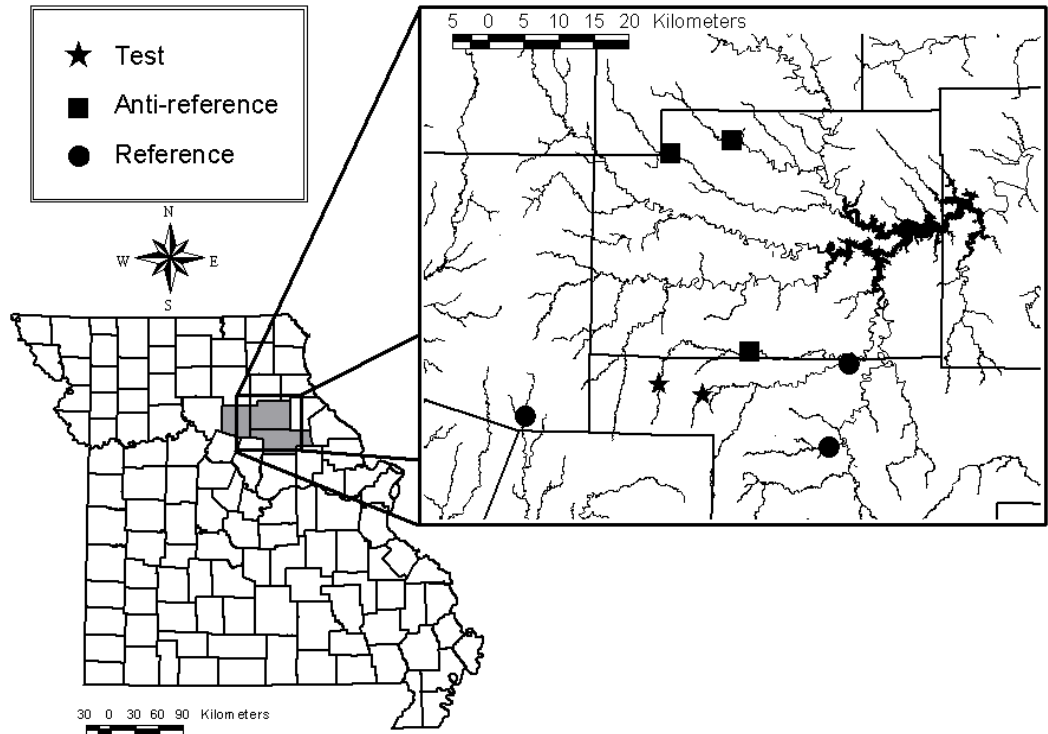


Figure 2. Scoring criteria for a metric that decreases in response to increasing impact. The end of the top and bottom whiskers represent the 95th and 5th percentiles, respectively. The top and bottom of the box represent the 75th and 25th percentiles, respectively. The dot in the middle of the box represents the median. For reference streams, the 95th percentile determines the maximum limit for this metric. For anti-reference streams, the 5th percentile determines the minimum limit.

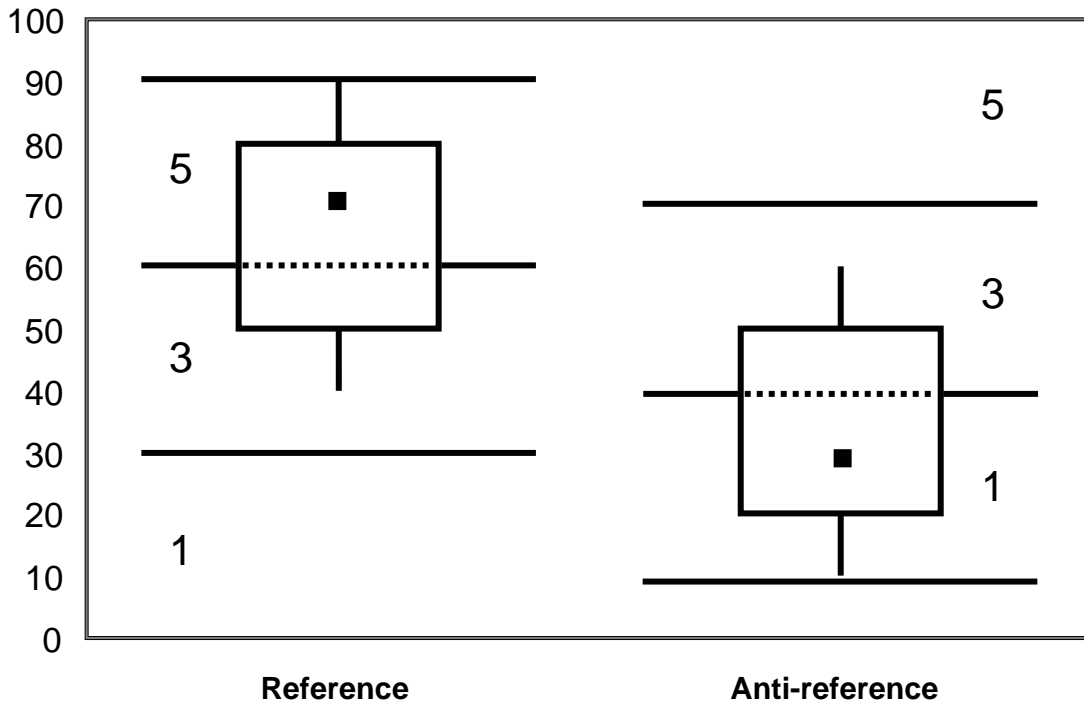


Figure 3. Scatter plots of reference and anti-reference sites for all seasons in 2-dimensional DFA space derived from environmental parameters. Open and filled symbols represent reference and anti-reference sites, respectively. Triangles, circles, and squares represent spring, summer, and fall, respectively.

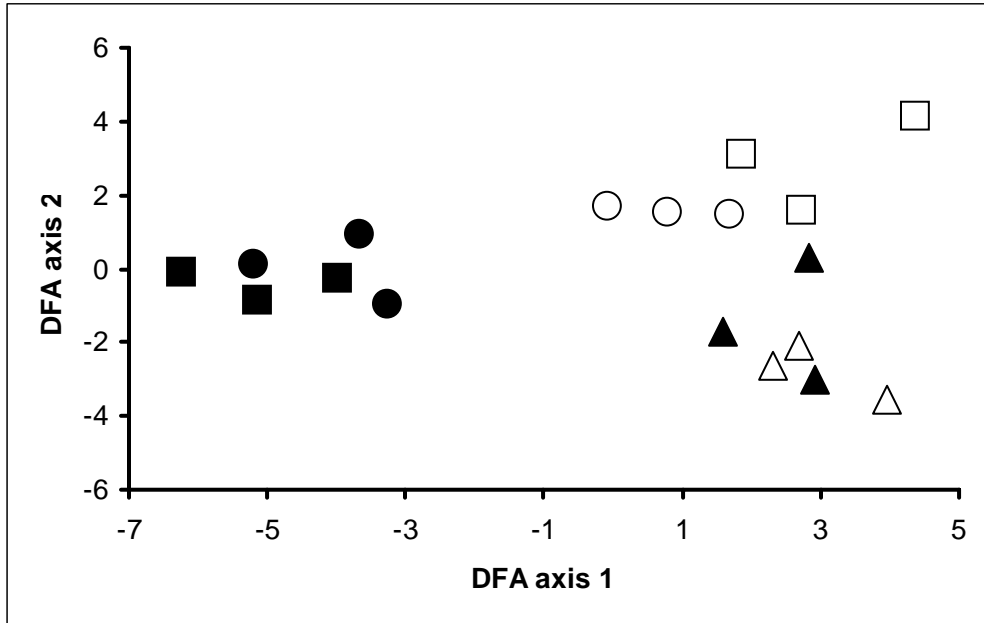


Figure 4. Scatter plots in 2-dimensional DFA space derived from environmental parameters for all seasons. Reference, anti-reference, and test sites are represented by circles, squares, and triangles, respectively.

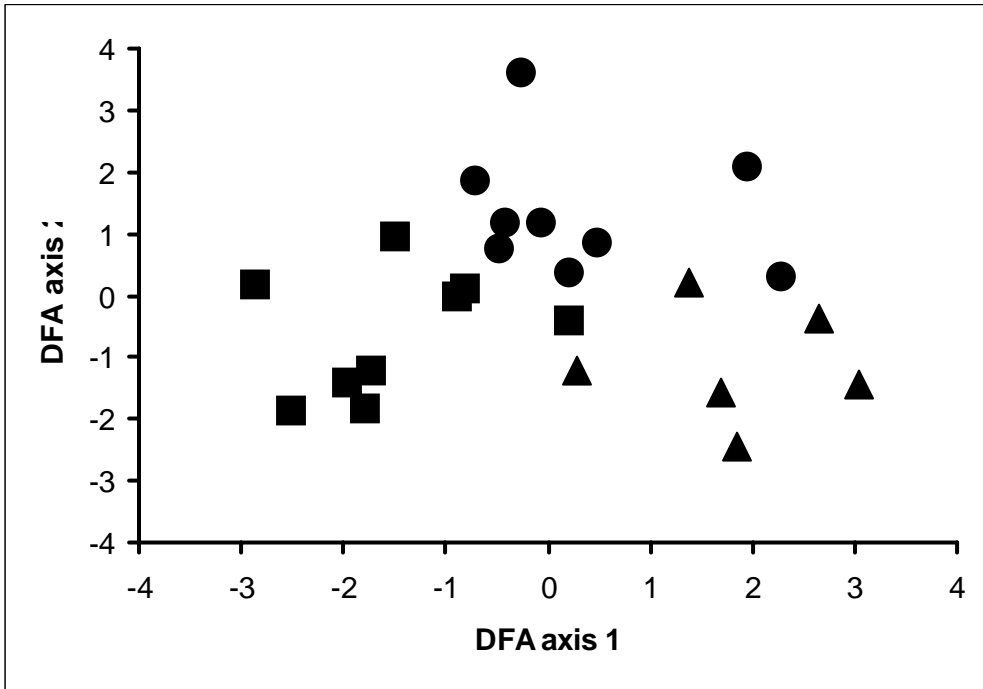
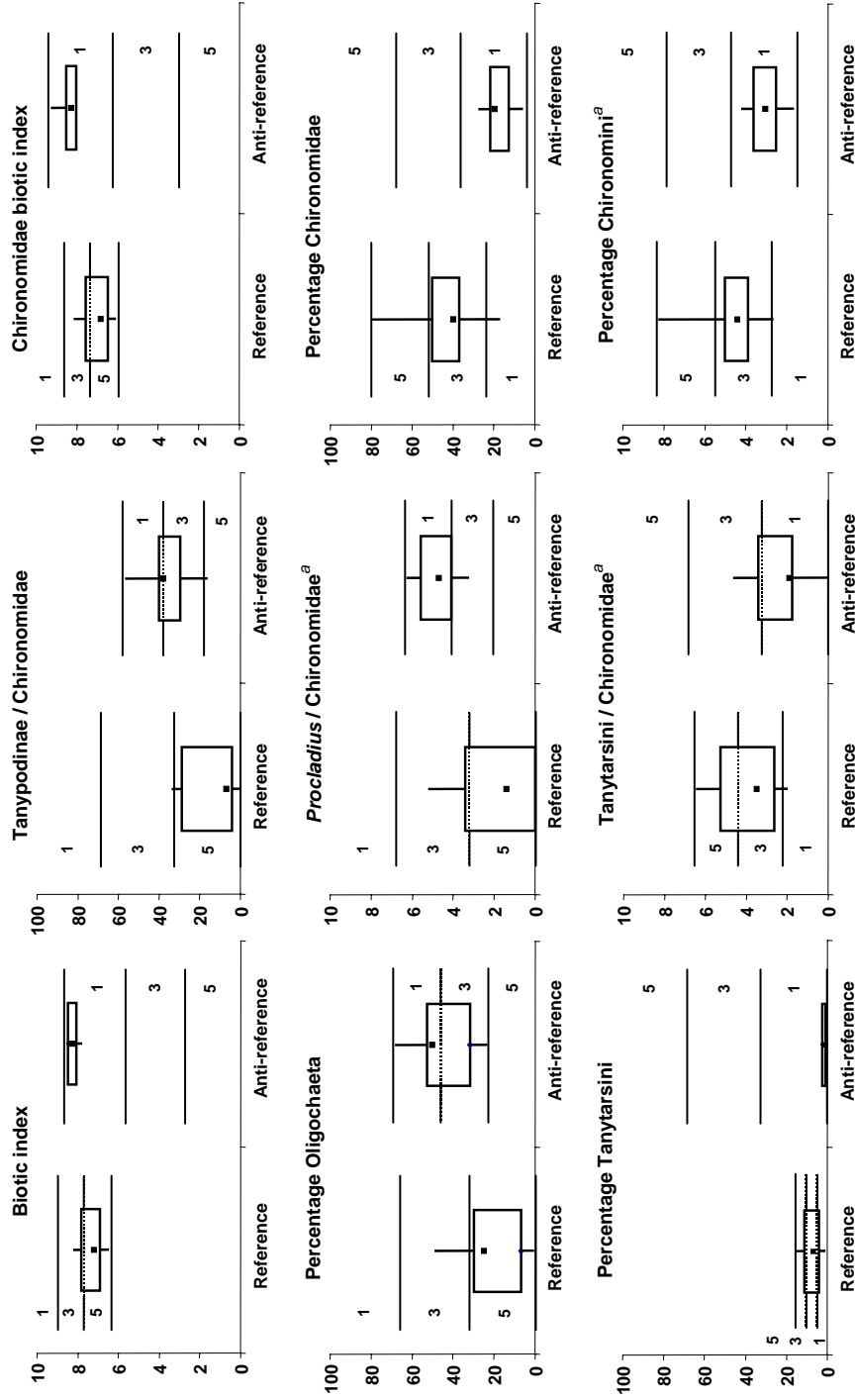


Figure 5. Reference and anti-reference scoring criteria for nine metrics. Square root transformed metrics are indicated by 'a'.



CHAPTER 4
Seasonal Predictability of a Benthic Macroinvertebrate Community in an Ozark Stream in Central Missouri, USA

4.1 Abstract

Benthic macroinvertebrates living in lotic habitats are influenced by a wide range of physical and chemical environmental factors that change over a temporal continuum. Within a year, different species can occupy the same space at different points in time. Thus, the community structure itself is in flux from season to season. This study focused on the structure of a riffle community in a single stream from a series of samples taken ca. monthly from December 2002 to December 2003. In addition to identifying sampled organisms, the maturity of each specimen was also considered. From this design, four objectives were addressed: 1) identification of rare and transient taxa; 2) usefulness of maturity data in analyzing community structure; 3) specific community measures that were least variable over the continuum and their predictability during a second year; 4) the construction of a River Invertebrate Prediction and Classification System (RIVPACS) model that classifies seasons based on biota and predicts an expected community at any point in time. Rare and two types of transient taxa were defined from observations of taxa over time. Rare taxa were considered residents of the community not properly accounted for by the sampling technique. Transient type I taxa were considered adventitious, possibly immigrating from nearby source habitats and transient type II taxa were short lived. Maturity data were useful in characterizing types of transient taxa and for weighting abundances. Metrics with standard abundances compared to their maturity weighted analogues revealed that measures based on finer taxonomic resolution or functional groups were more likely to differ. Paired t-tests showed that maturity weightings significantly modified the value of some metrics over the temporal continuum. Nine metrics representing five measurement categories displaying low variability over the annual continuum were selected for multiple regression analysis.

The modified MO Biotic Index was fairly predictable between years, regardless of season, whereas other measures were less so. Three seasons were biologically determined from cluster analysis toward the development of a RIVPACS model. Discriminant Function Analysis utilized temporal environmental variables to determine test date group membership. Comparisons of expected to observed communities revealed that 1 of 3 test dates were predicted fairly well by the model. Short term temporal changes within a single community offer challenging perspectives in benthic macroinvertebrate research. Definite patterns exist that can be observed and at times predicted. Greater consideration should be given to the short-term temporal dimension in lotic insect ecology.

4.2 Introduction

Aquatic macroinvertebrate communities in temperate lotic systems are influenced by seasonal weather patterns, annual disturbance regimes, and other dynamic forces. Due to seasonal changes in thermal conditions, resources become temporally partitioned (Cummins and Merritt 1996). Many aquatic insect life histories and development rates are influenced by temperature (Vannote and Sweeney 1980, Ward 1992, Williams and Feltmate 1992) and other physico-temporal factors (Wohl et al. 1995, Robinson and Minshall 1998). Annual precipitation has been shown to be a significant factor influencing community structure from year to year (McElravy et al. 1989). Differences in disturbance rates can dictate the number and types of species (obligate vs. specialist) that may coexist within a habitat (Ward 1989). Stream 'patches' change in quality over time (Wiens 2002) and thus, measurements taken at one time point may miss important events that could have affected the community prior to sampling (Cooper and Barmuta 1993). Thus, information related to lotic macroinvertebrates might be better understood

by evaluating temporal shifts in the community structure with regard to intra-annual environmental changes.

Biomonitoring programs commonly include processing protocols that retain rare taxa (Carter and Resh 2001) as a means to determine sample richness (Vinson and Hawkins 1996). It has been argued that rare taxa reveal relationships with habitat conditions that are not apparent with only common taxa (Faith and Norris 1989, Turak and Koop 2003). Other studies have highlighted the importance of removing rare taxa from data sets because they may be transient, thus accidental, inhabitants (Hawkins et al. 2000). Robinson et al. (2000) found that the elimination of rare taxa decreased the frequency distribution of taxa coefficients of variation. However, determining whether or not a taxon is a true resident of the community, or merely a transient, is usually left to expert opinion or pre-determined by a cutoff in relative abundance (e.g., Faith and Norris 1989). Although valid, this practice may overlook relevant information about the community. A population may exist for only a given time interval within a year. Sampling such a population near the beginning or end of its interval might capture a few individuals as compared to sampling the middle of its interval. Further, samples taken outside of its interval of occurrence would contain no trace of the taxon at all. Consider metapopulations moving from source to sink habitats. If the sample habitat acts as a sink for a particular taxon, then it is expected that this population will only be sustained by additional immigrants when the source is overcrowded and thus the taxon may not contribute reliable information about the habitat as compared to permanent members. Lastly, the abundance (or complete absence) of a taxon may be reflective of the sampling design; taxa occupying greater (or lesser) spatial extents than the sample area may result in underrepresentation (or over representation if sampling a concentrated patch) in the collected samples. Rare and transient taxa have the potential to influence the

development of community structure models and their effectiveness as predictors of environmental conditions.

Metrics are ecological measurements that characterize the biota in a predictable fashion to anthropogenic activities (Barbour et al. 1999) and are common components of biological assessment studies. In general, metrics require rigorous testing in order to provide the most meaningful understanding of their relationships with environmental factors (Norris 1995). Screening of metrics as a step in developing measures of biological condition usually includes examination of metric variability at reference sites as recommended in designing a Benthic Index of Biotic Integrity (B-IBI) (Barbour et al. 1999, Flotemersch et al. 2006). Some studies have investigated annual variation in community structure (McElravy et al. 1989, Robinson et al. 2000). However, few studies have investigated seasonal differences in metric values (Hilsenhoff 1977, Jones et al. 1981, Lenat 1993, Linke et al. 1999). Metrics with the least variability within a single year are most favorable as measures for biological assessment because they will be more consistent with regard to seasonal differences. It is important for a metric to be reliably predictable from year to year.

Some macroinvertebrates species change habitat preferences as they grow (Clifford 1970, White 1978, Minshall 1984, Buffagni et al. 1995, Lloyd and Sites 2000, Reich and Downes 2004) and may also change feeding strategies or prey items (Sheldon 1969, Norris 1995), even between immature stages (Snellen and Stewart 1979, Gibbs and Mingo 1986). Many aquatic macroinvertebrates are r-select and display high mortality, therefore high abundances of individuals can be observed during early stages of the population's development. It stands to reason that an early instar compared to a conspecific later instar will feed on less quantity of material and will represent less of a nutritive food source for predators. Thus, weighting individuals according to a set of maturity classifications can provide data with less emphasis to early instars and better

precision for analyzing the functional composition of the community. Classifications based on maturity (Clifford 1969, Bretschko 1985, Kosnicki and Burian 2003) and size (Corkum and Pointing 1979, Perry et al. 1986) have been used to characterize life history traits in macroinvertebrate populations, but this information has not been commonly utilized in bioassessment studies.

Multivariate techniques for predictive modeling in applied stream ecology have received more attention since the first accepted large scale application (Wright 1984). One of the more commonly used methods is known as the River Invertebrate Prediction and Classification System (RIVPACS). RIVPACS models predict an expected taxonomic assemblage of stream macroinvertebrates based on environmental factors in non-impacted habitats as a means to which test sites may be compared (Wright 1984, Hawkins et al. 2000).

The process of constructing a RIVPACS model involves clustering reference stream sites into groups based on their biological assemblages. Discriminant functions are created for each of these groups primarily using physical factors that are not greatly influenced by anthropogenic activities (Reynoldson et al. 1997, Clarke et al. 2003, Flotemersch et al. 2006). These functions are used to predict the probability of test sites belonging to each of the reference groups. The probabilities are then multiplied by the frequencies of each taxon for every reference group, referred to as a capture probability (P_c). The number of observed taxa from the test site is compared to the sum of taxa P_c 's (expected taxa frequencies) in what is known as an observed to expected (O/E) score. Test sites exhibiting O/E scores close to zero are considered poor quality habitats, whereas scores close to or over 1 indicate that they are similar to reference conditions. A modification known as an O/E_{50} , only includes taxa that have expected frequencies of occurrence ≥ 0.5 (Simpson and Norris 2000). Although this would seem to be a more

conservative approach, O/E_{50} models can be more sensitive to changes under certain conditions (Hawkins et al. 2000).

Contemporary RIVPACS type models utilize presence-absence data to obtain expected assemblages from each taxon's frequency of occurrence within classified groups, and thus the probability of any taxon occurring is between 0 and 1. Rather than predicting the expected assemblage based on the probability of a taxon's occurrence, it is also possible to use relative abundance data in constructing an expected community structure for evaluating test sites. This would allow the use of abundance based similarity indices in comparing an observed community at a test site to an expected community.

Designing a RIVPACS model for a temporal gradient involves clustering taxa of sampling dates as a means of biologically determining seasons. Discriminant functions may best utilize temporal variables (i.e., cumulative precipitation over a specified time interval, degree days) as a means of predicting group membership to season. Thus, the capture probability for test sites could be calculated based on the probability of belonging to each season and the taxonomic frequencies within those seasons.

The goal of this investigation was to use a case study to demonstrate the importance of seasonal effects on community structure. Specifically, we 1) define types of rare and transient taxa observed from our sampling regime; 2) identify metrics that exhibit minimal seasonal variation and attempt to predict their values; 3) demonstrate the potential utility of maturity structured data; 4) develop a temporal RIVPACS-type model where seasons, rather than regions, are determined biologically.

4.3 Methods and Materials

4.3.1 Sampling and Processing

The Burris Fork is a temperate 2nd order wadeable stream in the Inner Ozark Border subsection of the Ozark Highlands ecoregion (Omernik 1987, Nigh and Schroeder 2002). Although the primary land use in this region is agriculture, this stream was selected because it has been designated by the state of Missouri as a reference stream for development of regional biocriteria (Rabeni et al. 1997, Sarver et al. 2002). Samples were taken from a 300 m reach 11 km south of California, Missouri, USA (38° 33' 10.24" N; 92° 34' 13.66" W). Macroinvertebrates were sampled from 3 separate riffles with a D-frame kick net by physically disturbing 1 m of substrate upstream of the net for 1 min. Each sample was transferred to a container containing 95% ethanol and taken to the laboratory for identification and enumeration. Dissolved oxygen (mg/L and percent), conductivity, pH, temperature, and current velocity were measured at each sample point. Two StowAway TidbiT temperature data loggers (Onset computer corporation, Bourne, Massachusetts, USA) were bolted to 1 meter long metal stakes and hammered into the substrate at two points in the stream so that loggers were positioned just above the substrate surface. Loggers were programmed to record temperature in degrees Centigrade every 40 min starting at 12:01 a.m. on the winter solstice (21 December 2002). Daily precipitation data were obtained from the United State Department of Commerce, National Oceanic and Atmospheric Administration, weather station in California, Missouri.

Samples were taken ca. monthly for 12 dates from 20 December 2002 to 28 November 2003. This time frame was designated as the Model Development Period (MDP). Three test samples were taken on 21 December 2003, 23 March 2004, and 3 October 2004 to represent different seasons for the purpose of validating models

constructed from data collected in the MDP and are referred to as t₁, t₂, and t₃, respectively.

Whole samples were sorted. Macroinvertebrates were identified to the genus or species level, when possible, except for oligochaetes which were left at class. Chironomids were counted at the family level, then at least 11% of the individuals were subsampled. The subsampled chironomids were cleared in 10% KOH for ca. 12 h and transferred to 100% ethanol, then slide-mounted with Euparal. Relative abundances from these identifications were calculated for each sample. Macroinvertebrates from each kick sample were numerically pooled in order to obtain a whole sample community profile of the stream reach for each date. Similarly, means of environmental variables were calculated for each date, except precipitation and Julian Days for which there was only one measurement on each date. Cumulative degree days were calculated as the sum of mean daily temperatures > 0° C from the mean daily temperatures recorded from one or both data loggers, depending on the number in operation, starting from 20 December 2002. Due to logger malfunction and loss, in-stream temperature data were available only until 25 March, 2004. Degree days from this point on were estimated using linear regression with data from loggers at this site and loggers deployed in a nearby stream ($n = 461$, $r^2 = 0.99$). Fixed period cumulative precipitation and degree days were calculated for periods of 4, 7, and 14 days previous to the sampling dates. Back calculations for fixed count degree days for 20 December 2002 were estimated from a second order polynomial regression of daily mean water temperatures and daily mean air temperatures obtained from the weather station ($n = 461$, $R^2 = 0.92$). Because taxa were sampled at intervals, coarse levels of identification (i.e., family) were inspected for correspondence with finer levels from other periods in the study. When clear associations were made, the coarse level identifications were elevated to the finer level. Other taxa were collapsed into coarser level identifications (usually from species to

genus) when there were relatively few identified specimens at the fine level but many associated individuals at the coarse level. Operational taxonomic units (OTUs) were established thus allowing for control of ambiguous taxa between sample dates (Cuffney et al. 2007).

Taxa of the Elmidae genus *Stenelmis* were treated differently than other taxa. Adults of *Stenelmis lateralis* Sanderson and *Stenelmis sexlineata* Sanderson (Coleoptera: Elmidae) were distinct from each other and from other species of *Stenelmis*. Because other species of adult *Stenelmis* were difficult to identify, they were left at the genus level but maintained as a separate OTU from those of *S. lateralis* and *S. sexlineata*. Larvae could not be associated with adults and were proportioned into adult OTUs based on the abundance of each adult OTU in the sample. OTUs and their abundances for all taxa were entered into an Excel spread sheet and joined to an Access database (Microsoft Corporation, Redmond, Washington, USA) with functional feeding group, tolerance values, and other taxonomic information obtained from the Missouri Department of Natural Resources (Sarver 2001) and other sources (Merritt and Cummins 1996, Barbour et al. 1999, Bode et al. 2002) to create a master dataset for further processing and analysis.

During the identification process, each non-oligochaete individual was assigned to a maturity class. Four classifications of maturation were used: early, middle, late, and pupae/adult, designated as class I, II, III, and IV, respectively. Thus, for certain groups (e.g., mayflies, stoneflies) there were only three available classifications because they do not have pupae or aquatic adult forms. Individual classifications were determined, based on qualitative observation of size and morphological development of each specimen. Guidelines for the placement of the major groups are given in Table 7. The classes were used to weight abundances in thirds; the total number of individuals in class

I was multiplied by 0.33, class II by 0.67, and classes III and IV were counted 1 for 1. Thus, a second master dataset was created with maturity corrected abundances.

4.3.2 Rare and Transient Taxa

After all taxa were placed into OTUs, each OTU was examined for its occurrence over the entire study. In this way, criteria were created to classify taxa as rare or transient (see below). All rare and transient taxa were eliminated from both master datasets before analysis.

4.3.3 Metric Assessment

From both master datasets (maturity weighted and non-weighted abundances), 120 metrics were calculated for each date. These were reduced to a suite of metrics each having a coefficient of variation (CV) < 20 over the MDP. This suite was further reduced with the goal of retaining metrics representing the categories of functional feeding group, tolerance, diversity, richness, and composition. When more than one derivative of a metric met the criteria (e.g., percentage of 3 dominant taxa, percentage of 4 dominant taxa), only one was chosen. Further, standard metrics were selected for model construction when both the standard and maturity weighted analogues met the variability criteria. Stepwise multiple regression was used for each of these metrics to find models that explained the metric values for each sampling date with physico-chemico-temporal factors, their squares, and the single interactions of all predictors with each other (Table 8). Observed and expected values for each metric were plotted for sampling dates taken over the MDP and for each test sample. The mean squared prediction error (MSPE) of the test samples was compared to the mean square error (MSE) of each regression model as a means of validation.

4.3.4 Maturity Weighted vs. Standard Abundance

Paired t-tests were used to compare standard metrics to their maturity analogues for tolerance, diversity, percentage functional feeding group, and relative abundance metrics

within the MDP. The Wilcoxon Signed-Rank Test was used for paired differences that were not normally distributed. For these analyses, standard metrics were recalculated to only include non-oligochaete taxa that had a maturity weighting factor so that the direct effect of maturity weighting could be shown. Dates that did not contain target taxa of a particular metric (e.g., percentage Plecoptera) were eliminated from analysis. The difference of the standard metric subtracted from its maturity analogue was taken for each sampling date to determine if maturity weighting tended to increase or decrease the metric value. Lastly, maturity weightings were examined to determine if the metric value changed by more than 5, 10, and 20% as compared to the standard for each date.

4.3.5 Multivariate Assessment

The construction of RIVPACS models considers many processes (Moss et al. 1987, Wright 1995, Moss et al. 1999, Hawkins et al. 2000, Simpson and Norris 2000, Clarke et al. 2003, Van Sickle et al. 2006) but basically follows 3 main steps (Fig. 6). Step 1 involves cluster of reference sites, or in our case sample dates, into discrete groups using biological data. In step 2, Discriminant Function Analysis (DFA) is performed with physico-chemico-temporal variables on the groupings delineated in step 1 in order to create linear functions used for estimating the probability of group membership of test sites. The expected assemblage for each given test site is calculated in step 3 by multiplying the frequency of taxa from each group delineated in step 1 by the probability of the given test site belonging to the related group as determined in step 2. The sums of these products are used to find the expected taxonomic richness at a site and is compared to the actual richness observed at the site. An excellent explanation of the procedure can be found in Flotemersch et al. (2006). A brief description of designing the temporal RIVPACS models of this study is given here.

The STEPDISC procedure in SAS was used to reduce OTUs into a suitable set of taxa for the purpose of classifying dates into discrete temporal groups (biologically

determined seasons). Maturity weighted and standard datasets were considered, separately. Cluster analysis (Ward method) of the final reduced OTU set was performed with the SAS CLUSTER procedure to classify dates of the MDP into discrete seasons (**d**). The taxonomic frequency of occurrence (**f**) and relative abundance (**a**) of pre-STEPDISC OTUs (**x**) was found for each seasonal grouping **d**.

The STEPDISC procedure was also used to reduce the set of physico-chemico-temporal variables into a discrete set of predictors suitable for DFA. DFA of the seasonal groupings **d** was performed with the SAS DISCRIM procedure using the reduced set of predictor variables to create discriminant functions for estimating the probability of sampling date group membership (**p_d**) individually for MDP and test sampling dates.

Sampling date probability of group membership for each OTU (**p_d, x**) was multiplied by the expected OTU occurrence frequency **f** and relative abundance **a** for corresponding seasonal groups, separately. The products of each group **d** were added to find the probability of occurrence frequency capture (**P_{fc}**) and relative abundance capture (**P_{ac}**) of each OTU, respectively. The summation of **P_{fc}** for all OTUs was taken for each date where **P_{fc}** > 0 and **P_{fc}** ≥ 0.5, separately, to determine two sets of expected scores. The summation of all observed taxa for each **P_{fc}** threshold was divided by the corresponding expected scores in order to obtain O/E₀ and O/E₅₀ scores for each date. The **P_{ac}** of each OTU **x** was the predicted relative abundance of that OTU expected to occur at each given date. The percentage similarity index (Whittaker and Fairbanks 1958) and non-weighted Pinkham and Pearson's index (Pinkham and Pearson 1976) were used to compare the similarity of the observed community to the expected, for all sampling dates.

4.4 Results

4.4.1 Rare and Transient Taxa

Seventy-two of 113 OTUs were determined to be rare or transient (Table 9). Rare taxa were considered to be part of the community but present in low abundances. Many of these taxa were thought of as permanent residents but only occurred in half or fewer of the sampling dates because the sampling area was considered a fragment of their overall habitat; they occurred in habitats that our sampling method did not target. Two types of transient taxa were identified. Type I transient taxa were those that infrequently occurred usually in low numbers and often in early stages of maturity without any clear associations to season. Type II transient taxa were defined as being present for up to 3 consecutive sampling periods and were considered short-lived, temporally transient members of the community associated with a season.

4.4.2 Metric Assessment

Fourteen metrics representing richness, diversity, and tolerance measurement categories had a CV < 20 over the MDP (Table 10). Because functional feeding group and composition metrics did not generate CVs less than 20, percentage mature gatherers (CV = 31.7) and percentage mature Diptera and non-insects (CV = 27.1) were added to this dataset so that representatives of these categories could be included for model development. Maturity abundance weighted metrics were chosen because they had CVs closer to the criteria compared to their standard analogues for percentage gatherers (CV = 36.7) and percentage Diptera and non-insects (CV = 33.0). Nine of these metrics were selected for multiple regression with physico-chemico-temporal variables.

As a result of the use of squares and interactions among predictors, stepwise multiple regression produced many complicated models. For each metric, the simplest significant model was selected from those produced in the analyses. However, if an additional factor improved the R² by ca. 0.10 or more, then the more complicated model

for that metric was favored (Table 11). Observed and expected metric values for all sampling dates tended to be fairly close for all metrics over the MDP (Figs. 7-10). The MSPE of the modified MO Biotic Index test samples was not substantially different from the MSE of the model, indicating that the predictive ability of the model is unbiased (Table 12). The MSPE for EPT richness was about twice that of the MSE. The MSPE compared to the MSE for the rest of the metrics was 3 to 10 times higher, indicating that the predictive ability of the models is not reliable (Neter et al. 1996).

4.4.3 Maturity Weighted vs. Standard Abundance

Paired t-tests of metrics calculated with standard abundances and their maturity weighted analogues revealed 6 metrics that changed significantly, and 3 others that approached significance, as a result of having maturity weighted data over a temporal continuum (Table 13). Ten pairwise comparisons showed that 75% or more of the sampling dates had at least a $\pm 5\%$ change in value. Four pairwise comparisons increased in value for 75% or more sampling dates when maturity structured data were applied, whereas 7 other pairwise comparisons decreased in value for 75% or more sampling dates.

4.4.4 Multivariate Assessment

Several cluster analyses were performed with different combinations of stepwise reduced OTUs and with the removal of sampling dates that did not classify into a distinguishable group. A choice cluster dendrogram was produced (Fig. 11) with the set of maturity weighted abundances for the OTUs given in Table 14 and was obtained after the removal of the 30 June sampling date (see Discussion). The cluster analysis classified dates into 3 seasonal groups, roughly described as winter, summer, and autumn. Winter was primarily characterized by the presence of *Isoperla* sp. (Plecoptera: Perlodidae) and a low abundance of *Rheotanytarsus* sp. (Diptera: Chironomidae). Summer was distinguished by a high abundance of *Bezzia/Palpomyia* sp. (Diptera: Ceratopogonidae),

a low abundance *Cricotopus/Orthocladius* sp. (Diptera: Chironomidae), and the absence of *Stenonema femoratum* (Ephemeroptera: Heptageniidae). Autumn was characterized by a high abundance of Planariidae and *Rheotanytarsus* sp.

The STEPDISC procedure of the physico-chemico-temporal variables identified degree days, degree days₁₄, and precipitation₇ (Table 8) as environmental variables suitable for DFA. DFA correctly classified the samples of the MDP into their designated seasonal groups (Table 15) (Wilks $\lambda < 0.001$). Functions 1 and 2 accounted for 75.82% and 24.18% of the variation, respectively. The TEST and TESTLIST options in the DISCRIM procedure of SAS produced the probability of group membership to seasons for dates of the MDP and each test date, respectively (Table 15). Both t₁ and t₂ plotted well within the range of the MDP winter group, whereas t₃ showed slight departure from autumn towards the summer MDP group (Fig. 12).

The community structure of the MDP test sites was evaluated by two measures of similarity. The percentage similarity index is a measure more sensitive to structural-functional similarity whereas the Pinkham and Pearson's index tends to be more sensitive to changes in less common taxa (Brock 1977, Washington 1984). The percentage similarity index showed moderate changes among the sampling dates of the MDP with date 14 April showing a marked deviation from expected community composition. The Pinkham and Pearson's index indicated that considerable changes in the less common taxa occurred throughout the sampling dates of the MDP with the most notable differences at 18 January and 22 May. The O/E₀ and O/E₅₀ models had similar results; sampling dates of the MDP were similar to the expected assemblage of their respective season. With both similarity indices and O/E models, t₁ was similar to the individual MDP sampling dates with regard to its model response. Test dates t₂ and t₃ showed considerable departure from expected community relative abundances and frequencies of occurrence, except for the t₂ O/E₀ model which indicated moderate

departure. Departures were more extreme for relative abundances than they were for frequencies of occurrence.

4.5 Discussion

The temporal regime represents a fourth dimension in ecological studies (Ward 1989). The physico-chemical properties of a habitat change over time, as does the community of biota that resides within it. Just as a sampling strategy must account for the spatial extent of its target community, the temporal context must also be considered. Theoretically, taxa that are present in the community for the majority of the year are more likely to be sampled compared to those that are present for only a few months. Therefore, the frequency of sampling or the sample period itself will ultimately determine if a taxon is a member of the target community.

Numerically rare taxa are typically excluded from analyses because they tend to create noise that distracts from relationships of interest. However, the reason why a taxon is numerically rare can yield other important ecological information. We distinguished rare taxa as those not adequately targeted by the spatial sampling regime; they exist spatially at a finer or coarser extent than that employed by the sampling methodology, and thus should be eliminated from the dataset. Transient type I taxa were considered adventitious individuals not associated with the target community. Some of these taxa were thought of as frequent invaders that were unable to become established members of the community; it was surmised that metapopulations originated from a source and the sample habitat was their sink. Transient type II taxa were found for only a short period of time in the sampled habitat. These organisms could be targeted for studying season-specific conditions. The sampling regime will ultimately define a transient type II taxon in that more frequent samplings will encounter the taxon more often, accounting for more of its life cycle.

Identifying rare and transient taxa based on seasonal presence and maturity in addition to abundance offers a few important advantages over using relative abundance alone. Taxa that reside over a greater (or lesser) extent to the sampling protocol should be removed because the sampling design may not adequately capture their true representation in the community. Sometimes large numbers of transients, or metapopulations, may invade the sample habitat for a short period of time. Long term monitoring or frequent sampling throughout a year can help reveal the true members of the target community.

The identity of rare or transient taxa may be useful in adding new ecological information to the community. The presence of transient type II taxa can be thought of as seasonal check points, marking time intervals that signify a shift in the community structure. Knowledge of such organisms could be useful in seasonally calibrating metrics or B-IBI scores, depending on their presence or absence due to adult emergence. Transient taxa, may also signify ecological importance. If the identity of transient taxa is reliable, information about the habitat could be revealed and act as a complement to information generated from “weedy” taxa (e.g., Scarsbrook and Townsend 1993). For instance, the development of metrics such as “transient taxa richness” may be useful in identifying the disturbance regime of a habitat; a stream reach with more transient taxa compared to a different reach with the same number of overall taxa may be in a post recovery stage from a disturbance.

Ideally, the best metrics would be those that show little to no change in relation to seasonal conditions, but show a distinct change along a gradient in response to an environmental stressor. However, most metrics are influenced by seasonal weather patterns, climate changes, and other biotic and abiotic influences. Therefore, it is important to identify metrics that are least susceptible to seasonal variation and account for those influences when they are present.

Short-term temporal sampling showed great variability in community structure over the continuum as shown in metric values. A few metrics exhibited low variation over the annual sampling regime and were chosen for multiple regression. The modified MO Biotic Index and richness metrics tended to be the most predictable and consistent metrics over the annual continuum, whereas diversity, composition, functional, and other tolerance measures were not. Although bioassessment studies are interested in reliable measures, the metrics we chose may not be suitable for B-IBI construction unless they are shown to have a graded response to disturbance. Our interest was not to identify metrics associated with disturbance, but to find ones that are least influenced by the variability within natural systems. Although models with higher R^2 could have been produced, the MSE of these models were so small that any validation would have been unrealistic. In general, the multiple regression models did not accurately predict metric values for test dates; they were over fit.

The taxonomic and EPT richness metrics were most variable between 14 April and 27 September (Fig. 7). Chironomidae richness values tended to be consistent, in part because many chironomid taxa were considered rare or transient and were eliminated from the dataset. The predictor coefficients for all the richness measures were less influential than the Y-intercept (Table 11), indicating that the temporal environmental variables had low influence. Some species within a family (Hauer and Resh 2006) or unrelated species with similar functional traits (Cummins et al. 1989, Townsend 1989, Wiens 2002) may replace one another over the temporal continuum, tending toward consistent richness measures. Thus, as environmental conditions change over the temporal continuum, taxa replace one another, yielding a fairly consistent number of species. *Simulium* sp. (Diptera: Simuliidae) was scarce or absent in the late summer and early autumn samples, during the period when *Hydropsyche* sp. (Trichoptera: Hydropsychidae) was present. Although representing different functional

groups, Plecoptera were replaced by members of the mayfly family Baetidae in the summer months, accounting for some of the consistency in EPT richness. Richness measures are some of the most common community measures and their ability to show consistency over the temporal continuum is a credit to their usefulness in ecological studies.

Both diversity measures had similar observed values over the study period (Fig. 8). This is not surprising considering that Shannon's Diversity Index is the numerator of Pielou's J (Washington 1984). Pielou's J is a measure of evenness, restricted to a value between 1 and 0, and thus tends to be more stable than Shannon's Diversity Index, which is not bound to a theoretical maximum. Regardless, the models did not predict well for the test dates.

The pattern of percentage 3 dominant taxa, from sampling date to sampling date, was opposite of the diversity indices (Fig. 9), which is expected because relative increases in dominant taxa will decrease the diversity and evenness of the community. This notion was even reflected in the model equation (Table 11). The predictive ability of the percentage 3 dominant taxa regression was not accurate, however. The observed values of the modified MO Biotic Index from date to date were the most consistent compared to those of other metrics. This metric was originally developed for South Africa (Chutter 1972), later modified for Wisconsin (Hilsenhoff 1977), and versions of this index are now utilized by many states in their biological water quality monitoring and assessment programs (e.g., Lenat, 1993, Sarver, 2001, Jessup and Gerritsen, 2002, Bode et al., 2002, Lawson, 2005). A noticeable trough occurred on 18 January and 19 February, and can be accounted for by the high abundance of *Allocapnia* sp. (Plecoptera: Capniidae), a winter stonefly. Adult males were collected on 19 February and identified as *A. vivipara* (Claassen). The OTU was maintained at the genus level because nymphs cannot be identified to species and a few adults from a single date were insufficient to support an

association of more than 2000 larval individuals collected over two years. However, *A. vivipara* has been observed to inhabit polluted conditions and is considered by some researchers as one of the more tolerant stonefly species (B. Poulton, per. com.). The tolerance value of 2.8 given for *Allocapnia* sp. in this study might be considered too low, and if a higher value was given, the trough in the MDP (Fig. 9) would not have been as great. Regardless, the predictive capabilities of this metric's regression were accurate.

The percentage mature gatherers and the percentage mature Diptera and non-insects represented some of the most variable metric types (Table 10, Fig. 10). Resources are destined to change over the temporal continuum and thus different feeding strategies will be displayed by the biota in response to those changes (Vannote et al. 1980). This appeared to be true particularly for the percentage mature gatherers as there were distinct troughs during which there was replacement by the shredders *Allocapnia* sp. and *Polypedilum* sp. (Diptera: Chironomidae) in winter and summer months, respectively.

The observed values for t₂ were outside of the MDP range for 7 of the 9 metric models. Closer inspection of the environmental variables shows that current velocity was higher on this test date than on any other date (Table 16). This value was not accounted for within the range of observed values of the MDP and, therefore, metric estimates may be unjustifiable (Zar 1999). Although current velocity was not a predictor for all models, it is implied that the environmental conditions experienced by the biota at t₂ were outside the MDP range of variability used to construct the multiple regression models, and therefore the models may not be valid for predicting metric values at this date. This demonstrates some of the difficulty in explaining environmental variability in field studies and emphasizes the challenges incurred by a dynamic environment.

The results of metric analysis from this study indicate that many metrics are variable over a year and that sampling protocols that are designed with a designated

sampling period should be cautious of seasonal variability. Samples taken during the early part of the sampling interval may not be comparable to samples taken near the late portion of the sampling interval and samples may not be comparable year to year. For instance, the Missouri assessment protocols (Sarver et al. 2002) recommends that macroinvertebrate samples be taken from mid-March to mid-April for evaluating stream impairment. This time frame could allow for large numbers of Capniidae and Leuctridae (Plecoptera), stonefly groups typically thought of as intolerant to pollution, to be captured at the beginning of the sampling period during cold years but not at the later end of the sampling period, or not at all from samples taken during warm years. In this study, 144 nymphs of *Allocapnia* sp. were collected on 15 March 2003, but only 2 individuals were collected from 23 March 2004. As mentioned above, this taxon had a substantial influence on the modified MO Biotic Index, but also contributes to the EPT richness metric, two of the four primary metrics used by Missouri for evaluating aquatic life impairments. A more reliable sampling protocol could utilize ambient temperature data to predict a more optimal sampling period based on accumulated degree days. Alternatively, biological delineation of seasons with cluster analysis demonstrated that there are unique community structures that can be temporally partitioned. Knowledge of when shifts in community structure are least likely to occur could help establish a more reliable period to conduct sampling. Our data suggests a sampling period for winter (mid-January to mid-February) might have more consistent results compared to a spring season, which we found to be more difficult to identify with cluster analysis.

Individual maturity data provided greater ecological resolution in measuring the structure of the community with respect to seasonal processes. More emphasis is given to taxa that are abundant and in a state of moderate to late development. Our results indicated that maturity weighted data were better for multimetric and multivariate modeling compared to non-weighted data. In this light, the utility of different weighting

schemes to account for different life histories traits could offer much improved resolution for metric calculations and other types of community structure analysis.

Over the annual continuum, differences between metric values calculated with standard abundances versus maturity weighted analogues tended to average out (Table 13). However, metrics that were based on a more specific subset of the community tended to weigh differently when maturity data were used rather than standard data. For example, maturity weighted percentage Plecoptera increased for 7 of 10 sampling dates and changed > 5% in value compared to the standard weighted metric for all 10 sampling dates. However, paired comparisons did not reveal a significant difference between the standard calculation and the maturity analogue (Table 13). The percentage Elmidae (Coleoptera) showed significant differences between the standard metric and its maturity weighted analogue, changing > 20% for 8 of 12 pairwise comparisons.

This notion is more compelling when comparing metrics made up of several groups to the smaller subsets of those groups. For instance, comparisons of the percent change in metric value of the standard weighted to the maturity analogues of percentage EPT (Ephemeroptera, Plecoptera, Trichoptera) showed fewer changes than any of the orders considered as a metric on their own. Moreover, percentage Hydropsychidae and percentage Philopotamidae (Trichoptera) had a higher incidence of percent change in metric value when maturity structured data were applied as compared to changes in their parent metric percentage Trichoptera. Composition metrics based on the dipterous family Chironomidae were also more likely to change in value after maturity weighting when finer taxonomic groups of the family (tribes and subfamilies) were considered. Metrics based on percentages of dominant taxa complimented these findings; the percentage dominant taxon changed more frequently with maturity weighting than the percentage dominant 2 taxa, which changed more frequently compared to the percentage dominant 3, 4, and 5 taxa, respectively. Lastly, more specialized functional feeding

groups also tended to be more influenced by maturity weighting than generalist groups. The values for percentage predators and percentage scrapers changed by > 10% more frequently than the collector metrics percentage gatherers and percentage filterers.

One of the more notable metrics to show a significant difference between the standard and maturity analogue was the modified MO Biotic Index, a metric used in many state biomonitoring programs. Although only one of the sampling dates showed a change > 5%, all but one of the dates showed a decrease from the standard to the maturity weighted metric value, thus edging toward a better habitat quality evaluation. Similar results were exhibited by the standard and maturity analogues of Shannon's Diversity Index and Pielou's J.

These results show that percentage based metrics are less likely to be influenced by maturity structured data when a larger portion of the community is considered. Percentage metrics that are based on more finite or specialized groups are more likely to change with maturity weightings. Metrics that incorporate the entire community (e.g., Biotic Index, Pielou's J) were less likely to change > 5% with maturity weightings, but tended to be consistent in the direction they changed, and therefore tended to be significantly different. The use of maturity weighted data may add different information to ecological measurements.

Maturity weighting was intended to adjust for over-weighted abundances of immature individuals that do not contribute as much to ecosystem function compared to more mature individuals of the same taxon. The stepwise reduced maturity weighted data were the best for delineating seasonal classifications, suggesting that the weighted data can be effective at adjusting for seasonal influences.

There are theoretical notions to take into consideration with maturity data. First, maturity classification may be difficult for individuals that appear to be between classes. Second, weightings have a direct influence on the calculations of individual taxa

(numerator), but also on the relative abundance of the community (denominator). Thus, bias in weighting one of the more abundant taxa will bias all calculations in the sample that utilize relative abundance. Third, all taxa may not deserve equal weight. It might be better to use a different weighting scheme, or include an additional factor to account for taxonomic life histories and habits. For instance, consider the comparison of two shredders, *Polypedilum* sp. and *Pteronarcys* sp. (Plecoptera: Pteronarcyidae). Such a comparison may not be an equivalent 1 to 1 between taxa, considering an individual *Pteronarcys* sp. could be 1200 times the biomass of an individual *Polypedilum* sp. (based on dry mass estimates of *Polypedilum* sp. and *Pteronarcys dorsata* from Benke et al. 1999) and is longer lived. Thus, it might be more valid to weight *Pteronarcys* sp. 0.3:0.7:1.0 but *Polypedilum* sp. at 0.01:0.03:0.1, for instance, accounting for *Polypedilum*'s faster growth rate and overall lower individual biomass. Alternatively, an additional weighting factor could be used to place more emphasis on the presence of *Pteronarcys* sp. or less on *Polypedilum* sp. Such refinements could increase the precision of metric values and other measures in relation to ecological conditions.

Biologically delineating seasons offers insights into the fluctuation of community structure but presents challenges. Performing whole counts on samples tended to be cumbersome due to the high numbers of individuals (Fig. 13). Because total abundance was especially high during June-August, and that sampling date 30 June had over 14,000 individuals, additional summer samples were not pursued. With this regard to the cluster analysis results, it is probable that if more sampling dates in June and July were included in the analysis, a summer season would have been more finely resolved and a fourth season representing spring would have been elevated from the winter sampling dates. However, due to the underrepresentation of summer samples, 30 June was widely disparate from the other sampling dates within the MDP; when included in the cluster analysis it usually came out by itself. Thus, it was eliminated to enable

distinct biological classifications. The seasons as classified by macroinvertebrates were found to be adequate groupings for the purposes of this study.

Variables used for the DFA portion of RIVPACS are normally based on physical parameters that are not highly alterable by human activities. Clarke et al. (2003) pointed out that the predictors should 'fit the purpose' of the model. The STEPDISC procedure of SAS found three temporal variables (degree days, degree days₁₄, and precipitation₇) appropriate for the purpose of this study. Cumulative degree days and precipitation are ideal for studying short-term temporal community structure as they represent recent environmental changes that have had a direct influence on the community, yet are not directly influenced by human activities.

Both relative abundance and frequency of occurrence models showed that t₁ compared well to what was expected. Test dates t₂ and t₃ did not display relative abundances and frequency of occurrences expected by the models. Reasons given for t₂ departure from expected metric values are implicated as the same reasons for the difficulty of the multivariate models to predict an expected community similar to the observed. The difficulty of the models to predict an expected community similar to the observed for t₃ is unclear; more temporal information is needed to properly predict the community relative abundance or frequency of occurrence. Once a community changes outside of the variability range of expected results, as noted with test date t₂, it could be permanently affected in that future community structures will not be predictable.

Comparisons of observed to expected relative abundances were more conservative than frequencies of occurrence. Typically, RIVPACS are developed for comparison of presence-absence data (Reynoldson et al. 1997). The Benthic Assessment of Sediment (BEAST) is a similar multivariate model developed in Canada, however, in contrast to RIVPACS, abundances are used for group classifications and test site assessment (Reynoldson et al. 1995, 2001). Furthermore, BEAST model comparisons

are based on membership of a test site to a single group, whereas RIVPACS models evaluate test sites based on the probability of membership to every group. The methodologies of the BEAST model demonstrate that abundance data are useful. A consideration with using similarity indices compared to O/E models is that taxa not represented in the reference data are preserved in the model as opposed to O/E models in which they are discarded. Thus, inclusion of taxa that are not present from MDP dates adds more information for the comparison of the observed to expected community structures.

Both multimetric and multivariate temporally based analyses could have direct applications toward the study of global climate changes. Long term cyclic trends in the community structure with relation to seasons may become altered if ambient temperatures remain warmer for a longer period of time. Permanent shifts in metrics that are least variable, annually, may indicate a structural shift in the community related to a climatic change. Biologically classifying seasons from year to year may show a tendency for one season to shrink or disappear all together as the community responds to new environmental regimes. Long term annual sampling is sure to show trends in community structure as related to climate change. However, a better understanding of intra-annual variation will help negate the possibilities of seasonal influences for such data.

Table 7. Physical attributes per taxonomic group used for determining individual maturity classifications.

Taxonomic group	Attributes
Turbelaria	Presence or absence of gonadopores and relative size.
Bivalvia	Relative size; presence of immatures in the mantle.
Gastropoda	Relative size and shell morphology.
Crustacea	Relative size and morphological development.
Ephemeroptera	Development of wingpads.
Odonata	Development of wingpads.
Plecoptera	Development of wingpads and relative size.
Hemiptera	Development of wingpads and relative size.
Megaloptera	Relative size.
Trichoptera	Relative size of individual and its case.
Coleoptera	Relative size of immatures.
Diptera	Relative size and morphological development.
Other organisms ^a	Relative size.

^a Nematoda, Hydrozoa, Hirudinea

Table 8. Physico-chemico-temporal factors considered for metric multiple regressions and discriminant function analysis of RIVPACS models.

Symbol	Name	Definition
g	Dissolved oxygen	Dissolved oxygen (mg/L) taken at the time of sampling.
θ	Pctg Dissolved oxygen	Percentage of dissolved oxygen taken at the time of sampling.
h	pH	pH taken at the time of sampling.
f	Flow	Current velocity (m/s) taken at the time of sampling.
p ₄	Precipitation ₄	Cumulative precipitation (inches) 4 days before the sample was taken.
p ₇	Precipitation ₇	Cumulative precipitation (inches) 7 days before the sample was taken.
p ₁₄	Precipitation ₁₄	Cumulative precipitation (inches) 14 days before the sample was taken.
t	Temperature	Water temperature (°C) taken at the time of the sampling.
d	Degree days	Cumulative degree days (daily mean > 0 °C) from Julian day 1.
d ₇	Degree days ₇	Cumulative degree days (daily mean > 0 °C) 7 days before sampling.
d ₁₄	Degree days ₁₄	Cumulative degree days (daily mean > 0 °C) 14 days before sampling.
j	Julian days	Number of days starting 20 December.

Table 9. Operational taxonomic units (OTUs) considered rare or transient from samples taken from the Burris Fork in central Missouri. Rare and transient as defined in the text are given as tentative assignments. Some taxa are given more than one designation to account for uncertainty or their versatility.

OTU	Rare	Transient I	Transient II
Nematoda	X	X	
<i>Erpobdella punctata</i>	X		X
<i>Helobdella triserialis</i>	X		X
<i>Mooreobdella microstoma</i>		X	
<i>Hydra</i> sp.	X		X
<i>Amnicola limosa</i>	X		
<i>Helisoma trivolvis</i>	X		
<i>Lymnaea</i> sp.	X		X
Acari	X		
Cladocera		X	
Ostracoda		X	
Cyclopoida		X	
Amphipoda		X	
Asellidae		X	
<i>Orconectes</i> sp.	X		
<i>Procloeon</i> sp.		X	
<i>Choroaterpes</i> sp.		X	
<i>Leucrocuta</i> sp.			X
Gomphidae		X	
Libellulidae		X	
<i>Enallagma</i> sp.	X	X	
<i>Acroneuria evoluta</i>	X		X
<i>Isonychia</i> sp.	X		X
<i>Perlinella drymo</i>	X		X
<i>Clioperla clio</i>	X		X
<i>Hydroperla crosbyi</i>	X		X
<i>Amphinemura</i> sp.		X	X
<i>Taeniopteryx</i> sp.			X
Corixidae		X	
Gerridae		X	
<i>Mesovelia mulsanti</i>		X	
Saldidae		X	
<i>Rhagovelia knighti</i>		X	
<i>Corydalis cornutus</i>	X		

Table 9. Continued.

<i>Sialis</i> sp.		X	
Dytiscidae		X	
<i>Peltodytes sexmaculatus</i>		X	
<i>Scirtes</i> sp.		X	
<i>Dubiraphia</i> sp.		X	
<i>Tropisternus</i> sp.	X		X
<i>Helichus</i> sp.		X	
<i>Rhyacophila</i> sp.		X	X
<i>Nyctiophylax</i> sp.		X	
<i>Ceraclea</i> sp.		X	
<i>Tipula</i> sp.	X		
Forcipomyiinae	X	X	
<i>Atrichopogon</i> sp.		X	
<i>Culicoides</i> sp.		X	
<i>Monohelea</i> sp.	X	X	
<i>Ablabesmyia</i> sp.		X	
<i>Larsia</i> sp.		X	
<i>Zavrelymia</i> sp.		X	
<i>Pseudochironomus</i> sp.	X		
<i>Cryptochironomus</i> sp.	X		
<i>Glyptotendipes</i> sp.	X		
<i>Microtendipes</i> sp.	X	X	
<i>Parachironomus</i> sp.	X	X	
<i>Paratendipes</i> sp.			X
<i>Stictochironomus</i> sp.	X	X	
<i>Cladotanytarsus</i> sp.	X	X	X
<i>Paratanytarsus</i> sp.	X		
<i>Corynoneura</i> sp.	X	X	
<i>Cricotopus trifascia</i>			X
<i>Diplocladius</i> sp.			X
<i>Hydrobaenus</i> sp.	X		
<i>Nanocladius</i> sp.	X		
<i>Parametriocnemus</i> sp.	X		
<i>Paraphaenocladius</i> sp.	X	X	
<i>Tienemanniella</i> sp.	X		
<i>Clinocera</i> sp.	X	X	
Cyclorrhapha		X	
Dolichopodidae		X	

Table 10. Metrics with a coefficient of variation (CV) less than 20 calculated over the Model Development Period (20 December 2002 – 28 November 2003). Metrics with the lowest CV for functional feeding group and community composition measurements were added to represent these metric types.

Metric ^a	Metric type	CV
Taxonomic richness	Richness	10.0
EPT richness	Richness	16.8
Chironomidae richness	Richness	14.3
Shannon Diversity Index	Diversity	11.7
Mature Shannon Diversity Index	Diversity	11.1
Pielou's J	Diversity	11.9
Mature Pielou's J	Diversity	10.4
Modified MO Biotic Index	Tolerance	9.0
Mature Modified MO Biotic Index	Tolerance	10.1
pctg 3 dominant taxa	Tolerance	19.4
pctg 4 dominant taxa	Tolerance	15.5
pctg Mature 4 dominant taxa	Tolerance	16.7
pctg 5 dominant taxa	Tolerance	12.5
pctg Mature 5 dominant taxa	Tolerance	16.7
pctg Mature gatherers ^b	Functional feeding	31.7
pctg Mature Diptera and non-insects ^b	Composition	27.1

^a pctg = percentage

^b Did not meet criterion of CV < 20

Table 11. Multiple regression models ^a used for predicting test date metric values.

Taxonomic richness = $28.67 + 0.28h^2$
EPT richness = $9.48 + 0.0042jp_{14}$
Chironomidae richness = $5.11 + 0.14gp_{14} - 0.15gf$
Shannon Diversity Index = $4.89 + 0.91f^2 - 0.041h^2$
Pielou's J = $1.47 - 0.012h^2$
modified MO Biotic Index = $6.38 - 0.031g^2 + 0.94gf - 6.83f^2$
pctg 3 dominant taxa = $-32.47 + 1.37h^2$
pctg Mature gatherers = $25.45 + 0.54fd_7 - 0.66tp_4$
pctg Mature Diptera and non-insects = $23.35 + 0.014\theta t$

^a pctg = percentage

Table 12. Metrics used for multiple regression.

Metric name ^a	F-value	<i>p</i> -value	Error df	R ²	MSE	MSPE
Taxonomic richness	8.42	0.016	10	0.46	5.910	20.330
EPT richness	11.28	0.007	10	0.53	1.640	3.333
Chironomidae richness	7.80	0.011	9	0.63	0.312	2.000
Shannon Diversity Index	7.92	0.010	9	0.64	0.034	0.326
Pielou's J	11.55	0.007	10	0.54	0.003	0.011
modified MO Biotic Index	66.64	< 0.001	8	0.96	0.016	0.019
pctg 3 dominant taxa	6.44	0.030	10	0.39	77.130	267.990
pctg Mature gatherers	12.79	0.002	9	0.74	45.960	347.425
pctg Mature Diptera and non-insects	19.37	0.001	10	0.66	38.348	218.051

^a pctg = percentage

Table 13. Metrics calculated from standard data and their maturity-weighted analogues. Means and standard deviations (SD) were based on sampling dates for which the target groups of the metric were present (*n*). Paired comparisons were used for t-test and signed rank test. The number of *n* sampling dates that were positive as a result of the standard metric subtracted from its maturity weighted analogue and the number of sampling dates where the maturity analogue represented a $\pm 5\%$, 10% , and 20% change is given.

Metric ^a	<i>n</i>	Mean \pm SD		<i>t</i> -value	<i>p</i> -value ^b	+/5% ^c	10%/20% ^c
		Standard	Maturity				
pctg EPT	12	44.7 \pm 17.3	44.4 \pm 18.1	-0.67	0.517	4/4	0/0
pctg Ephemeroptera	12	21.3 \pm 10.6	20.3 \pm 9.8	-2.57	0.026*	3/7	3/0
pctg Plecoptera ^d	10	9.9 \pm 14.4	12.3 \pm 17.1	17.50	0.084	7/10	8/4
pctg Trichoptera	12	14.3 \pm 8.1	13.8 \pm 8.2	-1.71	0.114	4/6	4/2
pctg Hydropsychidae	12	8.8 \pm 5.3	8.3 \pm 5.1	-1.71	0.115	4/8	4/3
pctg Philopotamidae	12	4.7 \pm 3.5	4.6 \pm 3.5	-1.61	0.134	3/9	4/2
pctg Chironomidae	12	30.1 \pm 12.0	29.1 \pm 12.7	-1.49	0.164	4/8	3/0
pctg Tanypodinae	12	4.2 \pm 3.5	4.3 \pm 4.2	0.12	0.905	5/10	7/5
pctg Orthoclaadiinae	11	7.2 \pm 6.9	7.4 \pm 7.4	0.63	0.540	5/8	8/4
pctg Chironomini	11	14.9 \pm 9.5	14.3 \pm 10.6	-1.16	0.274	3/9	6/4
pctg Tanytarsini	12	5.6 \pm 4.3	4.9 \pm 4.2	-2.19	0.051	4/10	8/6
Tanypodinae/Chironomidae	12	15.2 \pm 10.1	15.3 \pm 10.1	0.12	0.903	5/10	10/8
Orthoclaadiinae/Chironomidae ^d	11	27.5 \pm 23.0	30.6 \pm 27.5	12.00	0.320	7/7	5/2
Chironomini/Chironomidae	11	45.4 \pm 22.3	44.1 \pm 26.7	-0.78	0.453	5/6	6/3
Tanytarsini/Chironomidae	12	18.0 \pm 8.9	16.2 \pm 9.4	-2.44	0.032*	3/11	6/3
pctg Elmidae	12	14.2 \pm 12.4	15.8 \pm 13.2	3.15	0.009*	10/10	8/6
pctg Diptera and non-insects	12	38.8 \pm 9.3	37.4 \pm 10.1	-2.15	0.054	4/6	3/0
modified MO Biotic Index ^d	12	6.11 \pm 0.55	6.05 \pm 0.61	-35.00	0.003*	1/1	0/0
pctg dominant taxon	12	26.5 \pm 7.1	27.6 \pm 9.5	1.46	0.173	6/6	2/0
pctg dominant 2 taxa	12	42.5 \pm 8.6	44.0 \pm 11.2	1.49	0.164	7/5	2/0
pctg dominant 3 taxa	12	54.2 \pm 9.5	55.7 \pm 11.3	1.76	0.106	10/2	1/0
pctg dominant 4 taxa	12	63.3 \pm 9.0	64.4 \pm 10.7	1.31	0.217	9/2	1/0
pctg dominant 5 taxa	12	70.5 \pm 8.1	71.1 \pm 9.2	0.91	0.382	9/2	0/0
Simpson's Diversity Index ^d	12	0.14 \pm 0.04	0.15 \pm 0.05	17.00	0.203	8/5	2/1
Shannon Diversity Index ^d	12	2.39 \pm 0.22	2.36 \pm 0.26	-20.00	0.129	4/2	1/0
Pielou's J ^d	12	0.70 \pm 0.06	0.68 \pm 0.07	-35.00	0.003*	2/2	1/0
pctg shredder	12	27.5 \pm 12.1	28.6 \pm 14.8	1.18	0.264	6/7	5/0
pctg predator	12	8.2 \pm 4.6	8.4 \pm 5.3	0.48	0.638	6/10	8/2
pctg scraper	12	3.3 \pm 3.4	3.2 \pm 3.6	-0.96	0.356	3/9	6/1
pctg filterers	12	22.7 \pm 12.0	21.1 \pm 12.1	-3.16	0.009*	4/8	4/2
pctg gatherers	12	37.7 \pm 10.9	37.9 \pm 12.0	0.33	0.745	6/6	2/0

^a pctg = percentage

^b *Significant at $p < 0.05$ level.

^c the '+' indicates number of *n* for which the standard metric value subtracted from the maturity analogue was positive; the '5%', '10%', and '20%' indicate the number of *n* that changed $> 5\%$, 10% , and 20% , respectively, in metric value as a result of maturity weighting.

^d Non-parametric signed rank used.

Table 14. Maturity weighted abundances of macroinvertebrate taxa (listed by operational taxonomic unit) used for cluster analysis in the classification of seasons for the Burris Fork in Missouri. All dates of the Model Development Period (20 December 2002 – 28 November 2003) are shown.

Date	<i>Stenonema femoratum</i>	<i>Berosus</i>	<i>Cricotopus/Orthocladius</i>	Planariidae	<i>Rheotanytarsus</i>	<i>Isoperla</i>	<i>Bezzia/ Palpomyia</i>
20-Dec	30	21	55	23	6	0	0
18-Jan	17	3	171	18	21	2	0
19-Feb	13	2	176	4	15	0	3
15-Mar	57	70	69	7	6	1	5
14-Apr	11	7	246	2	17	6	3
22-May	0	0	35	18	15	0	11
6-Aug	0	4	6	1	188	0	10
30-Aug	0	137	0	12	66	0	13
27-Sep	12	50	32	50	116	0	0
24-Oct	14	61	13	47	92	0	0
28-Nov	22	70	130	46	142	0	2

Table 15. Group membership probabilities, similarity, and O/E model results for communities sampled from the Burris Fork in central Missouri. Discriminant function analysis was used to find the probability of group membership to seasons (winter, summer, autumn) determined from temporal variables from all dates covering the Model Development Period (MDP) (20 December 2002 – 28 November 2003). Probabilities of season membership for test dates (t1, t2, and t3) were determined from the MDP discriminant functions.

Date	Predicted Probability			Probability			Pinkham		O/E ₀	O/E ₅₀
	season	winter	summer	autumn	autumn	Pearson's	Percentage similarity			
20-Dec-02	Winter	1.00	0	0	0	0.48	70.50	1.02	1.06	
18-Jan-03	Winter	1.00	0	0	0	0.35	68.55	0.88	0.88	
19-Feb-03	Winter	1.00	0	0	0	0.42	70.61	0.95	1.02	
15-Mar-03	Winter	1.00	0	0	0	0.40	71.90	0.99	0.95	
14-Apr-03	Winter	0.99	0.01	0	0	0.40	56.78	1.16	1.09	
22-May-03	Summer	0.01	0.99	0	0	0.34	70.67	0.93	0.87	
6-Aug-03	Summer	0	1.00	0	0	0.44	83.21	1.04	1.06	
30-Aug-03	Summer	0	1.00	0	0	0.49	80.53	1.04	1.06	
27-Sep-03	Autumn	0	0	1.00	1.00	0.53	74.19	1.01	1.02	
24-Oct-03	Autumn	0	0	1.00	1.00	0.54	86.79	0.92	0.96	
28-Nov-03	Autumn	0	0	1.00	1.00	0.54	78.92	1.07	1.02	
21-Dec-03 (t1)	Winter	1.00	0	0	0	0.47	75.56	1.12	1.09	
23-Mar-04 (t2)	Winter	1.00	0	0	0	0.18	30.26	0.71	0.66	
3-Oct -04 (t3)	Autumn	0	0.01	0.99	0.99	0.16	30.44	0.64	0.58	

Table 16. Physico-chemico-temporal variable measurements from Burris Fork in central Missouri used for metric multiple regressions and discriminant function analysis of RIVPACS models. Dates covering the Model Development Period (20 December 2002 – 28 November 2003) for test dates (t1, t2, and t3) are given.

Date	Dissolved oxygen $\mu\text{g/l}$	percentage Dissolved oxygen	pH	Flow m/sec	Precip 4 ins	Precip 7 ins	Precip 14 ins	Temp $^{\circ}\text{C}$	Degree days $^{\circ}\text{C}$	Degree days $^{\circ}\text{C}$	Degree days $^{\circ}\text{C}$	Julian days
20-Dec-02	8.8	70.3	7.7	0.39	0.98	0.98	0.98	5.8	5.0	66.7	105.8	1
18-Jan-03	13.9	101.1	8.0	0.46	0.11	0.11	0.11	2.0	101.0	19.4	44.5	29
19-Feb-03	10.6	87.6	8.2	0.26	0.29	1.00	1.00	7.2	195.3	25.6	50.1	61
15-Mar-03	9.7	87.6	8.0	0.60	1.19	1.19	1.34	10.8	324.6	56.4	91.5	85
14-Apr-03	6.7	76.9	8.7	0.30	0.00	0.92	1.37	22.3	744.5	93.9	191.6	116
22-May-03	6.2	68.9	8.0	0.52	0.21	0.47	1.11	19.9	1407.7	137.8	275.3	153
30-Jun-03	5.0	66.3	8.2	0.37	2.10	2.10	2.12	29.7	2322.9	189.8	373.8	192
6-Aug-03	4.0	52.8	8.0	0.15	0.06	0.06	0.06	29.2	3372.2	199.0	398.6	228
30-Aug-03	2.9	35.9	7.6	0.14	0.46	0.46	0.51	25.4	4076.5	194.1	402.2	253
27-Sep-03	3.9	42.5	7.8	0.40	0.41	2.75	3.61	19.7	4692.2	139.8	288.4	281
24-Oct-03	6.1	63.0	7.6	0.30	0.00	0.91	1.54	17.1	5127.9	108.5	221.7	308
28-Nov-03	6.9	53.4	8.1	0.49	0.00	0.32	1.75	3.8	5500.1	49.7	123.8	343
21-Dec-03 (t1)	7.7	56.5	8.0	0.43	0.11	0.40	1.56	3.1	2.5	18.0	42.6	1
23-Mar-04 (t2)	6.5	56.6	8.3	0.69	0.02	0.26	0.51	9.1	406.2	71.7	129.4	94
3-Oct-04 (t3)	7.9	78.1	8.3	0.30	0.34	0.34	0.34	14.7	4664.3	121.6	273.5	288

Figure 6. Flow chart depicting the steps to construct a RIVPACS model showing different pathways for constructing expected communities based on occurrence frequency and relative abundances. Taken after Flotemersch et al. (2006).

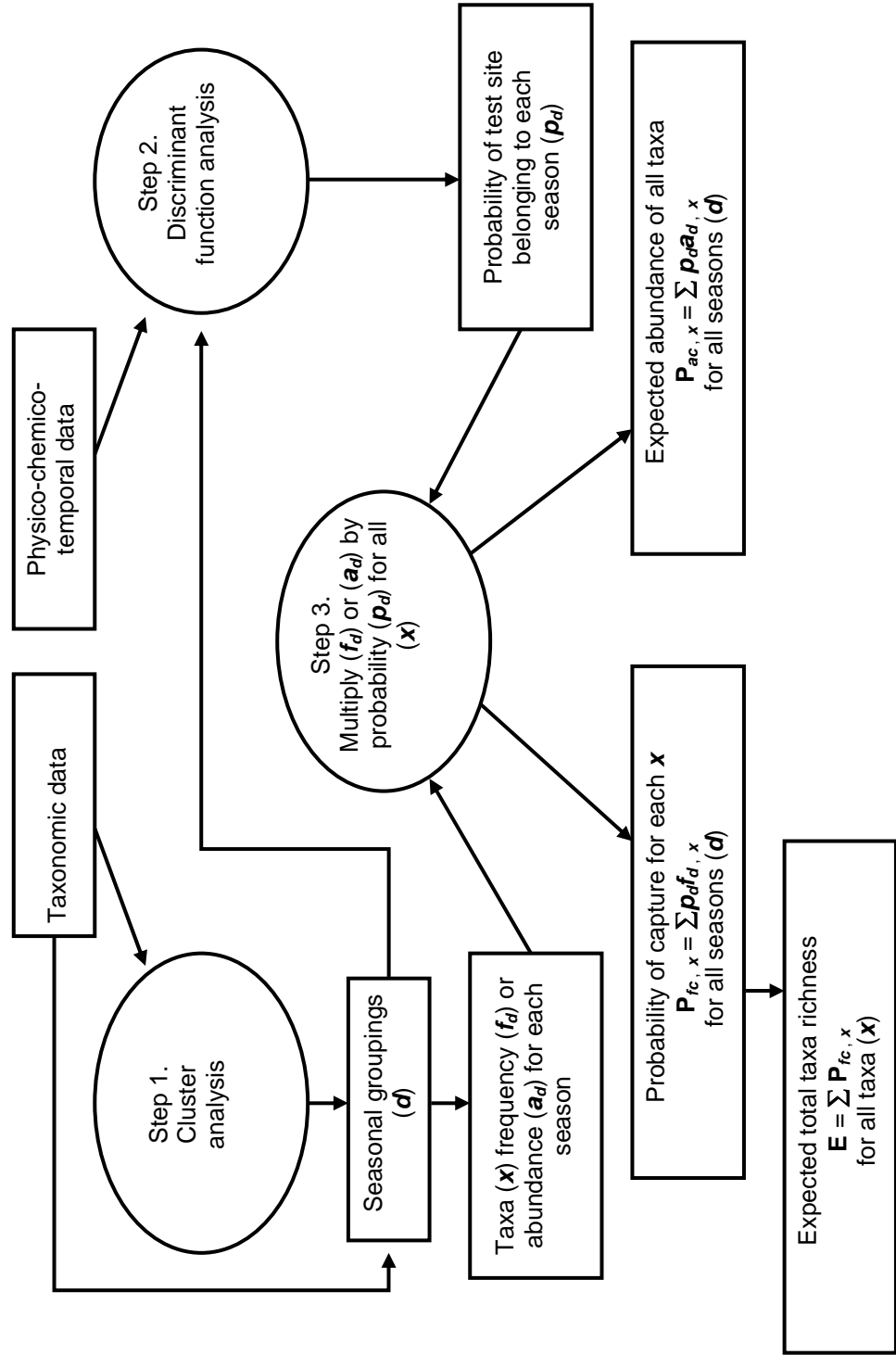


Figure 7. Observed and expected multiple regression trends for selected richness metrics. Vertical lines indicate the end of the Model Development Period (20 December 2002 – 28 November 2003) and the beginning of the test dates.

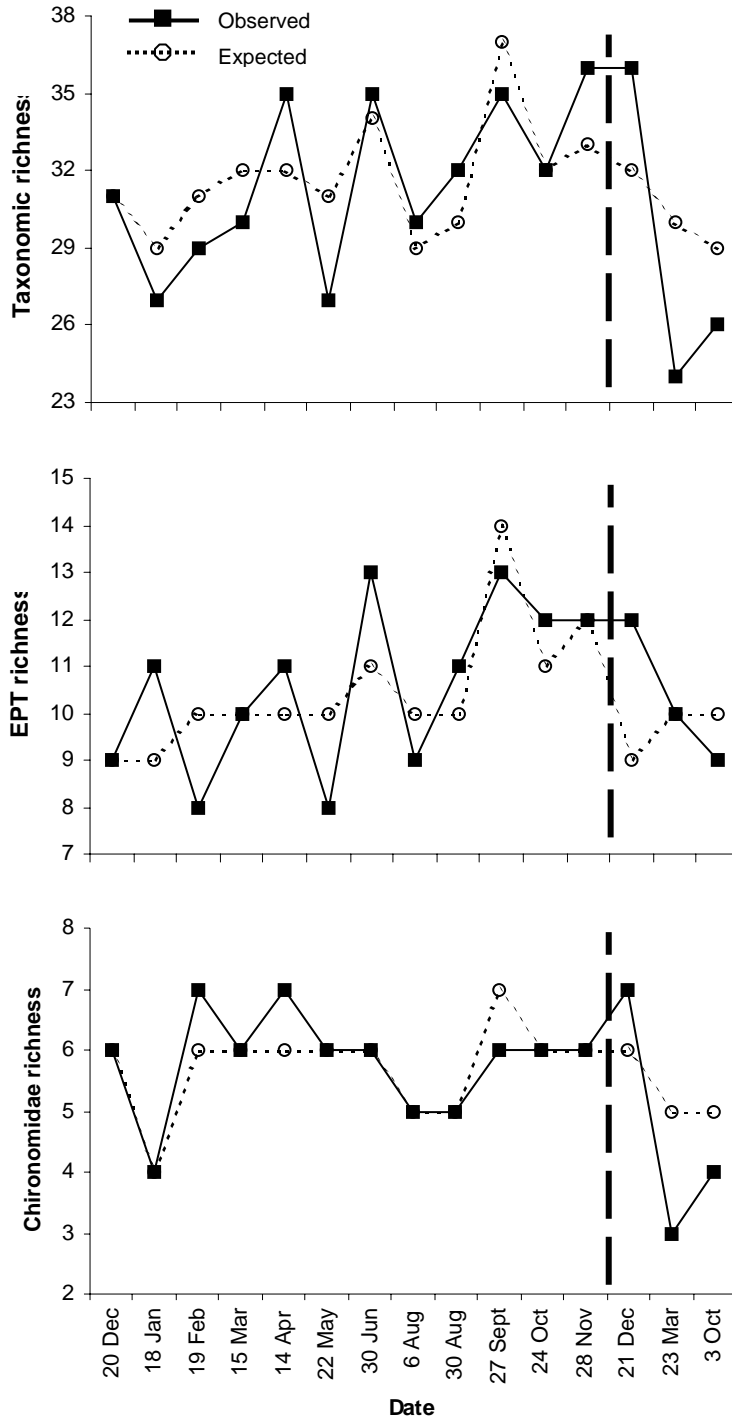


Figure 8. Observed and expected multiple regression trends for selected diversity metrics. Vertical lines indicate the end of the Model Development Period (20 December 2002 – 28 November 2003) and the beginning of the test dates.

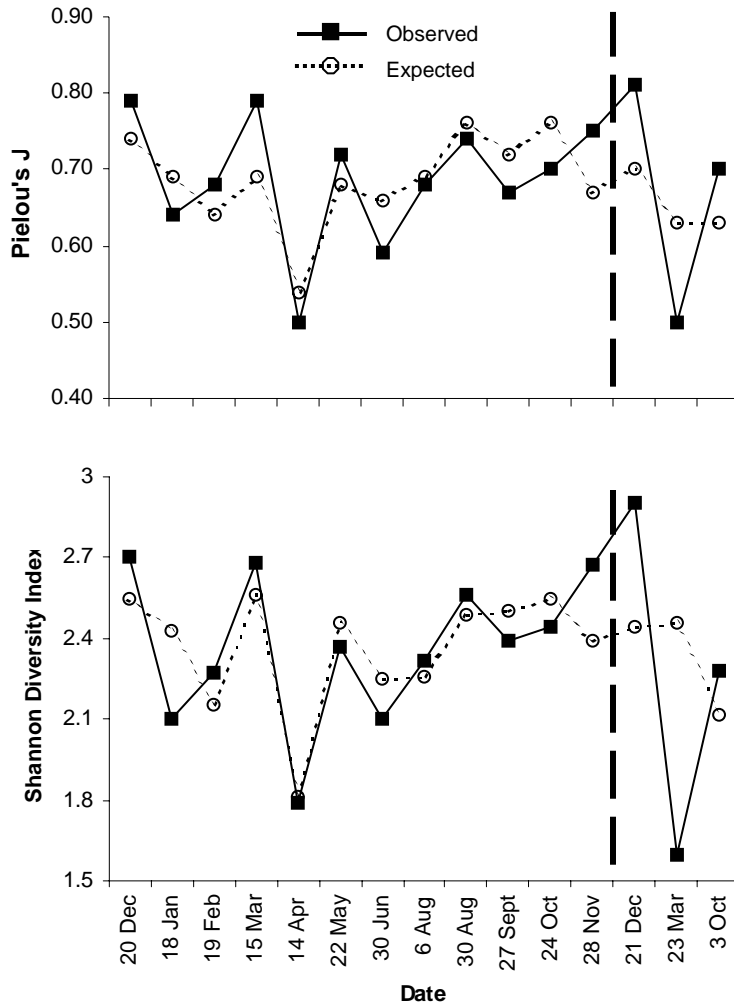


Figure 9. Observed and expected multiple regression trends for selected tolerance metrics. Vertical lines indicate the end of the Model Development Period (20 December 2002 – 28 November 2003) and the beginning of the test dates.

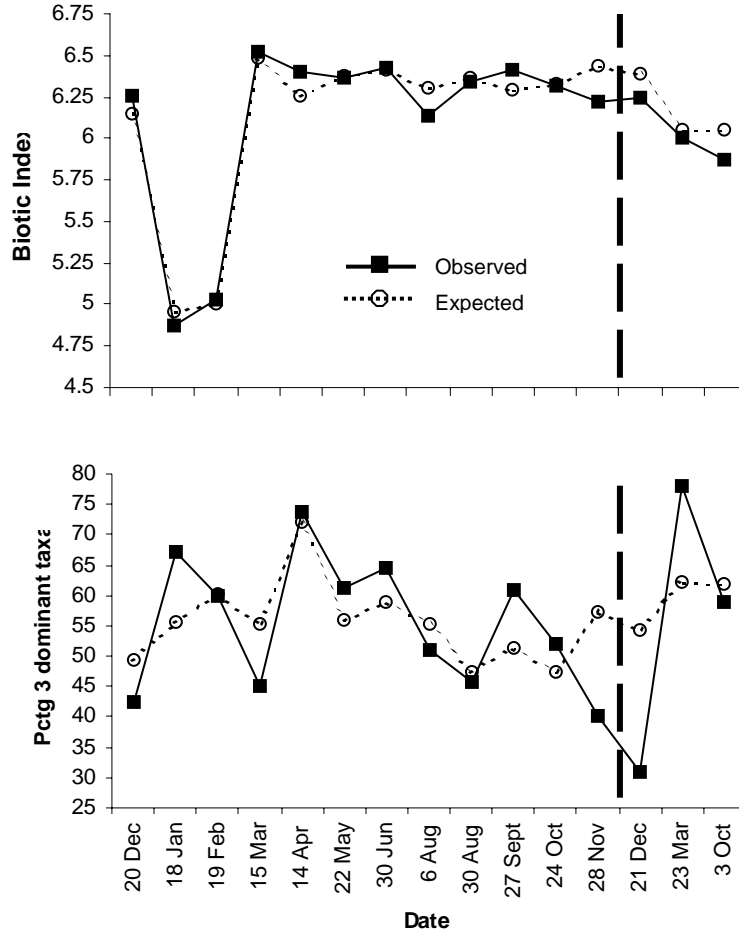


Figure 10. Observed and expected multiple regression trends for selected functional feeding and composition metrics. Vertical lines indicate the end of the Model Development Period (20 December 2002 – 28 November 2003) and the beginning of the test dates.

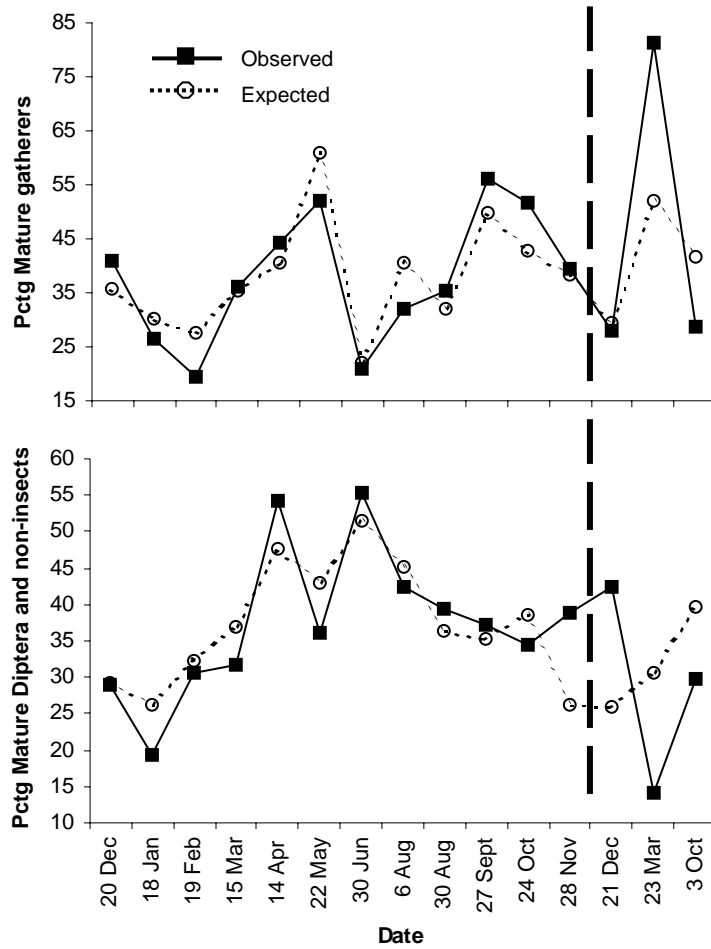


Figure 11. Cluster dendrogram showing sampling date classifications and biologically derived seasons from select Operational Taxonomic Units, based on semi-partial R^2 dissimilarity distance.

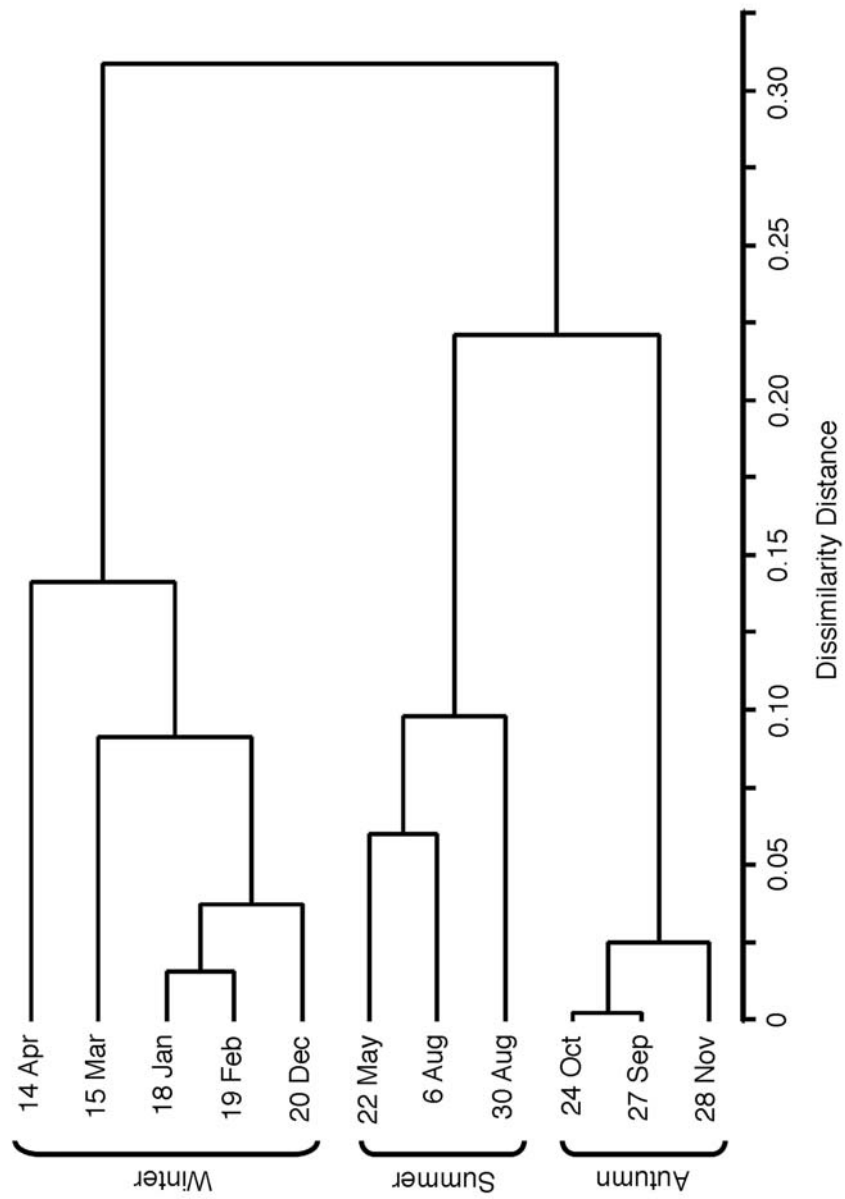


Figure 12. Discriminant function analysis scatter plot of Model Development Period sampling dates (20 December 2002 – 28 November 2003) and test dates (t1, t2, and t3).

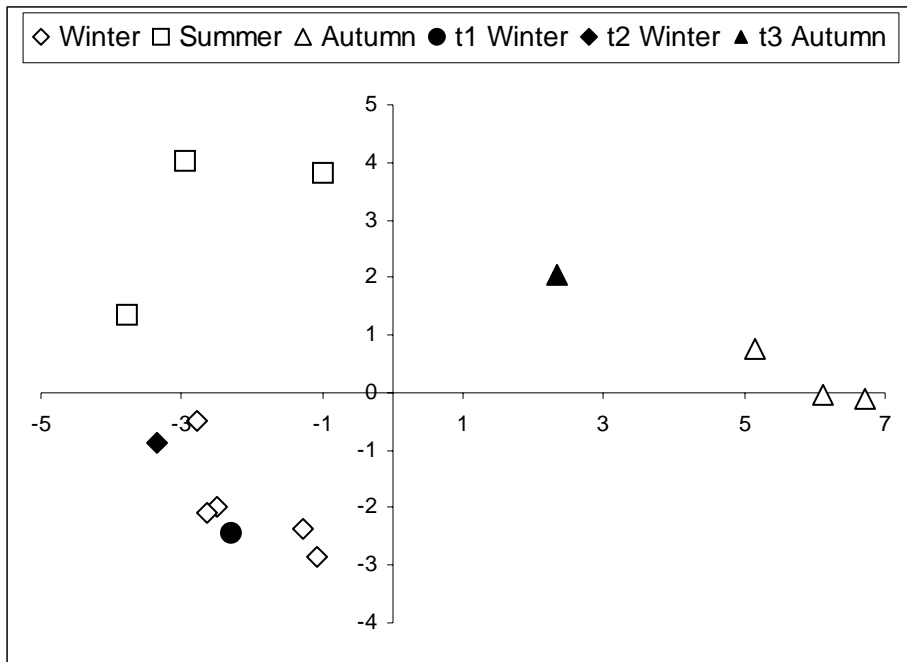
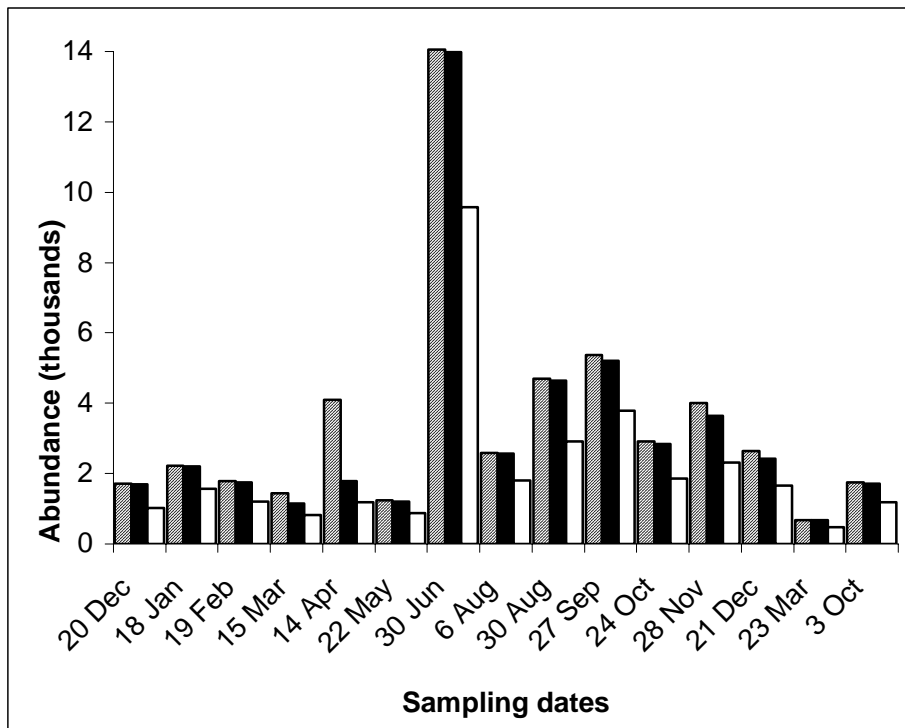


Figure 13. Abundance in thousands per sampling date. Striped bars indicate total abundance, solid black bars indicate total abundance minus taxa without maturity weightings (e.g. Oligochaeta), and white bars indicate maturity weighted abundance.



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Appendix A. Mean number of individuals for taxa of reference and anti-reference streams for sample periods I, II, and III. Only taxa with mean numbers ≥ 1 are included.

Class	Order	Family	Taxon	Reference			Anti-reference		
				I	II	III	I	II	III
Hydrozoa	Hydroida	Hydridae	<i>Hydra</i>				4-3	5	
Turbellaria	Tricladida	Planariidae		61	28-3	1-7	366.7	118	157
Oligochaeta							1		
Hirudinea	Arhynchobdellida	Erpobdellidae	<i>Mooreobdella microstoma</i>				1	2-3	2-3
	Rhynchobdellida	Glossiphoniidae	<i>Helobdella stagnalis</i>				1		
			<i>Helobdella triseriatis</i>				1-3	27	15.7
Gastropoda	Mesogastropoda	Hydrobiidae	<i>Amnicola limosa</i>				4-3		7-3
	Pulmonata	Ancylidae	<i>Ferrissia</i>						2-7
		Planorbidae	<i>Gyraulus parvus</i>				2		4-3
			<i>Micromenetus dilatatus</i>			4	30.7	6.7	11
		Physidae	<i>Physa</i>	2.7		3-7			1-7
Pelecypoda	Veneroidea	Sphaeriidae	<i>Pisidium</i>			2-7	4.7	6	14
			<i>Sphaerium</i>	3	2	2-3			14
Crustacea	Amphipoda	Hyalellidae	<i>Hyalella azteca</i>						14
Insecta	Odonata	Calopterygidae	<i>Calopteryx</i>			1			
		Coenagrionidae	<i>Argia apicalis</i>				3		3
			<i>Enallagma divagans</i>						4-7
		Gomphidae	<i>Progomphus obscurus</i>	1		9-3			2-7
		Libellulidae	<i>Erythemis</i>						1-3
			<i>Epicordulia princeps</i>						3-3
			<i>Perithemis tenera</i>			1-7			
			<i>Plathemis lydia</i>				1		
Ephemeroptera	Caenidae	Caenidae	<i>Caenis</i>	20	15-7	37	105	7.7	49
	Baetidae	Baetidae	<i>Procloeon</i>		1-3	3-3			
	Heptageniidae	Heptageniidae	<i>Stenacron</i>		1	1-7			
			<i>Stenonema femoratum</i>			7			
Hemiptera	Corixidae	Corixidae	<i>Palmacorixa nana</i>			1	4.7		15.3

Appendix A continued.

Class	Order	Family	Taxon	Reference			Anti-reference		
				I	II	III	I	II	III
Megaloptera	Sialidae		<i>Trichocorixa</i>				3.6		12.3
			<i>Stalis</i>		1.3				
Coleoptera	Dytiscidae	Halplidae	<i>Neoporus dimidiatus</i>				1.3		
			<i>Peltoodytes duodecimpunctata</i>						1
			<i>Peltoodytes edentulus</i>						1
			<i>Peltoodytes litoralis</i>						1
			<i>Peltoodytes pedunculatus</i>						1
			<i>Peltoodytes sexmaculatus</i>						1
			<i>Berosus pantherinus</i>			12.3			
Hydrophilidae	Elmidae		<i>Dubiraphia</i>	1.7	1	4			1
			<i>Oecetis</i>			5.3	1		21.7
Trichoptera	Leptoceridae		<i>Bezzia/Palpomyia</i>	4.7	3	6.7	1.3		
			<i>Bezzia</i>	2			9.3		1.7
Diptera	Ceratopogonidae		<i>Ceratopogon</i>	1			7.3		
			<i>Culicoides</i>			1.3	10.7	1.3	1.3
			<i>Dasyhelea</i>				1		
			<i>Probezzia</i>	3.3		3.3	4.7		2
			<i>Sphaeromyias</i>				2		
			<i>Chaoborus</i>			1		2	1

Appendix A continued.

Class	Order	Family	Taxon	Reference			Anti-reference		
				I	II	III	I	II	III
Chironomidae		<i>Ablabesmyia</i>			1	15.3	2		
		<i>Chironomus</i>	11	2.7	1.3	91	5.3	1	
		<i>Cladopelma</i>	1						
		<i>Cladotanytarsus</i>		1.7	7.3			2.3	
		<i>Clinotanytus</i>				2.7		5.3	
		<i>Cricotopus</i>				2			
		<i>Cryptochironomus</i>			1		1	1	
		<i>Dicrotendipes</i>			2.3	1	6.7		
		<i>Glyptotendipes</i>			8		7.7		
		<i>Parachironomus</i>						1.3	
		<i>Paratanytarsus</i>				3	3.7		
		<i>Phaenopsectra</i>	1.3	8.7	2	1	1.3		
		<i>Polypedilum</i>	2.7	30.7	16.3	6	12.3	8	
		<i>Procladius</i>		6.3	11.7	40.3	11	15.7	
		<i>Stictochironomus</i>	8	12	1.7	1.3	2.3		
		<i>Tanytus</i>						13.7	
		<i>Tanytarsus</i>	1	3.7	14.7			5.3	
Tabanidae		<i>Thienemannimyia</i> grp.				1			
		<i>Chrysops</i>				1			

Appendix B. Raw data excluding rare and transient taxa for Chapter 4.

Class	Order	Family	OTU	20-Dec	18-Jan	19-Feb	15-Mar	14-Apr	22-May	30-Jun	6-Aug
Turbellaria	Tricladida	Planariidae	Planariidae	37	29	5	9	3	30	284	3
Gastropoda	Lymnophila	Planorbidae	<i>Micromenetus dilatatus</i>	0	0	0	2	2	1	0	0
	Pulmonata	Ancylidae	<i>Ferrissia fragilis</i>	4	8	3	0	12	0	11	10
		Physidae	<i>Physa</i>	80	32	53	53	20	3	4	15
Pelecypoda	Veneroidea	Sphaeriidae	<i>Sphaerium</i>	135	1	4	76	6	15	312	8
Oligochaeta			Oligochaeta	26	4	50	298	2306	36	68	26
Insecta	Ephemeroptera	Leptophlebiidae	<i>Choroterpes</i>	0	0	0	0	0	0	1	0
		Baetidae	<i>Aeripenna</i>	0	0	0	0	8	3	21	0
			<i>Baetis</i>	0	0	0	0	12	37	514	3
		Caenidae	<i>Caenis</i>	303	92	179	210	169	0	6	23
		Tricorythidae	<i>Tricorythodes</i>	288	446	117	57	134	16	944	199
		Heptageniidae	<i>Stenacron</i>	3	2	0	10	8	0	1	1
			<i>Stenonema femoratum</i>	52	30	22	77	16	0	7	0
Odonata		Calopterygidae	<i>Hetaerina</i>	1	2	0	0	0	0	2	10
		Coenagrionidae	<i>Argia</i>	30	8	5	3	10	0	1	3
Plecoptera		Capniidae	<i>Allocapnia</i>	136	787	691	144	0	0	0	0
		Perlidae	<i>Neoperla harpi</i>	0	1	0	0	0	26	5	0
			<i>Perlesta decipiens</i>	0	0	0	8	32	27	0	0
		Perlidae	<i>Isoperla</i>	0	3	0	2	6	0	0	0
Trichoptera		Hydropsychidae	<i>Cheumatopsyche</i>	133	252	75	18	24	132	2572	375
			<i>Hydropsyche</i>	0	0	0	0	0	0	9	26
		Hydroptilidae	<i>Hydroptila</i>	28	17	2	6	30	16	95	6
		Leptoceridae	<i>Oecetis</i>	1	5	1	0	0	0	4	1
		Philopotamidae	<i>Chimarra</i>	128	89	31	15	7	7	1077	268

Appendix B continued.

Class	Order	Family	OTU	20-Dec	18-Jan	19-Feb	15-Mar	14-Apr	22-May	30-Jun	6-Aug
	Coleoptera	Elmidae	<i>Stenelmis</i>	7	5	4	4	27	63	110	59
			<i>Stenelmis lateralis</i>	6	2	0	10	21	35	30	26
			<i>Stenelmis sextineata</i>	40	40	10	98	351	375	1094	375
	Hydrophilidae		<i>Berosus</i>	34	4	2	74	7	0	0	6
Diptera	Tipulidae		<i>Hexatoma</i>	4	0	0	9	1	1	19	5
	Simuliidae		<i>Simulium</i>	9	13	26	0	5	4	7	1
	Ceratopogonidae		Ceratopogonidae	1	0	2	7	7	1	4	0
			<i>Bezzia/Palpomyia</i>	0	0	5	7	3	12	11	14
	Chironomidae		<i>Cricotopus/Orthocladius</i>	75	195	206	111	352	65	170	10
			<i>Eukiefferiella</i>	0	18	45	9	55	0	40	0
			<i>Dicrotendipes</i>	6	0	18	0	30	9	0	0
			<i>Polypedilum</i>	33	0	63	53	157	251	4583	571
			<i>Rheotanytarsus</i>	12	45	36	9	20	37	1936	339
			<i>Tanytarsus</i>	19	0	18	18	10	18	30	19
			<i>Thienemannimyia</i> grp.	72	82	108	33	228	9	90	169
	Tabanidae		<i>Hybomitra</i>	5	0	7	11	4	5	4	8
	Empididae		<i>Hemerodromia</i>	3	0	3	0	3	0	9	7

Appendix B continued.

Class	Order	Family	OTU	30-Aug	27-Sep	24-Oct	28-Nov	21-Dec	23-Mar	3-Oct
Turbellaria	Tricladida	Planariidae	Planariidae	17	73	69	72	21	5	67
Gastropoda	Lymnophila	Planorbidae	<i>Micromenetus dilatatus</i>	0	1	1	1	6	0	0
	Pulmonata	Ancylidae	<i>Ferrissia fragilis</i>	78	12	5	26	53	0	5
		Physidae	<i>Physa</i>	85	17	3	8	89	0	0
Pelecypoda	Veneroida	Sphaeriidae	<i>Sphaerium</i>	27	490	370	318	217	7	68
Oligochaeta			Oligochaeta	63	159	72	378	206	6	25
Insecta	Ephemeroptera	Leptophlebiidae	<i>Choroterpes</i>	0	5	8	0	0	0	0
		Baetidae	<i>Acerpenna</i>	2	23	1	4	0	1	0
			<i>Baetis</i>	1	60	30	1	0	0	33
		Caenidae	<i>Caenis</i>	173	76	59	304	254	1	6
		Tricorythidae	<i>Tricorythodes</i>	523	822	802	803	141	0	24
		Heptageniidae	<i>Stenacron</i>	4	12	15	0	10	2	50
			<i>Stenonema femoratum</i>	0	16	21	41	35	3	1
Odonata		Calopterygidae	<i>Hetaerina</i>	17	20	0	1	1	0	2
		Coenagrionidae	<i>Argia</i>	54	16	13	4	7	0	0
Plecoptera		Capniidae	<i>Allocapnia</i>	0	0	0	162	289	2	0
		Perlidae	<i>Neoperla harpi</i>	4	6	0	7	2	0	1
			<i>Perlستا decipiens</i>	0	0	0	0	2	12	0
		Perlidae	<i>Isoperla</i>	0	0	0	0	6	23	0
Trichoptera		Hydropsychidae	<i>Cheumatopsyche</i>	533	194	221	355	269	10	489
			<i>Hydropsyche</i>	22	30	1	3	1	0	6
		Hydroptilidae	<i>Hydroptila</i>	21	3	2	8	8	0	0
		Leptoceridae	<i>Oecetis</i>	8	24	12	2	0	1	0
		Philopotamidae	<i>Chimarra</i>	438	174	136	203	64	5	326

Appendix B continued.

Class	Order	Family	OTU	30-Aug	27-Sep	24-Oct	28-Nov	21-Dec	23-Mar	3-Oct
	Coleoptera	Elmidae	<i>Stenelmis</i>	55	145	14	8	11	4	18
			<i>Stenelmis lateralis</i>	111	145	29	15	18	77	45
			<i>Stenelmis sextineata</i>	581	1459	320	118	159	420	214
	Hydrophilidae		<i>Berosus</i>	209	69	68	107	62	5	0
Diptera	Tipulidae		<i>Hexatoma</i>	3	1	0	1	4	1	4
	Simuliidae		<i>Simulium</i>	0	0	9	25	66	20	2
	Ceratopogonidae	Ceratopogonidae		1	1	0	6	4	0	0
			<i>Bezzia/Palpomyia</i>	16	0	0	3	0	2	1
	Chironomidae		<i>Cricotopus/Orthocladius</i>	0	39	30	212	242	29	9
			<i>Eukiefferiella</i>	0	0	10	19	10	0	0
			<i>Dicrotendipes</i>	29	10	0	0	10	0	0
			<i>Polypeditum</i>	1031	988	343	437	120	21	147
			<i>Rheotanytarsus</i>	119	155	182	203	90	0	146
			<i>Tanytarsus</i>	140	58	9	48	101	12	0
			<i>Thienemannimyia</i> grp.	318	58	57	108	30	0	49
	Tabanidae		<i>Hybomitra</i>	19	9	4	5	7	5	1
	Empididae		<i>Hemerodromia</i>	2	4	2	4	7	0	5

Appendix C. Raw maturity weighted data excluding rare and transient taxa for Chapter 4.

Class	Order	Family	OTU	20-Dec	18-Jan	19-Feb	15-Mar	14-Apr	22-May	30-Jun	6-Aug
Gastropoda	Tricladida	Planariidae	Plathyhelminthes	23	18	4	7	2	17	194	1
	Lymnophila	Planorbidae	Micromenetus dilatatus	0	0	0	2	2	1	0	0
	Pulmonata	Ancylidae	Ferrissia fragilis	2	5	2	0	9	0	7	6
Pelecypoda	Veneroida	Physidae	Physa	53	22	37	38	8	1	1	9
		Sphaeriidae	Sphaerium	72	1	3	44	4	9	222	5
Insecta	Ephemeroptera	Leptophlebiidae	Choroterpes	0	0	0	0	0	0	1	0
		Baetidae	Acerpenna	0	0	0	0	7	2	17	0
	Caenidae	Tricorythidae	Baetis	0	0	0	0	9	33	367	3
			Caenis	147	51	105	135	101	0	3	21
			Tricorythodes	179	285	74	38	88	10	605	149
	Odonata	Heptageniidae	Stenacron	2	1	0	6	5	0	1	1
			Stenonema femoratum	30	17	14	57	12	0	4	0
			Hetaerina	1	2	0	0	0	0	1	8
			Argia	11	4	2	2	4	0	0	1
			Allocapnia	96	613	565	120	0	0	0	0
Plecoptera	Perlidae	Neoperla harpi	0	1	0	0	0	0	19	4	0
		Perlesta decipiens	0	0	0	3	11	25	0	0	
		Isoperla	0	2	0	1	5	0	0	0	
		Cheumatopsyche	84	163	41	16	15	76	1618	251	
Trichoptera	Hydropsychidae	Hydropsyche	0	0	0	0	0	0	0	8	18
		Hydroptila	25	16	2	4	22	9	70	4	
		Oecetis	1	4	1	0	0	0	3	1	
		Chimarra	76	55	16	12	5	4	795	188	

Appendix C continued.

Class	Order	Family	OTU	20-Dec	18-Jan	19-Feb	15-Mar	14-Apr	22-May	30-Jun	6-Aug	
Coleoptera	Elmidae	Stenelmis		7	5	4	4	17	50	69	50	
		Stenelmis lateralis		6	2	0	8	13	28	19	22	
Diptera		Stenelmis sexlineata		40	40	10	79	223	299	683	320	
		Hydrophilidae	Berosus	21	2	1	70	7	0	0	6	
		Tipulidae	Hexatoma	3	0	0	9	1	1	1	12	4
		Simuliidae	Simulium	6	8	14	0	3	2	2	7	1
		Ceratopogonidae	Ceratopogonidae	1	0	1	2	6	1	1	4	0
		Ceratopogonidae	Bezzia/Palpomyia	0	0	3	5	3	11	9	10	
		Chironomidae	Cricotopus/Orthocladius	57	172	176	69	244	35	105	6	
			Eukiefferiella	0	12	27	6	34	0	20	0	
			Dicrotendipes	2	0	6	0	20	6	0	0	
			Polypedilum	13	0	21	23	96	200	3233	373	
			Rheotanytarsus	6	21	15	6	17	16	1400	188	
			Tanytarsus	11	0	6	9	7	6	20	9	
	Thienemannimyia grp	42	43	48	26	183	3	56	141			
	Tabanidae	Hybomitra	4	0	5	9	3	4	3	6		
	Empididae	Hemerodromia	2	0	2	0	2	0	7	5		

Appendix C continued.

Class	Order	Family	OTU	30-Aug	27-Sep	24-Oct	28-Nov	21-Dec	23-Mar	3-Oct
	Tricladida	Planariidae	Plathyhelminthes	12	50	47	47	14	5	48
Gastropoda	Lymnophila	Planorbidae	Micromenetus dilatatus	0	1	1	1	4	0	0
	Pulmonata	Ancylidae	Ferrissia fragilis	48	8	4	12	33	0	4
		Physidae	Physa	44	11	2	4	61	0	0
Pelecypoda	Veneroida	Sphaeriidae	Sphaerium	18	291	228	198	146	2	45
Insecta	Ephemeroptera	Leptophlebiidae	Choroterpes	0	5	8	0	0	0	0
		Baetidae	Acerpenna	1	23	1	2	0	1	0
			Baetis	0	46	29	1	0	0	24
		Caenidae	Caenis	116	48	30	188	128	1	3
		Tricorythidae	Tricorythodes	331	545	512	499	86	0	16
		Heptageniidae	Stenacron	1	8	8	0	6	1	25
			Stenonema femoratum	0	11	14	23	30	3	0
Odonata		Calopterygidae	Hetaerina	10	8	0	1	0	0	1
		Coenagrionidae	Argia	25	9	8	2	4	0	0
Plecoptera		Capniidae	Allocapnia	0	0	0	126	244	2	0
		Perlidae	Neoperla harpi	1	3	0	3	1	0	1
			Perlesta decipiens	0	0	0	0	1	6	0
		Perlodidae	Isoperla	0	0	0	0	3	12	0
Trichoptera		Hydropsychidae	Cheumatopsyche	361	113	141	230	196	6	315
			Hydropsyche	17	21	1	2	1	0	5
		Hydroptilidae	Hydroptila	15	2	2	4	7	0	0
		Leptoceridae	Oecetis	4	15	6	1	0	0	0
		Philopotamidae	Chimarra	270	114	82	121	38	2	222

Appendix C continued.

Class	Order	Family	OTU	30-Aug	27-Sep	24-Oct	28-Nov	21-Dec	23-Mar	3-Oct
Coleoptera	Elmidae	Stenelmis		35	113	12	8	10	3	15
		Stenelmis lateralis		70	113	25	15	16	58	37
Diptera	Hydrophilidae	Stenelmis sexlineata		367	1135	275	113	140	314	173
		Berosus		137	51	61	70	45	4	0
		Hexatoma		2	1	0	1	3	1	3
		Simulium		0	0	5	16	45	10	1
		Ceratopogonidae		1	0	0	2	1	0	0
		Ceratopogonidae	Bezzia/Palpomyia	13	0	0	2	0	2	0
		Chironomidae	Cricotopus/Orthocladius	0	32	13	130	200	24	1
			Eukiefferiella	0	0	7	12	10	0	0
			Dicrotendipes	16	6	0	0	7	0	0
			Polypedilum	645	808	192	211	57	11	91
Tabanidae		Rheotanytarsus		66	115	92	141	44	0	116
		Tanytarsus		79	42	6	22	47	8	0
		Thienemannimyia grp		190	32	37	91	24	0	42
		Hybomitra		14	7	2	4	5	4	1
Empididae										
		Hemerodromia		1	3	1	2	3	0	2

Appendix D. SAS program and included documentation used to calculate metrics for Chapter 4.

```

/*This SAS program calculates the 64 metrics: taxa richness; Ephemeroptera richness;
Plecoptera richness; Trichoptera richness; Chironomidae richness; EPT richness; Elmidae
abundance; total abundance; %Elmidae; Diptera and noninsect abundance; %Diptera and
noninsect; EPT abundance; %Chironomidae; Ephemeroptera abundance; Plecoptera
abundance; Trichoptera abundance; %EPT; EPT/Chironomidae; %Ephemeroptera;
%Plecoptera; %Trichoptera; Hydropsychidae abundance; %Hydropsychidae; Oligochaeta
abundance; %Oligochaeta; Philopotamidae abundance; %Philopotamidae; Orthoclaadiinae
abundance; Tanypodinae abundance; Tanytarsini abundance; Chironomini abundance;
%Chironomidae; %Orthoclaadiinae; Orthoclaadiinae/Chironomidae;
Tanypodinae/Chironomidae; %Tanypodinae; %Tanytarsini; Tanytarsini/Chironomidae;
%Chironomini; Chironomini/Chironomidae; Shredder abundance; %Shredder; Predator
abundance; %Predator; Scraper abundance; %Scraper; Filterer abundance; %Filterer;
Gathering collector abundance; %Gathering collector; HBI; Simpson's diversity;
Shannon Diversity Index; Pielou's J; %Dominant taxon; %2dominant taxa; %3dominant
taxa; %4dominant taxa; %5dominant taxa; Dominant2 taxa abundance; Dominant3 taxa
abundance; Dominant4 taxa abundance; Dominant5 taxa abundance; Dominant taxon
abundance. The code can be easily manipulated to include maturity structuring. The
variables of the input statement include: date1=the date a sample was taken; grab=the
composite number taken; class=the class of the organism; order=the order of the
organism; family=the family of the organism; subfam=the subfamily of the organism;
tribe=the tribe of the organism; code=unique code for each OTU; num= number of
individuals counted; bi=tolerance value; ffg=functional feeding group; age1=maturity
class 1; age2=maturity class 2; age3=maturity class 3; age4=maturity class 4. Note that
that dates in the if/then statements read 'XXXXX' and should be replaced with
appropriate dates of the dataset so that dates are represented sequentially for all dates.*/

```

```

data truth;
input date1$ grab$ class$ order$ family$ subfam$ tribe$ code num bi ffg$ age1
      age2 age3 age4;
if date1='XXXXX' then date=1;
if date1=' XXXXX ' then date=2;
if date1=' XXXXX ' then date=3; /*etc...*/
cards;
;
/*data truth2; set truth; /*This term will allow for maturity weighting*/
      num=(.33*age1)+(.67*age2)+age3+age4;
run;*/
proc sort data=truth out=prenograb;
      by date code;
run;
proc means data=prenograb noprint; /*produces a dataset that has combined the
numbers of */
      var num; /*individuals for each unique taxon; removes grabs.*/
      by date code bi;
      output out=nograb sum=num2;
run;

```

```

proc sort data=truth out=filt; /*INITIAL CALCULATION FOR %FILTERERS*/
    by date ;                               /*Selects for filtering collectors*/
    where ffg='Fi' ; /*or ffg='Co,Fi'*/
run;
proc means data=filt noprint; /*calculates the sum of all filterers*/
    var num;
    by date ;
    output out=filtsum    sum=fsum;
run;
proc sort data=truth;
    by date ;
run;
proc means data=truth noprint; /*produces the total number of individuals*/
    var num;
    by date ; /*can be modified with grab to look at total numbers per grab*/
    output out=total    sum=total;
run;
proc sort data=filtsum;
    by date ;
run;
data filter;
    merge filtsum total;
    by date ;
    filter=100*fsum/total; /*takes the 100% ratio of filterers*/
run;
proc sort data=truth out=pred; /*INITIAL CALCULATION FOR %PREDATORS*/
    by date ;                               /*Selects for Predators*/
    where ffg='Pr,Co' or ffg='Pr' /*or ffg='Co,Pr' */or ffg='Pr,He';
run;
proc means data=pred noprint; /*calculates the sum of all Predators*/
    var num;
    by date ;
    output out=predsum    sum=predsum;
run;
proc sort data=predsum;
    by date ;
run;
data predator;
    merge predsum total;
    by date ;
    predator=100*predsum/total; /*takes the 100% ratio of Predators*/
run;
proc sort data=truth out=col; /*INITIAL CALCULATION FOR %COLLECTORS*/
    by date ;
    where ffg='Co' or ffg='Co,Sh' or ffg='Co,Fi' or ffg='Co,Sc' or ffg='Co,Pr';
run; /*Selects for Primary Collectors*/
proc means data=col noprint; /*calculates the sum of all Primary Collectors*/
    var num;
    by date ;
    output out=colsum    sum=colsum;
run;

```

```

proc sort data=colsum;
    by date ;
run;
data collect;
    merge colsum total;
    by date ;
    collect=100*colsum/total;          /*takes the 100% ratio of Primary
Collectors*/
run;
proc sort data=truth out=scrap;      /*INITIAL CALCULATION FOR %SCRAPERS*/
    by date ;
    where ffg='Sc' or ffg='Sc,Co' /*or ffg='Co,Sc'or ffg='He,Sc'*/ ;
run;                                  /*Selects for Scrapers*/
proc means data=scrap noprint;      /*calculates the sum of all Scrapers*/
    var num;
    by date ;
    output out=scrapsum sum=scrapsum;
run;
proc sort data=scrapsum;
    by date ;
run;
data scraper;
    merge scrapsum total;
    by date ;
    scraper=100*scrapsum/total;      /*takes the 100% ratio of Scrapers*/
run;
proc sort data=truth out=shred;      /*INITIAL CALCULATION FOR %SHREDDERS*/
    by date ;
    where ffg='Sh' /*or ffg='Co,Sh' */ or ffg='Sh,Co';    /*Selects for Shredders*/
run;
proc means data=shred noprint;      /*calculates the sum of all shredders*/
    var num;
    by date ;
    output out=shredsum sum=shredsum;
run;
proc sort data=shredsum;
    by date ;
run;
data shredder;
    merge shredsum total;
    by date ;
    shredder=100*shredsum/total;    /*takes the 100% ratio of shredders*/
run;
proc sort data=truth nodupkey out=prerich; /*This eliminates redundant taxa*/
    by date code;                    /*so that richness measures can*/
run;                                  /*be calculated.*/
proc means data=prerich noprint;
    by date;
    output out=rich    n=rich;
run;
proc means data=prerich noprint;

```

```

        by date;
        where order='Ephemero';
        output out=Erich    n=Erich;
run;
proc means data=prerich noprint;
    by date;
    where family='Chironom';
    output out=chirich    n=Chirich;
run;
proc means data=prerich noprint;
    by date;
    where order='Plecopte';
    output out=Prich    n=Prich;
run;
proc means data=prerich noprint;
    by date;
    where order='Trichopt';
    output out=Trich    n=Trich;
run;
data richness; merge rich Erich Prich Trich chirich;
    by date;
    if Prich=. then Prich=0;
    EPTrich=Erich+ Prich +Trich;
run;
data prebi;    set nograb;           /*Takes out all taxa that do not have a bi value*/
    by date;
    if bi=. then delete;
run;
proc means data=prebi noprint;      /*Calculates the total individuals with a bi value*/
    var num2;
    by date;
    output out=totalbi    sum=totalbi;
run;
data biprep;    merge prebi totalbi; /*pre calculates the biotic index*/
    prebi=num2*bi/totalbi;
    by date;
run;
proc means data=biprep noprint;     /*Finalizes the calculation of the biotic index*/
    var prebi;
    by date;
    output out=bi    sum=HBI;
run;
data predives; merge nograb total rich; /*This bit of code combines preps for the
Diversity indices*/
    by date;
    preD=(num2*(num2-1))/(total*(total-1));
    rat=num2/total;
    lograt=log(num2/total);
    presdi=-rat*lograt;
    prepJ=log(rich);
    preJ=presdi/prepJ;

```

```

run;
proc means data=predives noprint;
    var preJ;
    by date;
    output out=J sum=J;
run;
proc means data=predives noprint;
    var preD;
    by date;
    output out=D sum=D;
run;
proc means data=predives noprint;
    var presdi;
    by date;
    output out=sdi sum=sdi;
run;
proc sort data=nograb out=predom; /*preps data for calculation of %dominanat taxon*/
    by date num2;
run;
proc means data=predom noprint;
    var num2;
    by date;
    output out=domtax max=domtax;
run;
proc sort data=nograb out=nograbdm;
    by date descending num2;
run;
/*This next section deals with 15 sample dates, but more code can be added to account
for new sampling dates*/
data dateI dateII dateIII dateIV dateV dateVI dateVII dateVIII dateIX dateX
dateXI dateXII dateXIII dateXIV dateXV;
    set nograbdm; /*separates each of the dates as their own dataset*/
    if date = 1 then output dateI;
    if date = 2 then output dateII;
    if date = 3 then output dateIII;
    if date = 4 then output dateIV;
    if date = 5 then output dateV;
    if date = 6 then output dateVI;
    if date = 7 then output dateVII;
    if date = 8 then output dateVIII;
    if date = 9 then output dateIX;
    if date = 10 then output dateX;
    if date = 11 then output dateXI;
    if date = 12 then output dateXII;
    if date = 13 then output dateXIII;
    if date = 14 then output dateXIV;
    if date = 15 then output dateXV;
run;
/*these next 4 datasets are created with only the highest values from that of each of the
dates merged back into a single data set.*/

```

```

data top2; merge dateI(obs=2) dateII(obs=2) dateIII(obs=2) dateIV(obs=2)
dateV(obs=2)
dateVI(obs=2) dateVII(obs=2) dateVIII(obs=2) dateIX(obs=2) dateX(obs=2)
dateXI(obs=2)
dateXII(obs=2) dateXIII(obs=2) dateXIV(obs=2) dateXV(obs=2);
by date;
run;
data top3; merge dateI(obs=3) dateII(obs=3) dateIII(obs=3) dateIV(obs=3)
dateV(obs=3)
dateVI(obs=3) dateVII(obs=3) dateVIII(obs=3) dateIX(obs=3) dateX(obs=3)
dateXI(obs=3)
dateXII(obs=3) dateXIII(obs=3) dateXIV(obs=3) dateXV(obs=3);
by date;
run;
data top4; merge dateI(obs=4) dateII(obs=4) dateIII(obs=4) dateIV(obs=4)
dateV(obs=4)
dateVI(obs=4) dateVII(obs=4) dateVIII(obs=4) dateIX(obs=4) dateX(obs=4)
dateXI(obs=4)
dateXII(obs=4) dateXIII(obs=4) dateXIV(obs=4) dateXV(obs=4);
by date;
run;
data top5; merge dateI(obs=5) dateII(obs=5) dateIII(obs=5) dateIV(obs=5)
dateV(obs=5)
dateVI(obs=5) dateVII(obs=5) dateVIII(obs=5) dateIX(obs=5) dateX(obs=5)
dateXI(obs=5)
dateXII(obs=5) dateXIII(obs=5) dateXIV(obs=5) dateXV(obs=5);
by date;
run;
proc means data=top2 noprint;                                /*The total number of numbers of the
dominant taxa are calculated*/
    var num2;
    by date;
    output out=predom2 sum=predom2;
run;
proc means data=top3 noprint;
    var num2;
    by date;
    output out=predom3 sum=predom3;
run;
proc means data=top4 noprint;
    var num2;
    by date;
    output out=predom4 sum=predom4;
run;
proc means data=top5 noprint;
    var num2;
    by date;
    output out=predom5 sum=predom5;
run;
data dom2to5; merge predom2 predom3 predom4 predom5 total;
    by date;                                /*the dominant taxa percents are calculated.*/

```

```

dom2=100*predom2/total;
dom3=100*predom3/total;
dom4=100*predom4/total;
dom5=100*predom5/total;
run;
proc sort data=truth; /*INITIAL CALCULATION OF EPTCHI*/
  by date ;
run;
proc means data=truth noprint; /*calculates the sum of all EPT*/
  var num;
  by date ;
  where order='Plecopte' or order='Ephemero' or order='Trichopt';
  output out=ept sum=ept;
run;
proc means data=truth noprint; /*calculates the sum of all Ephemeroptera*/
  var num;
  by date ;
  where order='Ephemero' ;
  output out=Esum sum=Esum;
run;
proc means data=truth noprint; /*calculates the sum of all Plecoptera*/
  var num;
  by date ;
  where order='Plecopte' ;
  output out=Psum sum=Psum;
run;
proc means data=truth noprint; /*calculates the sum of all Trichoptera*/
  var num;
  by date ;
  where order='Trichopt';
  output out=Trsum sum=Trsum;
run;
proc sort data=truth;
  by date ;
run;
proc means data=truth noprint; /*produces the total number of Chironomidae*/
  var num;
  by date ; /*can be modified with grab to look at total numbers per grab*/
  where family='Chironom';
  output out=chi sum=chi;
run;
proc means data=truth noprint; /*produces the total number of individuals*/
  var num;
  by date ; /*can be modified with grab to look at total numbers per grab*/
  output out=total sum=total;
run;
data eptchi;
  merge ept chi total Esum Psum Trsum ;
  by date ;
  if Psum=. then Psum=0;
  perept=100*ept/total; /*calculates percent EPT*/

```

```

    eptchi=100*ept/chi;          /*takes the 100% ratio of EPT/Chironomidae*/
    E=100*Esum/total;  /*calculates percent Ephemeroptera*/
    P=100*Psum/total;  /*calculates percent Plecoptera*/
    Tr=100*Trsum/total; /*calculates percent Trichoptera*/
run;
proc sort data=truth out=dipanoin; /*INITIAL CALCULATION OF %DIPTERA AND
NONINSECTS*/
    by date code;
    where class not='Insecta' or order='Diptera';
run;
proc means data=dipanoin noprint; /*calculates the sum of all Diptera and
nonInsects*/
    var num;
    by date ;
    output out=noinsum sum=noinsum;
run;
proc sort data=noinsum;
    by date ;
run;
data noinsect;
    merge noinsum total;
    by date ;
    noinsect=100*noinsum/total; /*takes the 100% ratio of Diptera and non-
insects*/
run;
proc sort data=truth out=elm;          /*INITAIL CALCULATION OF %ELMIDAE*/
    by date code;                      /*Selects for Elmidae*/
    where family='Elmidae';
run;
proc means data=elm noprint;          /*calculates the sum of all Elmidae*/
    var num;
    by date ;
    output out=elmsum sum=elmsum;
run;
proc sort data=elmsum;
    by date ;
run;
data Elmidae;
    merge elmsum total;
    by date ;
    Elmidae=100*elmsum/total;          /*takes the 100% ratio of Elmidae*/
run;
proc sort data=truth out=hydro;       /*INITAIL CALCULATION OF
%HYDROPSYCHIDAE*/
    by date ;
    where family='Hydropsy';         /*Selects for Hydropsychidae*/
run;
proc means data=hydro noprint;        /*calculates the sum of all Hydropsychidae*/
    var num;
    by date ;
    output out=hydrosunsum=hydrosun;

```

```

run;
proc sort data=hydrosum;
    by date ;
run;
data perhydro;
    merge hydrosum total;
    by date ;
    perhydro=100*hydrosum/total;    /*takes the 100% ratio of Hydropsychidae*/
run;
proc sort data=truth;    /*INITAIL CALCULATION OF %OLIGOCHAETA*/
    by date ;
run;
proc means data=truth noprint;    /*calculates the sum of all Oligochaetes*/
    var num;
    by date ;
    where code=349;
    output out=oligo    sum=oligo;
run;
data peroligo;
    merge oligo total;
    by date ;
    peroligo=100*oligo/total;    /*takes the 100% ratio of Oligochaetes*/
run;
proc sort data=truth;    /*INITAIL CALCULATION OF
ORTHOCLADIINAE/CHIRONOMIDAE*/
    by date ;
run;
proc means data=truth noprint;    /*calculates the sum of all Orthoclaadiinae*/
    var num;
    by date ;
    where subfam='Orthocla';
    output out=ortho    sum=ortho;
run;
proc means data=truth noprint;    /*calculates the sum of all Tanypodinae*/
    var num;
    by date ;
    where subfam='Tanypodi';
    output out=tanypod    sum=tanypod;
run;
proc means data=truth noprint;    /*calculates the sum of all Tanytarsini*/
    var num;
    by date ;
    where tribe='Tanytars';
    output out=tany    sum=tany;
run;
proc means data=truth noprint;    /*calculates the sum of all Chironomini*/
    var num;
    by date ;
    where tribe='Chironom';
    output out=minichi    sum=cha;
run;

```

```

proc sort data=truth;
    by date ;
run;
proc means data=truth noprint;    /*produces the total number of Chironomidae*/
    var num;
    by date ;    /*can be modified with grab to look at total numbers per grab*/
    where family='Chironom';
    output out=chi    sum=chi;
run;
data chiros;
    merge ortho chi tanypod tany minichi total;
    by date ;
    if cha=. then cha=0;
    if tanypod=. then tanypod=0;
    if ortho=. then ortho=0;
    perchi=100*chi/total;    /*takes the ration of Chironomidae to Total*/
    orthotot=100*ortho/total;    /*takes the ratio of Orthocladiinae/Total*/
    orthochi=100*ortho/chi;    /*takes the ratio of Orthocladiinae/Chironomidae*/
    tpodchi=100*tanypod/chi;    /*takes the ratio of Tanypodinae/Chironomidae*/
    tpodtot=100*tanypod/total;    /*takes the ratio of Tanypodinae/Total*/
    tanytot=100*tany/total;    /*takes the ratio of Tanytarsini/Total*/
    tanychi=100*tany/chi;    /*takes the ratio of Tanytarsini/Chironomidae*/
    minitot=100*cha/total;    /*takes the ratio of Chironomini/Total*/
    minichi=100*cha/chi;    /*takes the ratio of Chironomini/Chironomidae*/
run;
proc sort data=truth out=philopot;    /*INITAIL CALCULATION OF
%PHILOPOTAMIDAE*/
    by date ;
    where family='Philopot';    /*Selects for Philopotamidae*/
run;
proc means data=philopot noprint;    /*calculates the sum of all Philopotamidae*/
    var num;
    by date ;
    output out=philsum    sum=philsum;
run;
proc sort data=philsum;
    by date ;
run;
data phil;
    merge philsum total;
    by date ;
    phil=100*philsum/total;    /*takes the ratio of Philopotamidae*/
run;
data metrics4; merge richness Elmidae noinsect eptchi perhydro peroligo
    phil chiros shredder predator scraper filter collect bi D sdi
J domtax dom2to5;
    by date;
    dom1=100*domtax/total;    /*This calculates the %dominanat taxon*/
run;
proc print data=metrics4;
run;

```

Vita

Ely Kosnicki was born on 23 March, 1973, in Turner's Falls Massachusetts on government welfare. He attended public and private schools across the country, graduating from Westbrook High School, in Westbrook Connecticut, with Honors in 1991. Ely paid his way through college, earning a Bachelors of Science and a Masters of Science, each in Natural Resources from the University of Connecticut, in 1997 and 2001, respectively. After completing his comprehensive exams in partial fulfillment of a Ph.D. in Entomology at the University of Missouri, Ely took a job with a consulting company for a year as a "production taxonomist" in Moscow Idaho, then for a second year as an environmental consultant in Woodland California. Ely returned to Missouri to receive his Ph.D. in Entomology in 2008.