EFFECTS OF TAXONOMIC RESOLUTION AND SPATIAL VARIATION ON METRICS USED IN BIOASSESSMENTS WITH MACROINVERTEBRATE ASSEMBLAGES OF THREE MIDDLE TENNESSEE STREAMS OF THE RED RIVER/SULPHUR FORK CREEK WATERSHED

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Effects of Taxonomic Resolution and Spatial Variation on Metrics Used in Bioassessments with Macroinvertebrate Assemblages of Three Middle Tennessee Streams of the Red River/Sulphur Fork Creek Watershed

A Thesis

Presented for the

Master of Science

Degree

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Rebecca Anne Houtman

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DEDICATION

To my children, Cassie and Jared, for their unconditional love, support, and patience so I could fulfill my academic dreams.

To my mother, Mary, for teaching by example.

To my siblings, Katie and Dave, for their cheers and friendship.

To my best friend, Heather, for the greatest friendship I've ever known.

To the Hamilton family for allowing Jared and me to be a part of their family while we worked towards the completion of this thesis.

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ABSTRACT

This study evaluated selected assumptions of the United States Environmental Protection Agency's Rapid Bioassessment Protocols (RBP) using benthic macroinvertebrate to assess the biotic integrity of streams. Three streams in the Red River/Sulphur Fork Creek (RR/SFC) watershed, Buzzard, Millers, and Spring creeks, were used to evaluate the selected RBP assumptions. Benthic macroinvertebrates were collected with Hess samplers from five riffles, within each of three reaches, within each of three streams. The macroinvertebrate samples were identified to three levels of taxonomic resolution: 1) all identified to family, 2) all identified to genus except for chironomids, and 3) all identified to genus, including chironomid larvae. Macroinvertebrates in phyla Nemertea and Nematoda, classes Turbellaria, Hirudinea, and Oligochaeta, order Hydracarina, and families Simuliidae, Tabanidae, Tipulidae, and Sphaeriidae were not further identified. Chironomid pupae were not identified below family. Metric scores were calculated for each of the data sets resulting from the three levels of taxonomic identification.

The RBP does not specify the taxonomic resolution required for accurate multimetric bioassessments using benthic macroinvertebrates. This study used graphical analyses of the three sets of 17 metric scores to evaluate the level of taxonomic resolution that seemed to produce the best bioassessment. Metric values calculated from the data set that included chironomids identified to genus consistently met two criteria assumed important for reliability: 1) the behavior of metrics were consistent with responses expected based on theory, and 2) the reference stream had the best value. Thus, genus-

level identification of macroinvertebrates, including the chironomids, is recommended for use in the RR/SFC watershed.

The RBPs suggest a sample from one stream reach is adequate for bioassessment. Field assessment of stream conditions suggested there were obvious differences among reaches within each of the three streams. Graphical analyses of the 17 metrics investigated revealed substantial differences among all reaches of all streams. Analysis of variance (ANOVA) detected significant differences among reaches within Buzzard Creek, the reference stream, for 36% of the metrics tested. Millers and Spring creeks had significant among-reach variance in 43% and 57% of the metrics tested, respectively. Thus, bioassessments based on samples from a single reach of a stream could be expected to yield inconsistent results.

Metrics were selected for inclusion in a multimetric index used for bioassessment based on their ability to discriminate among-streams within the RR/SFC watershed using both graphical and statistical analyses. The two criteria used in the evaluation of dot plots to select metrics were the same as those used to evaluate the effects of taxonomic resolution. The metrics taxa richness, Trichoptera taxa richness, EPT:Chironomid ratio, Biotic Index, percent scrapers, percent chironomids, percent predators, percent tolerant, percent omnivores, and intolerant taxa richness met these criteria. One-Way ANOVA of metric scores by stream using the data from the lowest reach only was also used to select metrics able to discriminate among-streams. Metrics were selected for inclusion in the multimetric index for bioassessment based on four criteria: 1) the metric responded as predicted by theory, 2) Buzzard Creek had the best metric scores, 3) ANOVA of the metric explained over half of the variation among streams ($R^2 \geq 50\%$) and was

significant ($P \leq 0.05$), and 4) Dunnett's test revealed Buzzard Creek to be significantly different from the treatment streams. The metrics Biotic Index, percent scrapers, percent predators, percent tolerant, and percent omnivores met these five criteria and were included in the multimetric index.

A multimetric bioassessment was performed on the study streams using ranks suggested in the 1989 RBP manual. By default, Buzzard Creek, the reference stream, ranked as "relatively nonimpaired." Millers Creek ranked "moderately impaired" with a similarity to the reference of 38%. Spring Creek ranked "moderately impaired" with a 33% similarity to the reference stream.

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CHAPTER I

INTRODUCTION

Description of Study Area

Red River/Sulphur Fork Creek Watershed

The Red River/Sulphur Fork Creek (RR/SFC) watershed in Robertson County, Tennessee is located in the Western Pennyroyal Plain subsection (designated 71e) of the Pennyroyal Karst ecoregion (Griffith et al. 1997). The geology of this subsection is characterized by limestone, chert, shale, siltstone, sandstone, and dolomite. Soils are of the Pembroke, Crider, and Baxter series, forming a thin, loess mantle over limestone (Miller 1974). Vegetation is dominated by *Quercus* and *Carya* and characterized as western mesophytic forest (Braun 1950).

Streams in Robertson County, Tennessee are targeted by the Environmental Protection Agency and U.S. Natural Resource Conservation Service for evaluation and improvement of water quality and implementation of best management practices. Nonpoint source (NPS) pollution has the most significant negative impact on this region's water quality (Finley et al. 1992). Nonpoint source pollution cannot be traced to a specific point such as industrial discharge.

Much NPS pollution in this region results from various agricultural practices that result in runoff to streams carrying soil particles, animal waste, pesticides, and fertilizers. Other significant sources of NPS pollution include roads, logging operations, urban development, lawns, rooftops, and parking areas, all of which contribute to runoff that may ultimately contaminate streams. Sedimentation is one of the most significant NPS pollutants in this watershed (Finley et al. 1992). Sources of sediment in streams of this watershed include urban development, logging, row crop agriculture, and unrestricted access to streams by livestock. Livestock destroy riparian zone vegetation through foraging and trampling, and thus destabilize stream banks.

Riparian zones are areas of transition between aquatic and upland ecosystems. Riparian zone vegetation is essential to healthy aquatic ecosystems due to its ability to stabilize stream banks, buffer the stream from storm water runoff, shade the stream, and provide cover, habitat, and allochthonous energy sources (Barbour et al. 1999). Riparian zone vegetation and the accumulated organic matter slows runoff water, allowing deposition of some of the sediment load before entering the stream. This depositional process also keeps some nutrients and pesticides from flowing into streams and lakes, and allows more time for water to percolate into the soil. The increased infiltration time replenishes groundwater, reduces peak flows during storm events, and maintains water levels in lakes and streams. Riparian zones also stabilize stream temperature through shading (Smith and Smith 1998). Allochthonous energy in the form of leaves and other associated organic matter are critical to stream function, especially in headwater streams (Merritt and Cummins, 1996). Thus, human-induced impact on riparian vegetation has

the potential to greatly influence stream ecosystem health with NPS pollution (Kerans and Karr 1994).

This study will evaluate potential problems associated with assessing the impact of NPS pollution using rapid bioassessment protocols (RBP) with macroinvertebrate community assemblages as developed by the Environmental Protection Agency (EPA) (Plafkin et al. 1989, Kerans and Karr 1994, Barbour et al. 1999). This study will also evaluate potential problems in attempting to apply RBPs to evaluate the biological integrity of streams in the RR/SFC watershed within Robertson County, Tennessee (Figure 1.1).

Buzzard Creek

Buzzard Creek, a third order tributary of Red River located in northwestern Robertson County, is designated by the Tennessee Department of Environment and Conservation (TDEC) as one of two 71e ecoregion reference streams in the RR/SFC watershed. This stream, which flows from a cave, was selected because of presumed minimal water quality impacts in the watershed and was validated as a reference stream through physicochemical analyses and macroinvertebrate biomonitoring (Arnwine and Denton 2001).

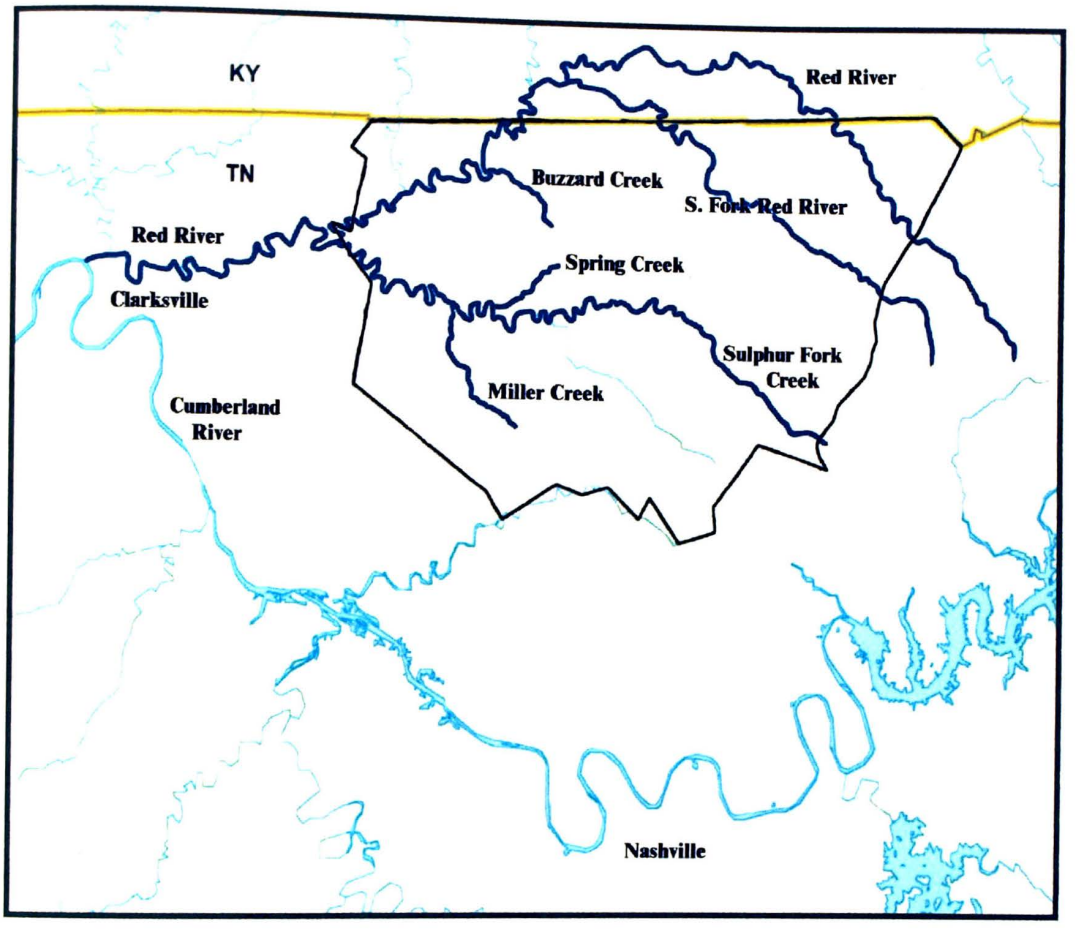


Figure 1.1. Map of study area with Robertson County, Tennessee in outline. Millers and Spring Creek are tributaries of Sulphur Fork Creek. Buzzard Creek and Sulphur Fork Creek are tributaries of the Red River.

Millers Creek

Millers Creek is a fourth order tributary of Sulphur Fork Creek (SFC) located in southwestern Robertson County. Millers Creek has a United States Geological Survey gauging station that records daily hydrologic levels in its middle region where it flows under Maxy Road at Turnersville. Millers Creek is believed to have reduced water quality due to sedimentation, unregulated cattle access, agricultural runoff, and other nonpoint source pollution (Hamilton and Finley pers. comm., Lebkuecher and Houtman 1999, Therrell and Taylor 1999).

Spring Creek

Spring Creek is a third order tributary of SFC located in central western Robertson County. Spring Creek is believed to have reduced water quality due to nonpoint source pollutants resulting from cattle access and agricultural runoff such as sediment, nutrients, and manure. Stream bank restoration occurred within the lower reach during 2000 (supervised by Dr. Mack Finley, The Center for Field Biology, Austin Peay State University). The approximately 270 m of restoration in the lower reach of Spring Creek included stream bank grade restoration; use of rip-raping, geotextiles, root wads and willow fascines to stabilize the toe of the bank; and planting of native willow (*Salix nigra*) and other trees on the upper bank. The stream bank restoration occurred after the samples for the present study was collected and therefore these data serves as a pretreatment baseline.

Importance and History of Biological Monitoring

Water quality has been a key scientific and political issue in the United States since the passage of the Rivers and Harbors Act in 1899. The laws regarding protection and improvement of water quality have changed much over the last 100 years as have the methods and requirements for monitoring (Resh and Jackson 1993). Biological monitoring uses living organisms, often entire elements of the biological community, to detect changes in streams caused by human activities and the impact of these changes have on the biological integrity of a stream (Karr and Chu 1999). Biological monitoring is important due to its ability to detect changes in a stream that are not induced by natural causes such as meteorological or seasonal variation, but by human induced habitat degradation. Often, water quality assessment has included chemical monitoring. However, chemical monitoring is analogous to a photograph; it provides a snapshot of what is occurring in a stream at a specific moment of time (Merritt and Cummins 1996). One problem with this method can occur because chemical monitoring cannot always discriminate between an increase of pollutants due to natural events such as flood, and long-term, negative changes in a system due to human impact (Karr and Chu 1999). Chronic exposure to certain pollutants at seemingly low levels may cause detectable changes in biological communities but be difficult to detect or seem insignificant with chemical techniques. Thus, the great advantage of biological monitoring is the ability of biological communities to integrate the effect of diverse pollutant sources that may vary in concentration over time. Karr (1981) was one of the first to apply a simpler, less expensive biological monitoring technique by evaluating fish communities. He dubbed

this the Index of Biological Integrity (IBI). The IBI is a compilation of several measures of community structure, i.e. metrics, referred to as a "multi-metric" index of biological integrity.

Since the 1980s, multimetric indices have been developed as a biological monitoring tool to interpret data gathered from benthic macroinvertebrate assemblages (Plafkin et al. 1989, Kerans and Karr 1994, Karr and Chu 1999, Barbour et al. 1999). Bioassessments using macroinvertebrate communities provides results analogous to a video (Merritt and Cummins 1996) in their ability to detect changes over time. Macroinvertebrate communities are usually not eliminated from a stream during a flood and other natural stream variations. Their evolutionary success in inhabiting streams results, in part, from resilience to such natural perturbations. However, human induced impacts, such as sedimentation or toxic pollutants, cause measurable changes in macroinvertebrate communities. Removal of riparian vegetation increases stream temperatures due to lack of shade and reduces input of allochthonous nutrients supplied by the vegetation. This can also be reflected in the biological community of streams, but not detected using water chemistry. This is because members of the benthic macroinvertebrate communities have specific niche requirements that can be greatly affected by human induced changes, such as removal of riparian vegetation. Bioassessments using benthic macroinvertebrates may be able detect significant, anthropogenic induced changes in community structure and function. Thus, a bioassessment, using macroinvertebrate assemblages, has the ability to detect long term human induced NPS pollutants (Kerans and Karr 1994).

Biological monitoring with multimetric indices was endorsed by the United States Environmental Protection Agency (EPA) with the publication of rapid bioassessment protocols (RBP) for determining water quality using benthic macroinvertebrate and fish assemblages (Plafkin et al. 1989). The EPA later issued a second edition of the RBP document that provides additional information for development of regionally specific RBPs and metrics, further evaluates RBP approaches to biomonitoring, and includes methods for using algal communities (Barbour et al. 1999). These RBPs are intended to provide guidance on the development of regionally specific, cost-effective methods to accelerate the use of biological monitoring in the United States. Suggested time and cost saving methods include collecting a 2 m^2 area from only a single reach that is characteristic of the stream. The minimum 2 m^2 area sampled consists of smaller, usually two-1 m^2, samples. Subsampling the macroinvertebrate sample (identifying only 100-300 organisms in a sample) and limiting taxonomic identification to family or genus are additional cost-saving measures (Barbour et al. 1999). The protocol specifies that the benthic sample is to be obtained as follows: "Using a 1 m kick net, 2 or 3 kicks are sampled at various velocities in the riffle or series of riffles. The area to be sampled can be anywhere within a 100 m reach of a stream that is representative of the characteristics of the stream" (Barbour et al. 1999).

Various studies have evaluated the methodology of the EPA protocols. Examples include the size of subsamples, metric selection, and habitats samples. One example includes an evaluation of the effects of enumerating a subsample of 100 organisms (Plafkin et al 1989) vs. enumerating a subsample of 300 organisms concluded that a 100-count subsample was biased when measuring taxa richness (Sovell and Vondracek 1999).

Another study evaluated methods for selection of metrics for use in a bioassessment based of types of NPS and point source pollution. The researches concluded that graphic representation of relationships between the extremely polluted and pristine ranges of data points "provided more insight into biology than a simple p-value could" on selection of metrics that could detect human impact (Fore et al. 1996). Other researchers analyzed the effect of sample size on metrics involving the structure of communities in sandy-bottom streams. They concluded that pollution tolerance and trophic organization metrics were robust regardless of sample size. However, metrics of community richness and structure were sensitive to sampling regimes that included spatial variation and/or multiple habitat types (Schiller and Hamilton 2000).

The EPA RBP has suggested that states develop their own set of metrics that are appropriate to ecoregions of that state (Barbour et al 1999). Two studies, one funded by the Tennessee Department of Environment and Conservation (TDEC) and the other by the Tennessee Valley Authority (TVA), have developed regionally-based multimetric indices for use in Tennessee streams. Both studies evaluated metrics that measured richness, composition, pollution tolerance, habit, and feeding groups (Arwine and Denton 2001, Kerans and Karr, 1994, Karr and Chu 1999). The study conducted by TDEC developed multimetric index criteria for bioassessment in each of the Level IV ecoregions of Tennessee (Arwine and Denton 2001, TDEC 2002). The Western Pennyroyal Karst ecoregion (Level IV ecoregion 71e) includes the RR/SFC watershed. Their sampling site was located near this study's lowest-most reach. The study conducted by Kerans and Karr (1994) for TVA, developed a benthic index of biological integrity (B-IBI) for rivers of the Tennessee Valley. My study will evaluate the reliability of some

of these methodologies in acquiring the results and the metrics chosen for inclusion in a multimetric index.

Statement of the Problem and Objectives

This study evaluated metrics using five samples from each of three reaches. Samples were collected from a single habitat (riffles). Specific objectives include: 1) determine the effect of taxonomic resolution on metrics; 2) determine if one reach can adequately represent a stream within the RR/SFC watershed; and 3) recommend a set of metrics for potential use in multimetric index bioassessments for the RR/SFC watershed.

CHAPTER II

MATERIALS AND METHODS

Sample Collection, Sorting, and Identification

Benthic macroinvertebrate samples were collected on 1 and 2 October 1999. Five reaches within each of three streams were sampled. A reach consisted of a sequence of 10 riffles. A riffle is a shallow area within a stream with hard substrate of cobble or larger sized rock and turbulent flow, and is usually the most biologically rich area of a stream (Karr and Chu 1999, Barbour et al. 1999). Sampled reaches chosen were distributed as equidistantly along the length of the stream as access allowed (Figure 2.1). A random numbers table was used to choose five of the ten riffles in each reach to sample. A modified Hess sampler with a 0.086 m^2 sample area and 500 µm mesh opening (Wildlife Supply Company, Buffalo, NY, USA) was used to collect benthic macroinvertebrates. A total of 25 macroinvertebrate samples (five riffles in each of five reaches) were collected from each stream. Only five samples from the first, third, and fifth (lower, middle and upper) reaches, totaling 15 samples, were included in this study. Samples were placed in 1 L jars and preserved in the field with 10% formalin. Samples were transferred to 80% isopropanol after one week.

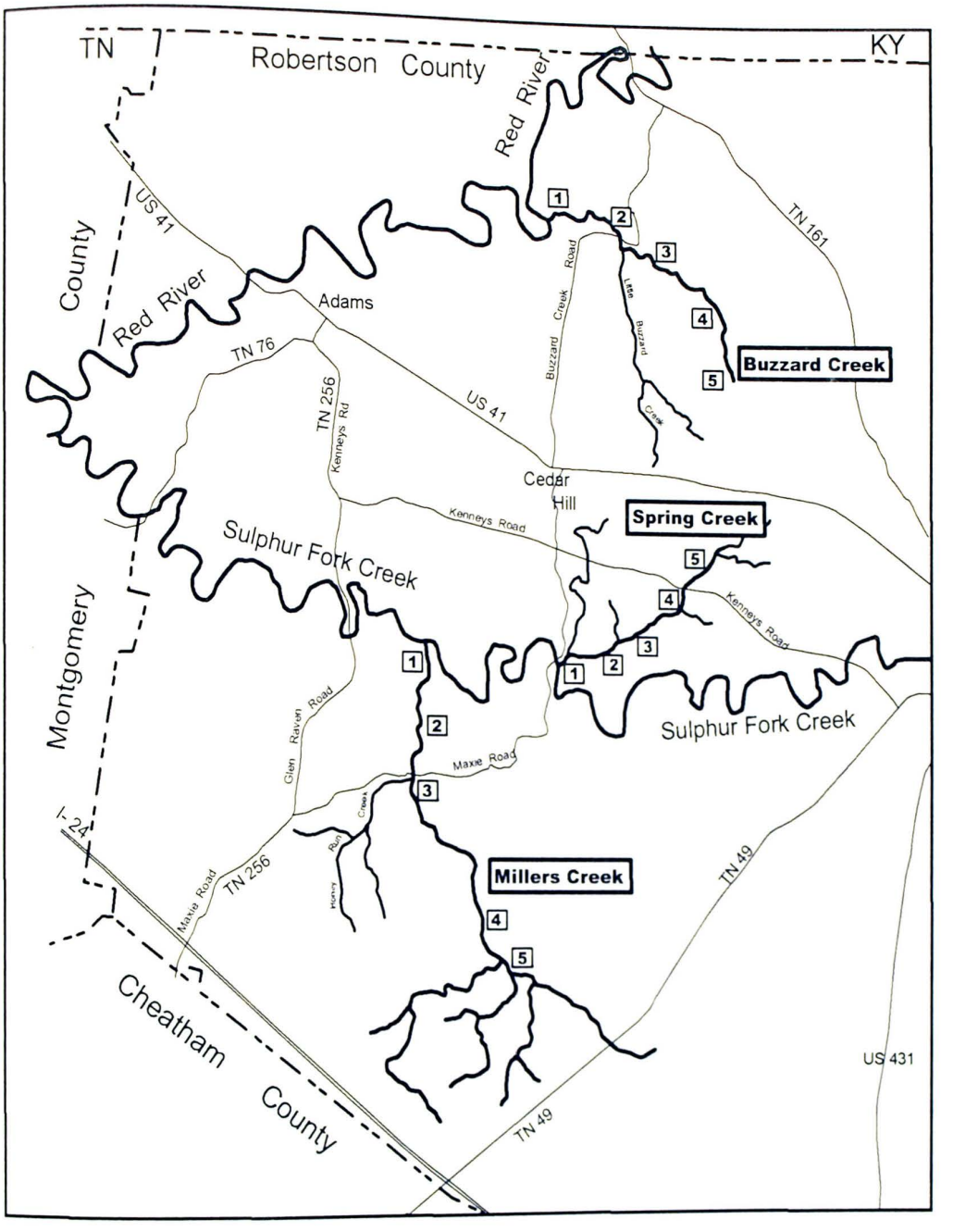


Figure 2.1. Map showing the three streams sampled for this study. Buzzard Creek is a tributary of the Red River. Spring and Millers creeks are tributaries of Sulphur Fork Creek, also a tributary of Red River. Numbers represent sampling reaches. Lowest reach within the stream = 1 and upper most reach = 5.

For a multimetric bioassessment, the EPA RBP recommends compositing at least two 1 m^2 kick net samples from a 100 m reach that is characteristic of the stream. This study collected 15 Hess samples, analyzed individually, from five riffles in each of three different reaches. The purpose of analyzing samples individually rather than compositing samples in a reach was to allow the determination of variance within and among-reaches. The Hess sampler was used rather than the 1 m^2 kick net because it collects from a specific area (0.086 m^2), which allows quantitative assessment of macroinvertebrate abundance and provides more accurate replication.

All macroinvertebrates were separated from detritus and identified to genus except for the following: Nemertea and Nematoda were identified to phylum; Turbellaria, Hirudinea and Oligochaeta to class; Hydracarina to order; and Chironomidae pupae, Simuliidae, Tabanidae, Tipulidae, Sphaeriidae to family. One large sample was split in half prior to removal of macroinvertebrates using a "box splitter." The macroinvertebrates from seven samples were split using a Folsom Plankton Splitter (Wildlife Supply Company, Buffalo, NY) after the specimens had been removed from the sample (Table 2-1).

Macroinvertebrates were identified using taxonomic identification keys in Merritt and Cummins (1996), Thorp and Covich (1991) and Wiggins (1977). Chironomid larvae were slide-mounted in CMCP-10 and identified to genus under a compound microscope using taxonomic keys by Coffman and Ferrington (1996), Epler (2001), and Wiederholm (1983). All sample sorting was conducted by undergraduate lab assistants and the author, and was checked by Dr. Steven W. Hamilton. Identifications were verified Dr. Hamilton and Debbie Hamilton, Clarksville, TN.

Table 2.1. Macroinvertebrate samples split pre- and post-sorting from debris. Reach 1 is furthest downstream, reach 3 intermediate and reach 5 furthest upstream. Riffles, numbered 1 through 5, indicate the order of sampling, downstream to upstream. Splitting pre-sort indicates halving the sample before the organisms were removed from the detritus. Splitting post-sort indicates halving the sample after macroinvertebrates were separated from detritus. Spring Creek, reach 5, sample 1, was split pre- and post-sorting due to its large sample size. Other samples were split only post-sorting.

Stream	***Reach***	***Riffle***	***Split pre-sort***	***Split post-sort***
Millers	3	4		X
Millers	3	5		X
Millers	5	3		X
Millers	5	4		X
Millers	5	5		X
Spring	5	1	X	X
Spring	5	3		X
Spring	5	4		X

Data Analysis

Enumeration of Macroinvertebrates at Three Levels of Taxonomic Resolution

All macroinvertebrates were identified to genus, with the exceptions noted previously. However, analysis of the macroinvertebrate data was performed at three different taxonomic resolutions: 1) all identification to family level, with previously noted exceptions, 2) all identifications to genus level, with chironomids to family and the previously noted exceptions, and 3) all identifications to genus, including chironomid larvae, and with previously noted exceptions. Analyzing these three levels of taxonomic resolution required construction of three discrete data sets, each consisting of the enumerated macroinvertebrates at each taxonomic resolution and the metric values calculated from these data sets. Identifying chironomid larvae to genus is time consuming due to slide preparation requirements for identification, the use of compound microscopic for identification and their abundance in samples. Analysis of the macroinvertebrate data with chironomids identified at the two different taxonomic levels was used to determine if genus-level taxonomic resolution of chironomids justifies the added effort for stream bioassessments in the RR/SFC watershed.

Metrics Used in Analyses

Macroinvertebrates in each taxon were enumerated to formulate and assess metrics for use in analyses. Metrics selected for evaluation were chosen due to their

robustness in other bioassessment studies (Plafkin et al. 1989, Barbour et al. 1999) or were recommended by the Tennessee Department of Environment and Conservation (Arwine and Denton 2001, TDEC 2002) or Karr and Chu (1999). All metrics evaluated, their mathematical calculation, and the rationale for their use are listed in Table 2.2.

The metric dominance varies in its method of calculation such that the number of individuals in the most abundant one, two, three, four or five taxa may be designated as the measure of dominance (Barbour et al. 1999). Dominance in this study was calculated based on the proportion of the sample represented by the single most numerically dominant taxon (Table 2.2).

Tolerance values used in deriving the Biotic Index, percent tolerant, and intolerant taxa richness metric scores ranged from 0.001 to 10, with lower values representing a lower tolerance to pollutants (Lenat 1993). Functional feeding groups were assigned according to Merritt and Cummins (1996) and TDEC (2002). The groups used were herbivores, detrivores, filter-feeders, scrapers, predators, omnivores and shredders.

Effect of Taxonomic Resolution on All Reaches

To determine which level of taxonomic resolution most accurately assessed streams, a set of metric scores was calculated from each of the three taxonomic data sets using Corel Quattro Pro 10 (2001). Dot plot graphs of each set of metric scores, using Microsoft Excel (2002), were examined to determine the level of taxonomic resolution at which the metric scores most consistently: 1) responded as expected based on theory, 2)

Table 2.2. Metrics evaluated organized by classification showing calculation method, and rational for use in a multimetric index for a bioassessment of the Red River/Sulphur Fork Creek watershed.

Category and Metric	Calculation	Rationale
RICHNESS METRICS		
Taxa Richness	The number of distinct taxa with all macroinvertebrates identified to the desired taxonomic level, genus where practical.	Taxa richness generally decreases with decreasing water quality (Weber 1973, Resh and Grodhaus 1983 as stated in Barbour et al1999). This water value is expected to decrease with lesser quality.
Abundance	All macroinvertebrate specimens are counted.	Numbers may increase or decrease due to certain types of stresses (Weber 1973). With fixed count subsamples this metric is not relevant. This value generally decreases with lesser water quality, but may increase with nutrient enrichment.
Ephemeroptera Taxa Richness	Number of Ephemeroptera (mayfly) taxa.	This order is generally sensitive to pollutants. This value is expected to decrease with lesser water quality (Barbour et al. 1999).
Trichoptera Taxa Richness	Number of Trichoptera (caddisfly) taxa	This insect order is generally sensitive to pollutants. This value is expected to decrease with lesser water quality (Barbour, et al. 1999).
EPT Taxa Richness	Total taxa in the orders Ephemeroptera, Plecoptera (stonefly), and Trichoptera.	In general, the majority of taxa in these three orders are pollution sensitive (Lenat 1988). This value is expected to decrease with lesser water quality (Barbour et al. 1999).
COMPOSITION METRICS		
Percent Shredders	All individuals in the "shredder" functional feeding group are counted and divided by the total number of individuals in the sample.	Shredder organisms and their microbial base are sensitive to toxicants and to modifications of the riparian zone that alter food inputs to the stream. A lower score indicates lesser water quality (Barbour et al. 1999).
Percent Oligochaetes	Total number of oligochaetes divided by total number of individuals in the sample.	Oligochaetes are known to dominate communities of lesser water quality particularly in cases of enrichment. An increased value indicates lesser water quality (Kerans and Karr 1994).

Table 2.2 continued.

Category and Metric	Calculation	Rationale
Percent Scrapers	All individuals of the "scraper" functional feeding group are counted and divided by the total number of individuals in the sample.	Scrapers are sensitive to the amount of sediment deposited in a stream. A lower value indicates lesser water quality (Karr and Chu 1999).
Percent Omnivores	Total number of organisms in the "omnivore" functional feeding group divided by total number of individuals in the sample.	This value is expected to increase with lesser water quality (Karr and Chu 1999).
TOLERANCE METRICS		
Biotic Index	The number of individuals of each taxon are multiplied by their tolerance value, summed for all taxa, and then divided by the total number of individuals.	A weighted average pollution tolerance of the stream community. Normally with a range of 0 to 10. A lower score indicates better water quality (Hilsenhoff 1988, Lenat 1988).
Dominance	Number of individuals in the numerically dominant taxon is divided by the total number of individuals.	A community in which one, or a few, taxa make up the majority of abundance indicates environmental stress and community imbalance. This value is expected to increase with lesser water quality (Plafkin et al. 1989, Barbour et al. 1999).
Percent Tolerant	Total number of individuals with a tolerance value between 8.0 and 10.	This metric is the percent of individuals with very high tolerant values. A higher value indicates lesser water quality.
Intolerant Taxa Richness	Total number of taxa with tolerance values between 0.001 and 2.0.	This metric is the number of very intolerant taxa. A lesser value indicates lesser water quality.

were best for the reference stream, and 3) resulted in the greatest number of metrics discriminating between streams.

Spatial Variability Within-Streams Using Genus-Level Data Set

Analyses of within-stream variation of metrics scores were performed to determine if a stream could be accurately assessed with only one reach, as suggested by the EPA. Significant variation among-reaches within a stream would suggest that more than one reach would need to be analyzed for a reliable assessment of a stream.

Field Observation of Reach Variability

Field observations provided several indicators that macroinvertebrates assemblages would differ among reaches. Indicators would include substrate stability, extent of riparian zones, agricultural practices adjacent to streams, turbidity, siltation and other obvious anthropogenic affects. Through field observation and photographs, the reaches of each stream were analyzed for differences. Photographs were taken of each reach from each stream, except the upper reach of Buzzard Creek. Discussion in the field and over photographs were conducted with Dr. Steven Hamilton, APSU, to identify habitat differences among reaches within streams.

Graphical Assessment of Among-Reach Variation of Metric Scores

Graphical analyses of among-reach variation were performed by plotting each metric's scores calculated from the genus-level macroinvertebrate data set against riffle number for each stream. The X-axis values (riffle number) ranged from 1 to 15 where samples 1-5, 6-10, and 11-15 were from the lower, middle, and upper reach, respectively. Among reach variations of metrics were subjectively recognized when the metrics for all or most samples in a reach responded, as a group, differently than in an adjacent reach or reaches. Dot plot graphs were used to confirm *a priori* assumptions developed from the field observations.

Statistical Analysis of Spatial Variation

The among reach variation in metric scores within each of the streams was evaluated using ANOVA to test the assumption that a stream can be adequately assessed by a 2 m^2 composite riffle sample from a single reach as suggested by the EPA (Barbour et al. 1999). This analysis used the genus-level data set for the 17 metrics evaluated. Statistical analyses using ANOVA provided a second method to confirm *a priori* assumptions developed from the field observations. All statistical analyses were performed with JMP version 4.03 (1989-2000 SAS Institution, Inc.). To meet the normality assumption of ANOVA, the scores of many metrics were transformed. Statistical results were considered significant for P values ≤ 0.05.

Visual Analysis of Dot Plot Graphs of Metrics

Dot plots, as described previously, were also used to determine if a metric had the ability to detect differences among streams. A metric was selected for inclusion in a multimetric index if it met the following criteria: 1) the metric responded according to theory, 2) the best score was obtained from the reference stream, and 3) the metric could discriminate among streams. Metrics were evaluated using samples from the lowest-most reach only. This is because both Millers and Spring creeks were clearly impacted in the lower reaches, and Buzzard Creek, which flows from a cave at its headwater, is only typical of the reference condition at the lower reach.

Statistical Analysis of Metrics

A one-way ANOVA was used to identify those metrics that could detect significant differences among-streams using data from the lowest most reach. To meet the normality assumption of ANOVA most metric's scores were transformed. The R^2 for each ANOVA was reported to indicate the proportion of the variability among streams accounted for by the metric. Statistical analyses were performed with JMP version 4.03 (1989-2000). All probability values of ANOVA analysis represented data that met the assumptions of ANOVA. Statistical results were considered significant for P-values $\leq$ 0.05.

Post-hoc differences from the reference stream using Dunnett's Test were performed when the ANOVA detected significant differences among streams. The genus-level data set was used for this analysis.

Metrics were selected for inclusion in the multimetric index for bioassessment based on five criteria: 1) the metric responded as predicted by theory, 2) the best score was obtained from the reference reach (Buzzard Creek), 3) the ANOVA of the metric was significant (≤ 0.05), 4) Dunnett's post-hoc mean separation test showed the reference stream was significantly different than the treatment streams, and 5) the metric explained a large proportion of the variance (R^2).

Multimetric Bioassessment Using Genus-Level Data Set

Multimetric Index Score

A multimetric index was compiled from the results of metric selection. The multimetric index of Buzzard Creek was derived from the five samples of the lowest reach only. Buzzard Creek values thus represented a "reference condition." The multimetric index scores from Millers and Spring creeks were means of the15 samples from all three reaches of each stream. The mean metric scores were converted to a percentage score by dividing each by the best value and multiplying by 100 [(metric/best metric value) x 100]. Converting metric scores to percentage scores normalized their values so that each metric was equally weighted. The multimetric index score was the mean of all the individual metric scores included in the multimetric index.

The multimetric index score was used in the bioassessment. The bioassessment score was the similarity of study stream multimetric index scores to the multimetric index score of the reference stream, i.e., the similarity of Millers and Spring creek's multimetric index score to that of the ecoregion reference stream, Buzzard Creek. The ranks used were "relatively nonimpaired" (>83% similar), "slightly impaired" (83≥54% similar), "moderately impaired" (53 ≥21% similar), and "severely impaired" (<21% similar) (modified from Plafkin et al. 1989).

CHAPTER III

RESULTS AND CONCLUSIONS

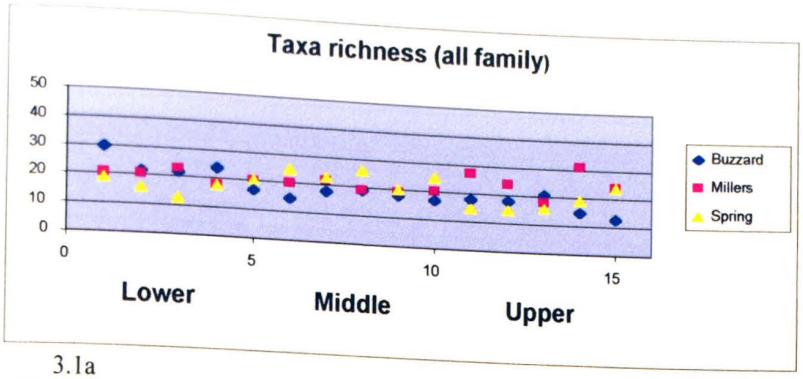
Effect of Taxonomic Resolution on Metrics

The effect of taxonomic resolution on the performance of metrics was analyzed with dot plots of each metric's scores calculated from three data sets that differed in taxonomic resolution: 1) family level, 2) genus, except chironomids at family, and 3) genus, including chironomids. Two criteria were used to assess the performance of the metrics at each taxonomic level: 1) metric responded as predicted by theory and consistently, and 2) the reference stream represented the best values.

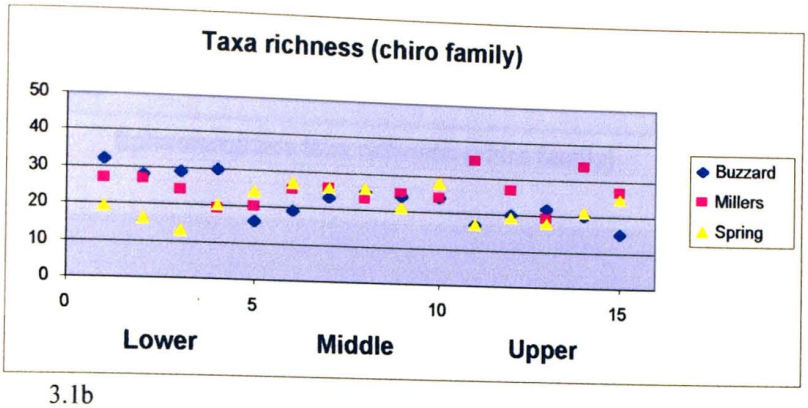
Figures 3.1-3.12 illustrate the effect of taxonomic resolution on the behavior of 12 of the 17 metrics evaluated. Only 12 metrics are affected by taxonomic resolution. The other five metrics consist of counts of individuals (such as abundance) or involve taxonomic groups not considered below the family level (such as percent oligochaetes).

Specific Metrics Affected With Chironomid Taxonomy

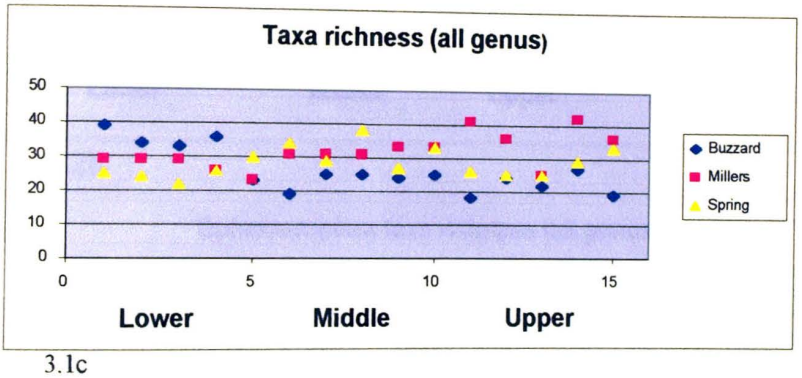
Pollution Tolerance Metrics. Metrics that measure pollution tolerance were more robust when chironomids were identified to genus. Chironomidae were given a mean family tolerance value of 5.70 on a 10 point scale (Lenat 1993). Identification of



3.1a

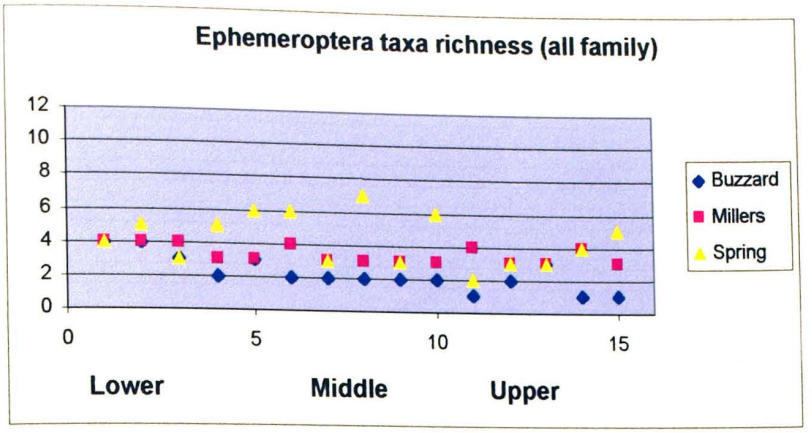


3.1b

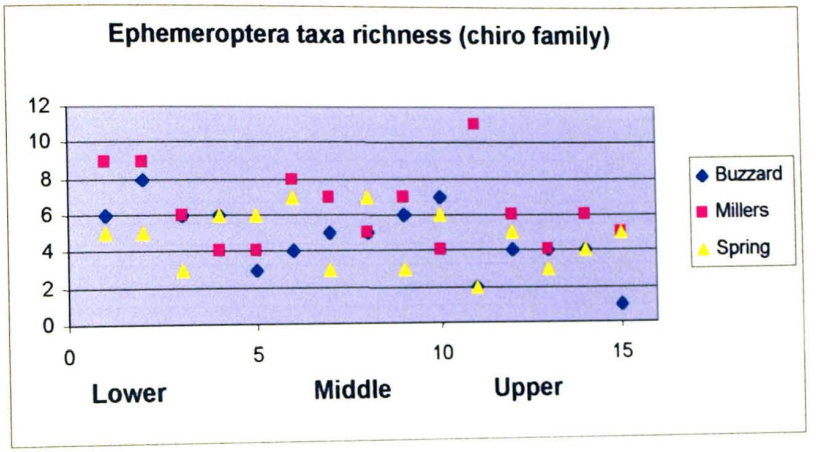


3.1c

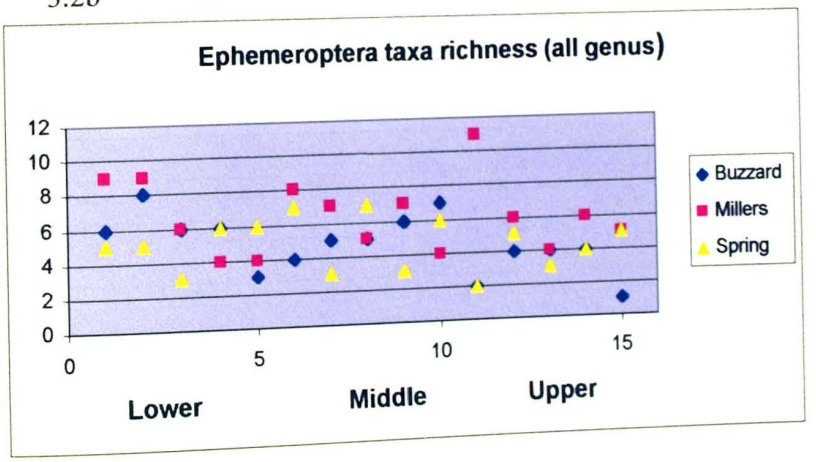
Figure 3.1. Effects of taxonomic resolution on the metric taxa richness. (a) Metric values calculated from the macroinvertebrate data set identified to family. (b) Metric values calculated from the data set that identified most taxa to genus, except chironomids. (c) Metric values calculated from the data set where all major taxa were identified to genus, including chironomids. X-axis refers to riffle samples. "Lower," "Middle" and "Upper" refer to reaches and correspond to riffles 1-5, 6-10 and 11-15, respectively.



3.2a



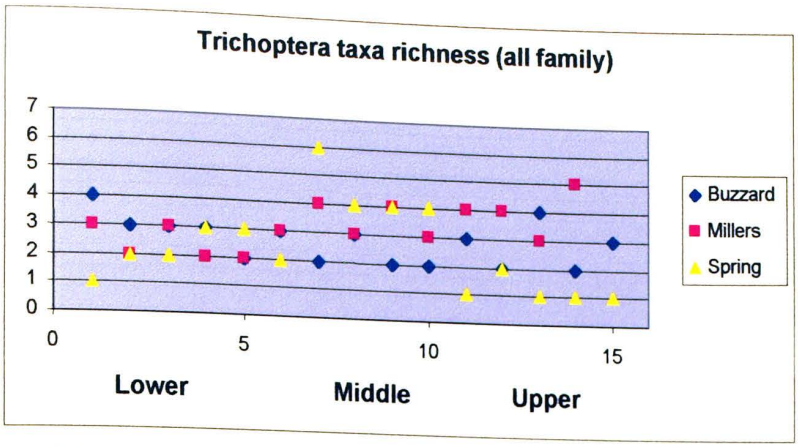
3.2b



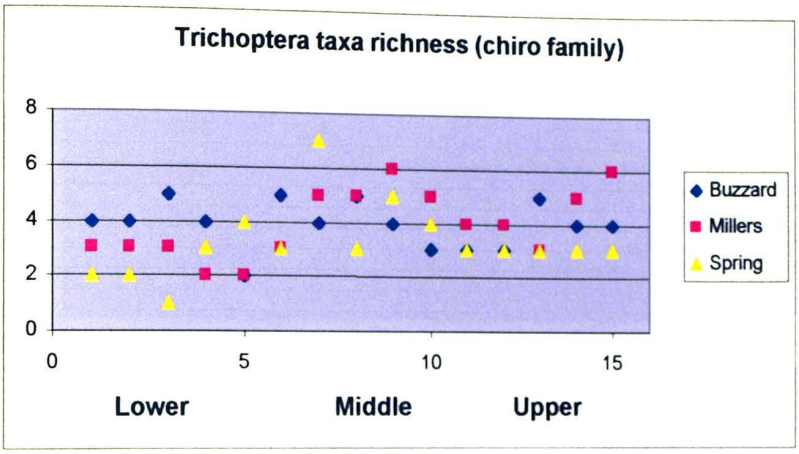
3.2c

Figure 3.2. Effects of taxonomic resolution on the metric Ephemeroptera taxa richness.

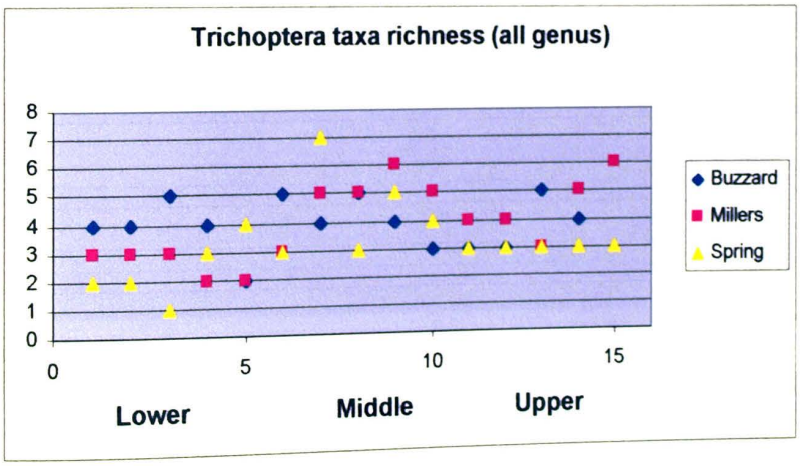
Refer to Figure 3.1 for description of figures.



3.3a



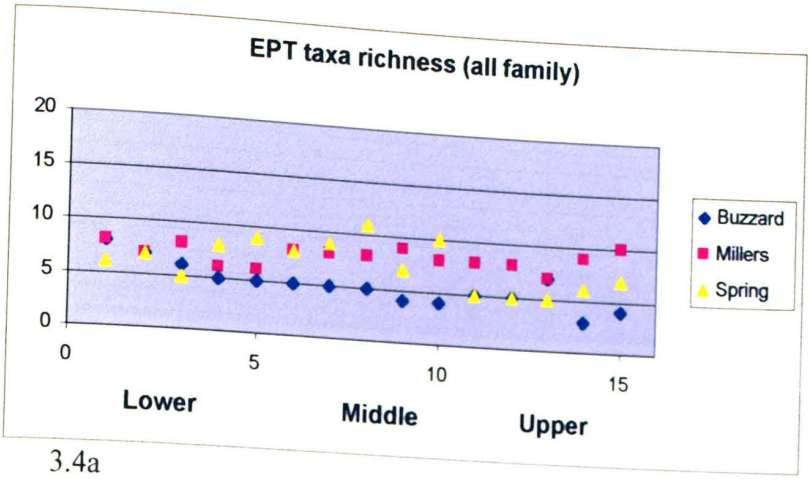
3.3b



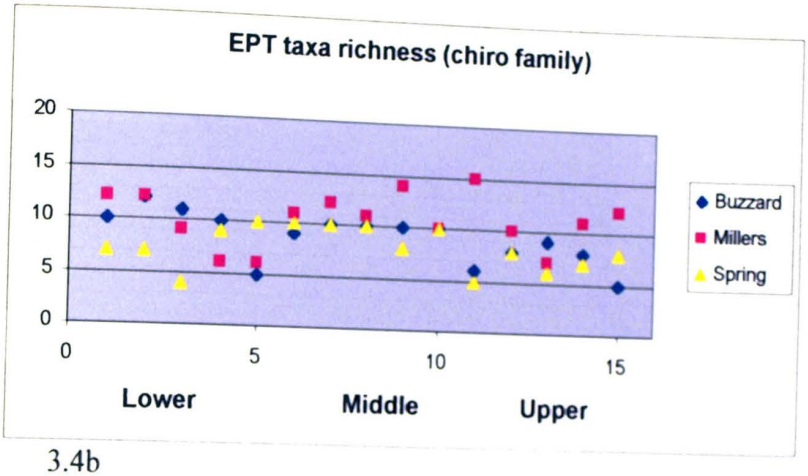
3.3c

Figure 3.3. Effects of taxonomic resolution on the metric Trichoptera taxa richness.

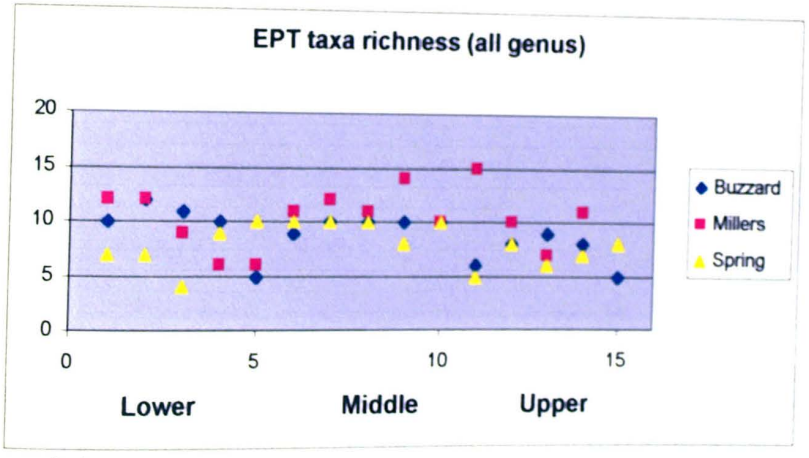
Refer to Figure 3.1 for description of figures.



3.4a

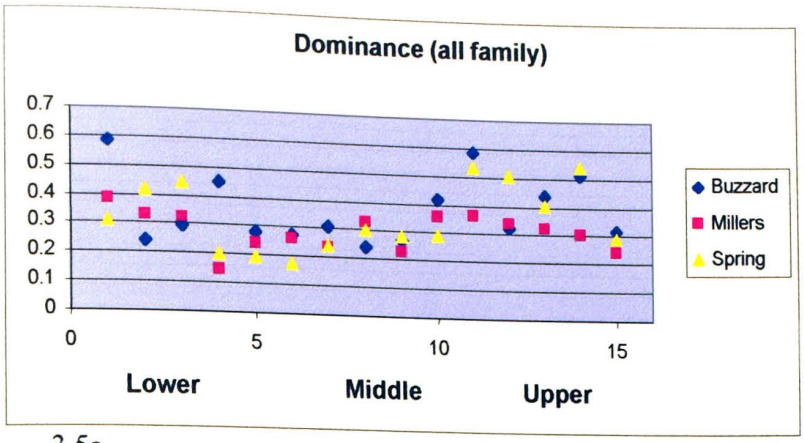


3.4b

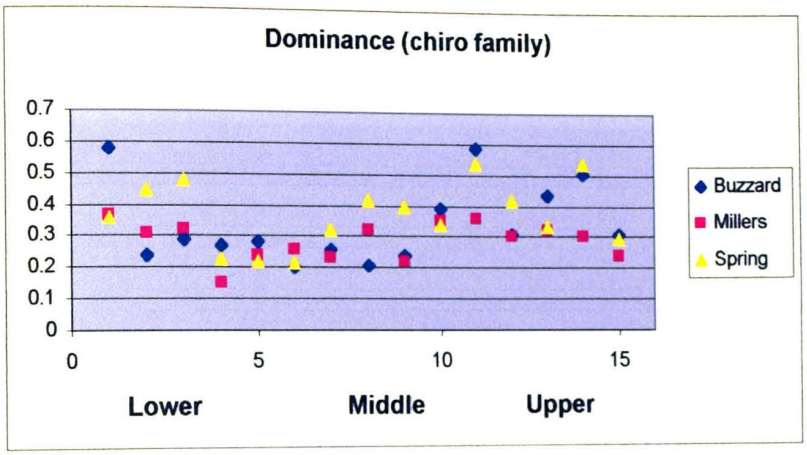


3.4c

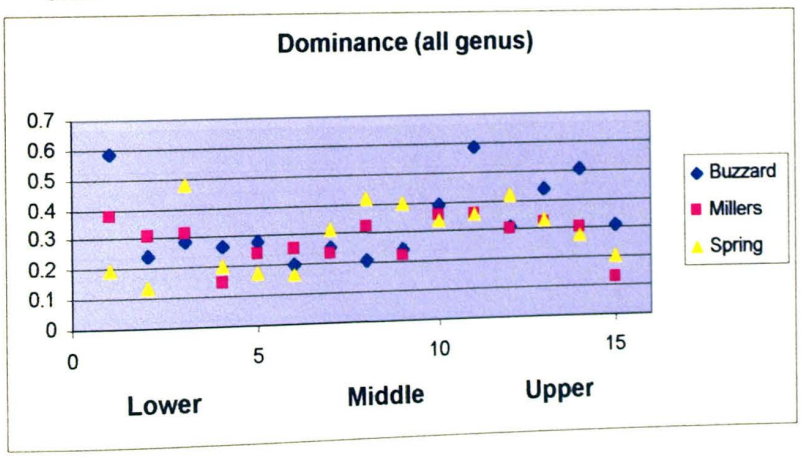
Figure 3.4. Effects of taxonomic resolution on the metric Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa richness. Refer to Figure 3.1 for description of figures.



3.5a

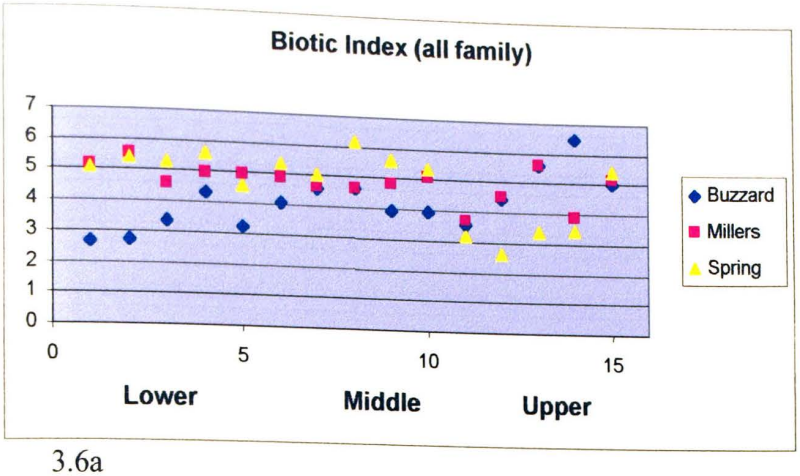


3.5b

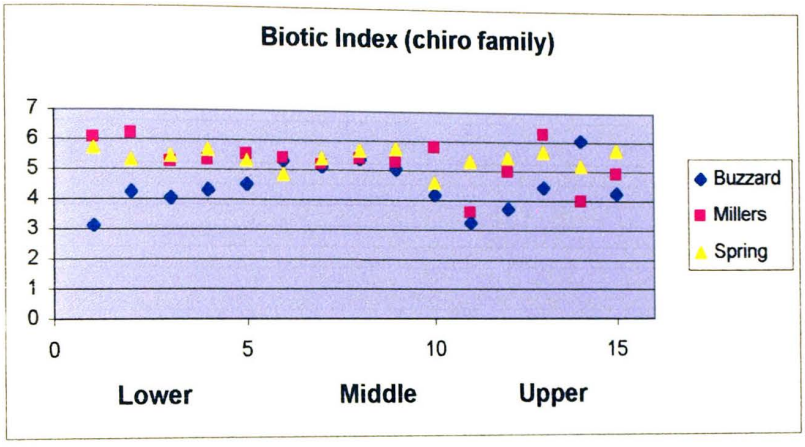


3.5c

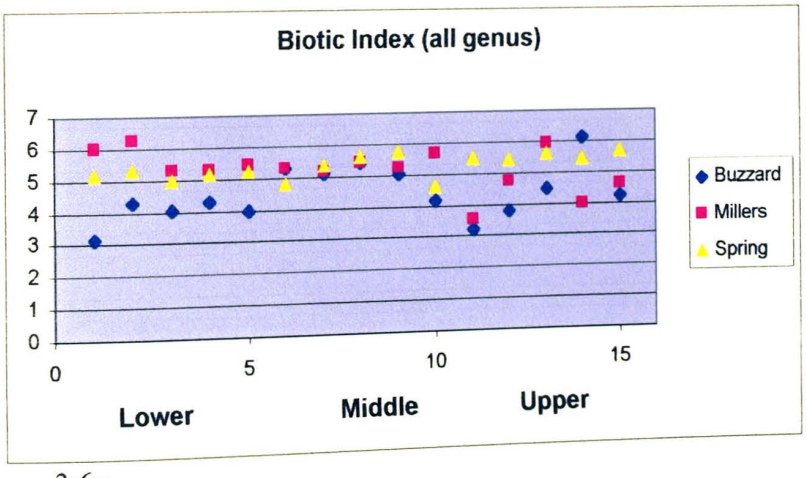
Figure 3.5. Effects of taxonomic resolution on the metric dominance. Refer to Figure 3.1 for description of figures.



3.6a

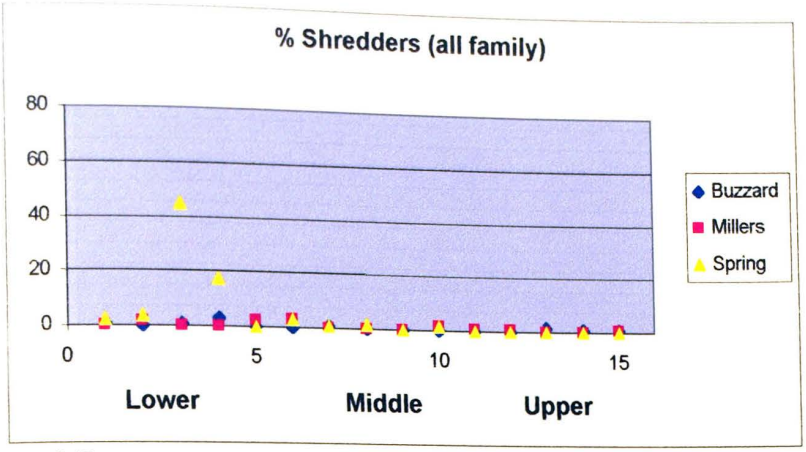


3.6b

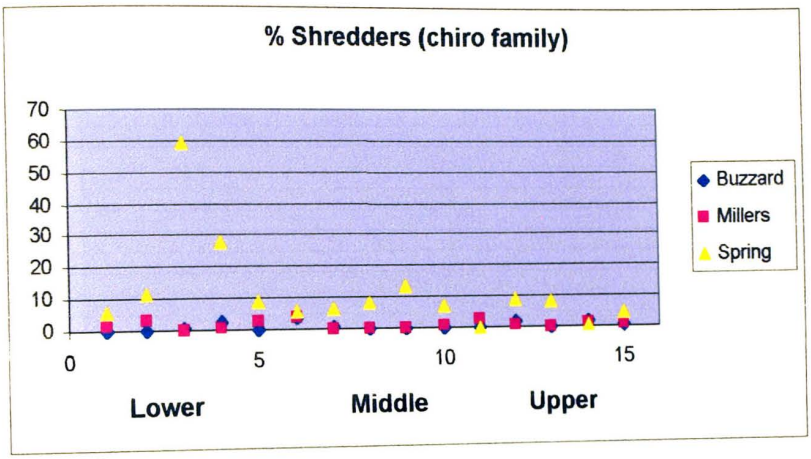


3.6c

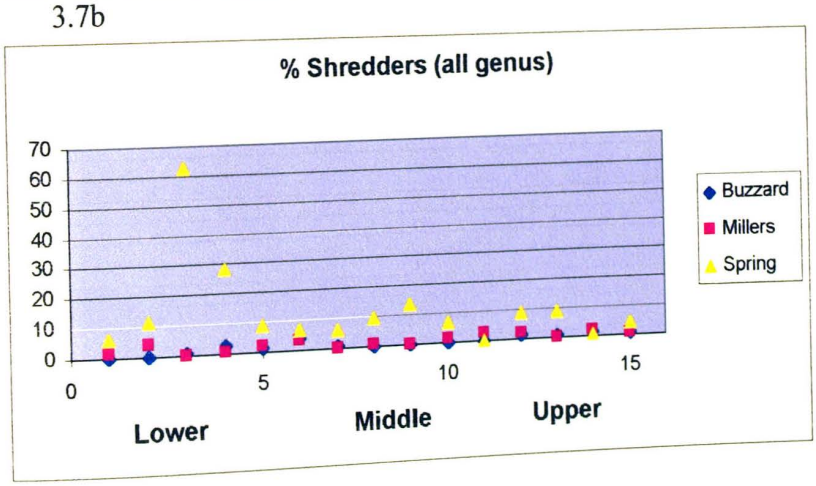
Figure 3.6. Effects of taxonomic resolution on the metric biotic index. Refer to Figure 3.1 for description of figures.



3.7a

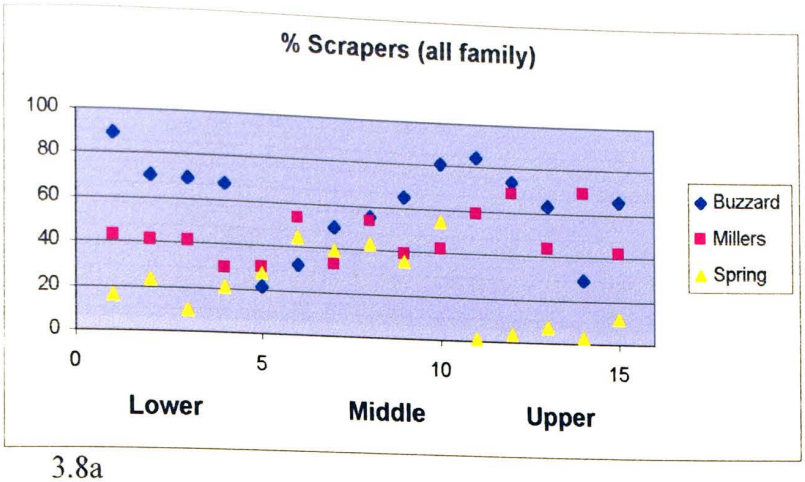


3.7b

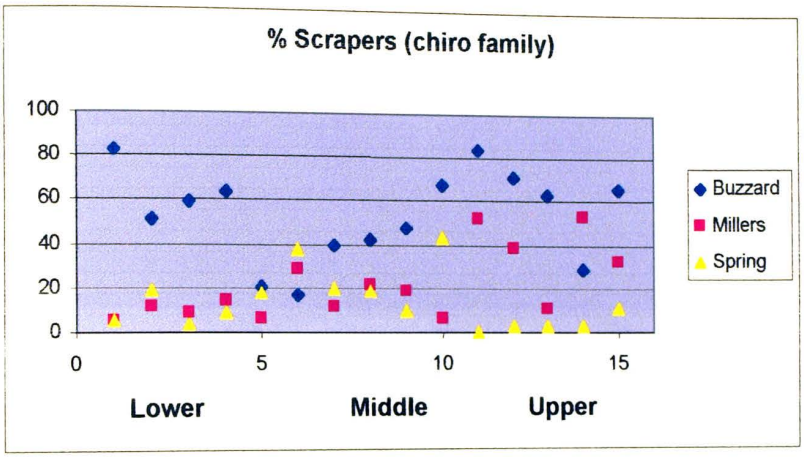


3.7c

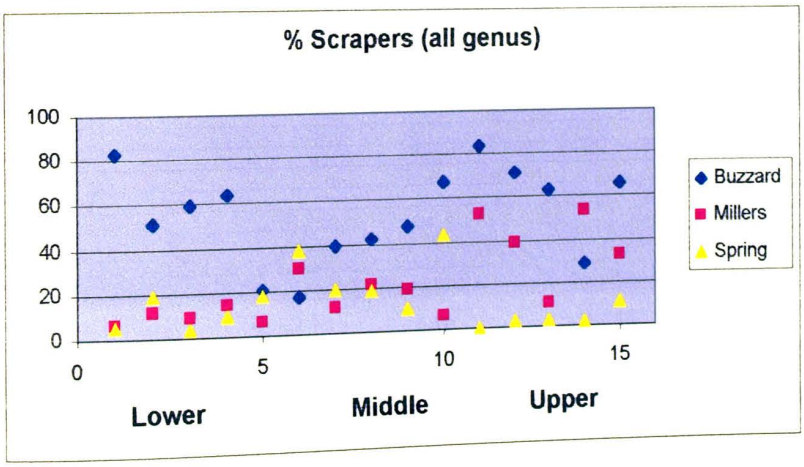
Figure 3.7. Effects of taxonomic resolution on the metric percent shredders. Refer to Figure 3.1 for description of figures.



3.8a

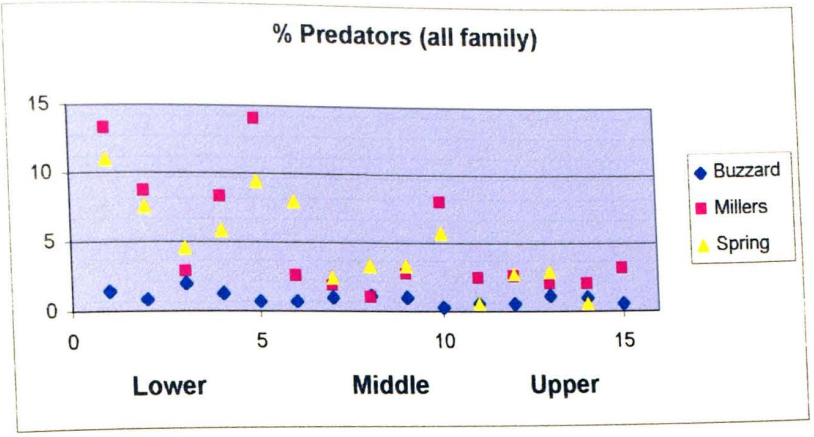


3.8b

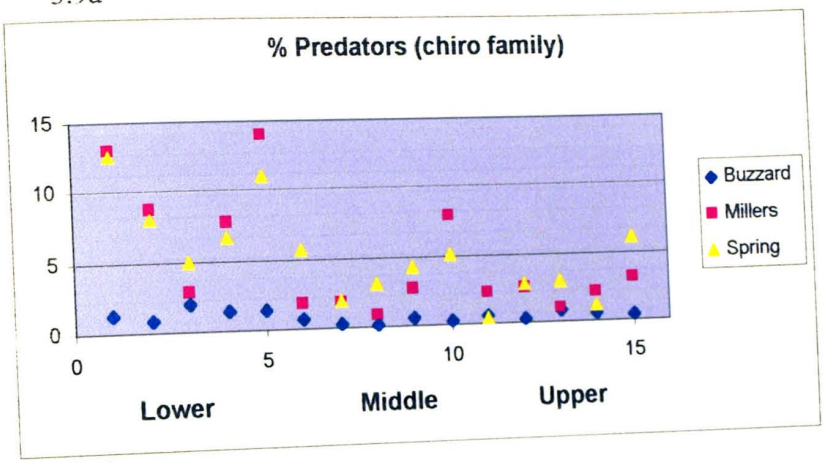


3.8c

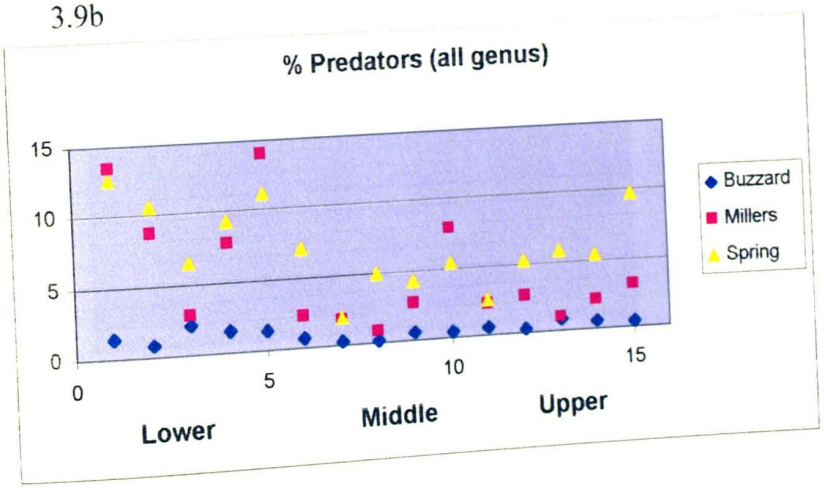
Figure 3.8. Effects of taxonomic resolution on the metric percent scrapers. Refer to Figure 3.1 for description of figures.



3.9a

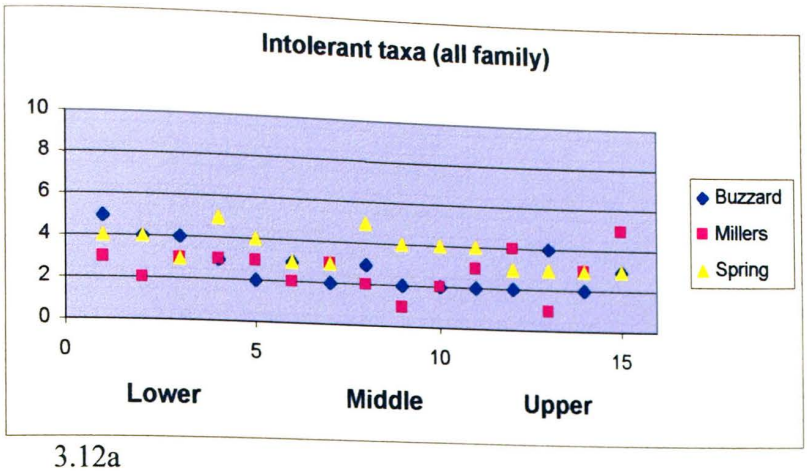


3.9b

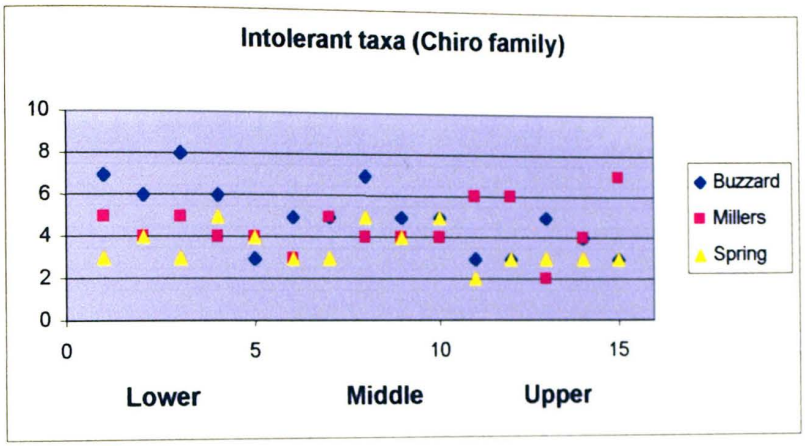


3.9c

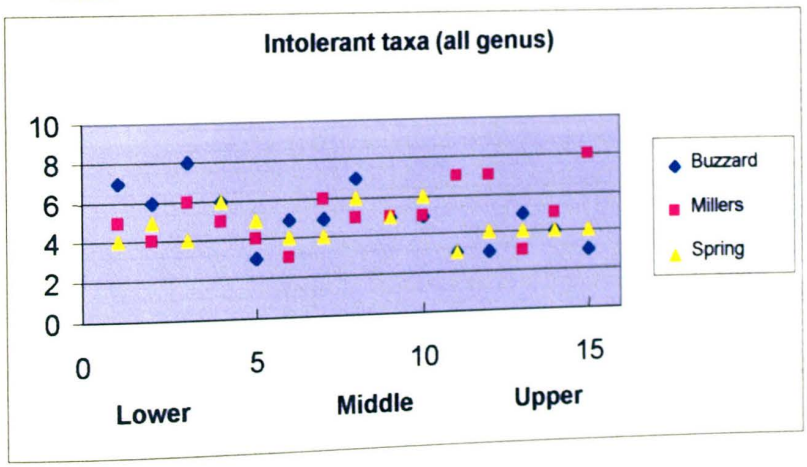
Figure 3.9. Effects of taxonomic resolution on the metric percent predators. Refer to Figure 3.1 for description of figures.



3.12a

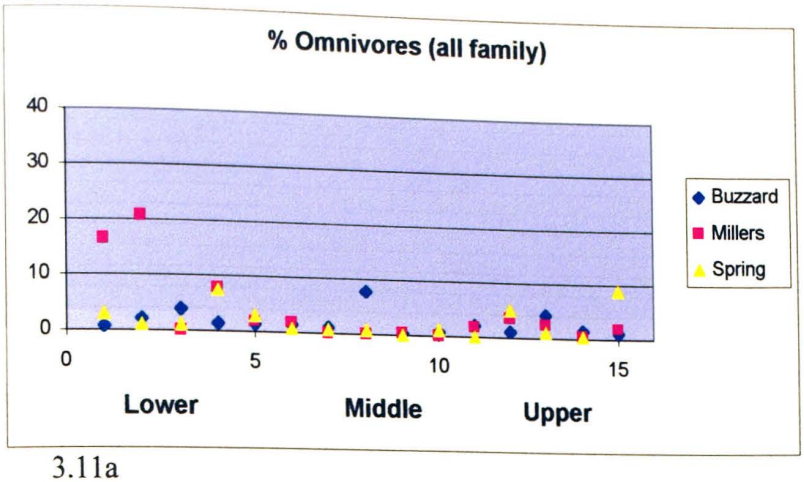


3.12b

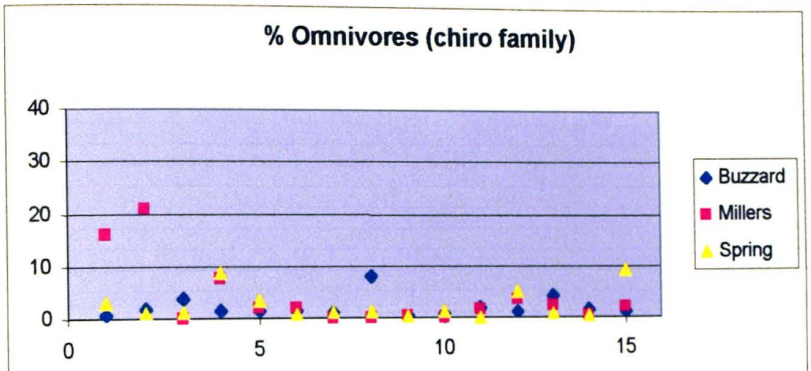


3.12c

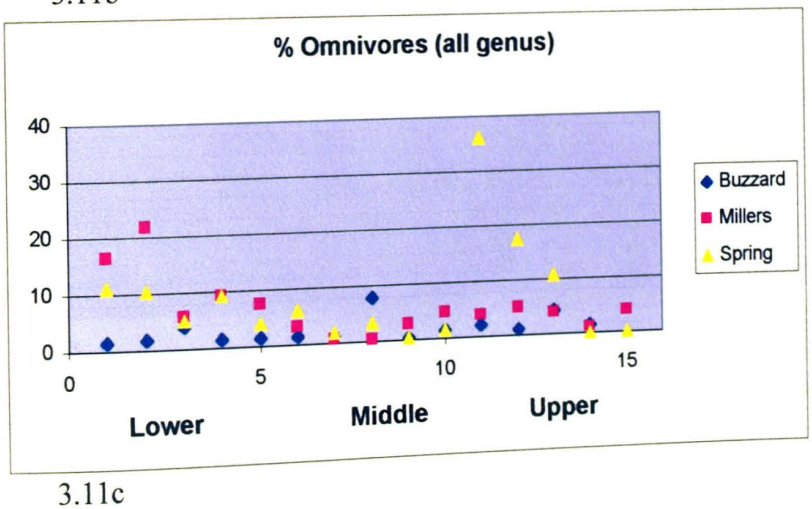
Figure 3.12. Effects of taxonomic resolution on the metric intolerant taxa. Refer to Figure 3.1 for description of figures.



3.11a

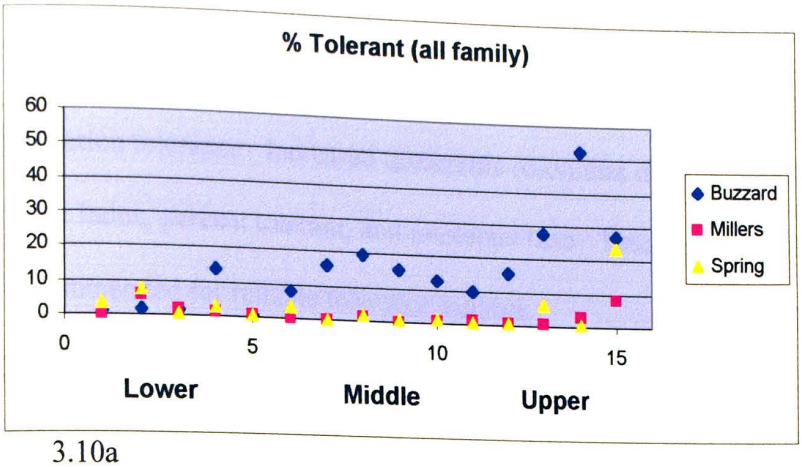


3.11b

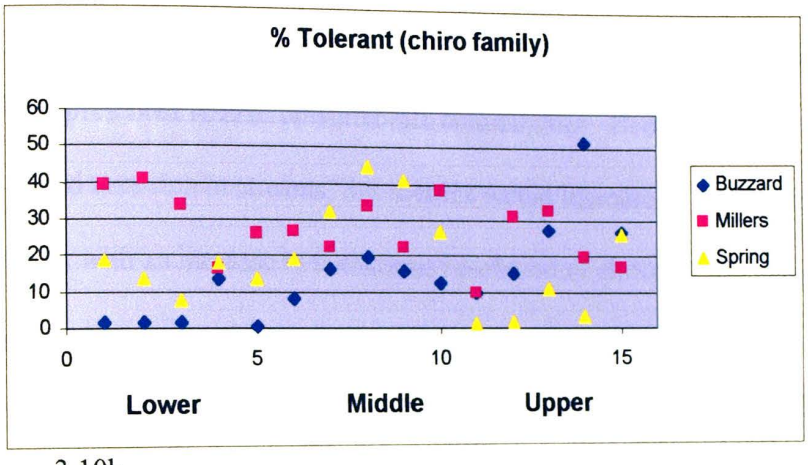


3.11c

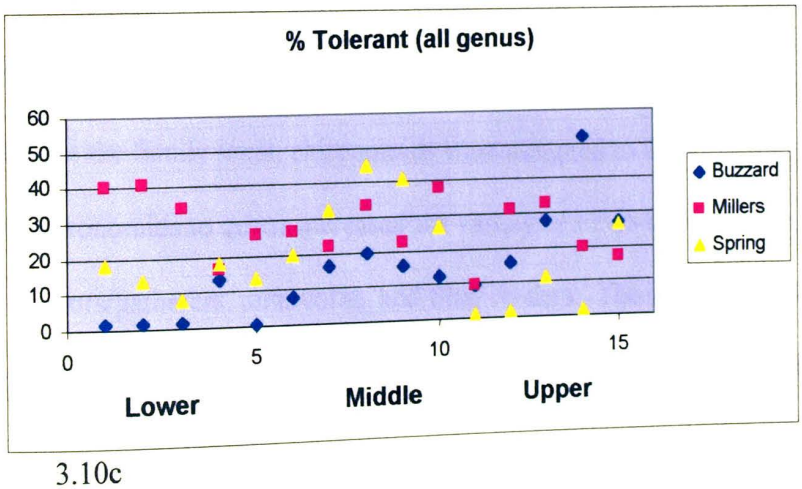
Figure 3.11. Effects of taxonomic resolution on the metric percent omnivores. Refer to Figure 3.1 for description of figures.



3.10a



3.10b



3.10c

Figure 3.10. Effects of taxonomic resolution on the metric percent tolerant. Refer to Figure 3.1 for description of figures.

chironomids to genus expanded the tolerance value range to 1.67 though 9.30 on the same 10 point scale. The increased range allowed for more precise bioassessment of streams with pollution tolerance. Increased taxonomic resolution of chironomids affects the metrics Biotic Index, percent tolerant, and intolerant taxa. Thus, genus-level taxonomy is recommended for reliable tolerance metrics.

Composition Metrics. The metric taxa richness increased in value, as expected, with genus-level identification of chironomids. This metric was dependent on the number of taxa represented in macroinvertebrate communities. Because chironomids are often abundant and taxa-rich in streams, this metrics would increase in sensitivity for stream assessment with an increase in taxonomic resolution of chironomids. Thus, genus-level taxonomy increased the robustness of this metric and is recommended.

Functional Feeding Group Metrics. Metrics scores of functional feeding groups, (FFG), such as percent predators, percent scrapers, and percent omnivores changed because at the family level, chironomids were assigned to the FFG scrapers, but identifying the chironomids to genus increased the variety of FFGs to include predators, herbivores, collectors/gatherers, omnivores, and filter feeders. The increased sensitivity of FFG metrics when chironomids are identified to genus strongly supports the need for increased taxonomic resolution of this family.

he metrics values generated from the family-level data set did not discriminate among streams as well as those generated from the two data sets with increased taxonomic resolution. Thus, family-level-only taxonomy is not recommended for metric evaluations of streams in the RR/SFC watershed.

The metrics Ephemeroptera taxa richness (Figure 3.2b), Trichoptera taxa richness (Figure 3.3b), EPT taxa richness (Figure 3.4b), percent shredders (Figure 3.7b), percent scrapers (Figure 3.8b), and percent tolerant, (Figure 3.10b) discriminated among streams better when all macroinvertebrates except chironomids were identified to genus as compared to the data set where all were identified to family only (Figures, 3.2a, 3.3a, 3.4a, 3.7a, 3.8a, 3.10a). However, the scores of these six metric were unchanged when calculated from the data set identifying all macroinvertebrates to genus including the chironomids (Figures 3.2c, 3.3c, 3.4c, 3.7c, 3.8c, 3.10c). For these six metrics, chironomid identification was irrelevant.

Taxonomic resolution to genus strongly affected the ability of the metrics taxa richness (Figure 3.1c), dominance (Figure 3.5c), Biotic Index (Figure 3.6c), percent predators (Figure 3.9c), percent omnivores (Figure 3.11c), and intolerant taxa (Figure 3.12c to discriminate among streams. For this set of six metrics, identification of chironomids to genus is important.

Therefore, both the "chironomids to family" and the "all to genus" data sets showed the greatest ability to discriminate among streams using six sets of metrics each. If using data where chironomids are to family, only six metrics met the criteria for

inclusion in the multimetric. However, if using data where chironomids are identified to genus, six additional metrics met the criteria for inclusion in the multimetric and increased its ability to discriminate among streams.

For 12 metrics, taxonomic resolution that included chironomids identified to genus consistently met the two criteria needed for reliability: 1) the behavior of metrics were consistent with responses expected based on theory and 2) the reference stream had the best value. These results suggest that benthic macroinvertebrates, including chironomids, should be identified to genus for use in multimetric bioassessments in the RR/SFC watershed.

Spatial Variability Within-Streams

Spatial variability was analyzed to determine if a stream would be accurately assessed with only one reach, as suggested by the EPA. Significant variation among-reaches within a stream, would suggest that more than one reach be analyzed for reliable stream bioassessment.

Field Observation

Reaches of the streams were compared with on-site observation and photography. Photographs were taken to compare the lower and middle reaches of Buzzard Creek (Figure 3.13). Buzzard Creek had stable gravel bars (indicated by vegetation), well-forested riparian zones, a closed canopy, and low turbidity and siltation in the lowest

reach (Figure 3.13a). The middle reach (Figure 3.13b) had a narrowly forested riparian zone and direct cattle access in some areas, some siltation, little turbidity, and a closed canopy in most locations. The upper reach of Buzzard Creek, which was not photographed, flows from a cave. It had a wide, well-forested riparian zone, a closed canopy, little siltation and turbidity, stable gravel bars and no cattle access. The upper reach of Buzzard Creek was different from Millers and Spring creeks in that its entire discharge issues from a cave. Millers and Spring creeks both have multiple spring sources, but both creeks flow as surface streams for some distance before the upper reach sampling sites.

The lower, middle, and upper reaches of Millers Creeks were photographed and evaluated (Figures 3.14). The lower reaches of Millers Creek had narrow, sparsely vegetated riparian zones, direct cattle access, siltation, some turbidity, unstable gravel bars, and an open canopy in many segments. The middle reach (Figure 3.14b) of Millers Creek had a wide, well-forested riparian zone, closed canopy, little to no siltation or turbidity, and stable gravel bars. The upper reach of Millers Creek (Figure 3.14c) had a narrowly forested riparian zone with a gravel road on the right bank, a mostly closed canopy, little sediment and turbidity, and stable gravel bars.

The lower, middle, and upper reaches of Spring Creek were photographed and evaluated. The lower reach of Spring Creek (Figure 3.15a) has many areas of non-forested riparian zone, unstable gravel bars, no canopy, direct cattle access, heavy siltation and turbidity. The middle reach of Spring Creek (Figure 3.15b) shows unstable stream banks and high turbidity after a few minutes of heavy rain. This reach had direct cattle access, a partially forested and often narrow riparian zone, a partially open canopy,

a. Lower Reach

b. Middle Reach

Figure 3.13. Photographs of the (a) lower and (b) middle reaches of Buzzard Creek, Robertson County, Tennessee.

a. Lower Reach

b. Middle Reach

c. Upper Reach

Figure 3.14. Photographs of the (a) lower, (b) middle and (c) upper reaches of Millers Creek, Robertson County, Tennessee.

a. Lower Reach

b. Middle Reach

c. Upper reach

Figure 3.15. Photographs of the (a) lower, (b) middle, and (c) upper reaches of Spring Creek, Robertson County, Tennessee.

and unstable gravel bars. Portions of the upper reach of Spring Creek (Figure 3.15c) have no forested riparian zone, no canopy cover, direct cattle access, with siltation and turbidity. Further downstream in this reach there is a narrow forested riparian zone that provides a full canopy.

Graphical Analyses

Graphical analysis was performed on the among-reach behavior of metrics. Dot plots of the 17 metrics calculated from the genus-level macroinvertebrate data sets, Figures 3.1c-3.12c and 3.16-3.20, illustrate the variation of metrics within each stream. The x-axis values range from 1 to 15, representing samples 1-5 from the lower reach, samples 6-10 from the middle reach, and samples 11-15 from the upper reach. Plots were evaluated graphically, comparing among samples 1-5, 6-10, and 11-15, to assess among-reach variation.

Graphical assessment of metric plots demonstrated among-reach variation of all metrics. For example, in Millers Creek average taxa richness (Figure 3.1c) ranged from ca. 28 in the lower reach, ca. 32 in the middle reach, and ca. 37 in the upper reach. Using this same metric and stream, the within reach values varied as well. In the lower reach, individual riffles 1-5 taxa richness ranged from 22-30 in the middle reach, riffle samples 6-10, ranged from 31-33 and in the upper reach, riffle samples 11-15, ranged from 25-43. Thus, any random riffle sample from any of the three reaches could yield misleading results. These results suggest that only two composited riffle samples from a single reach would not adequately represent a stream as suggested by the RPB (Barbour et al 1999).

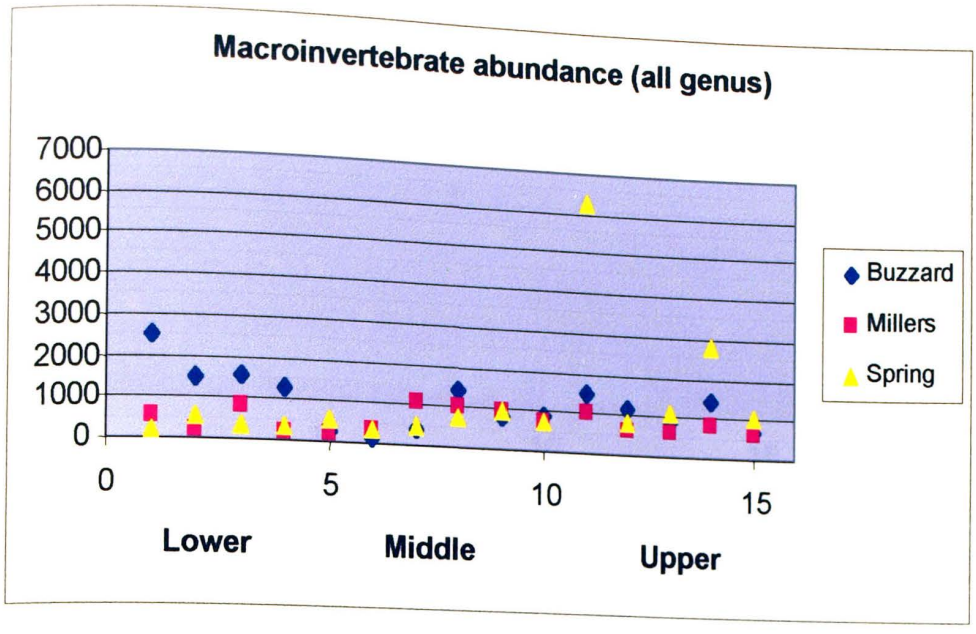


Figure 3.16. The metric macroinvertebrate abundance using the all genus data set.

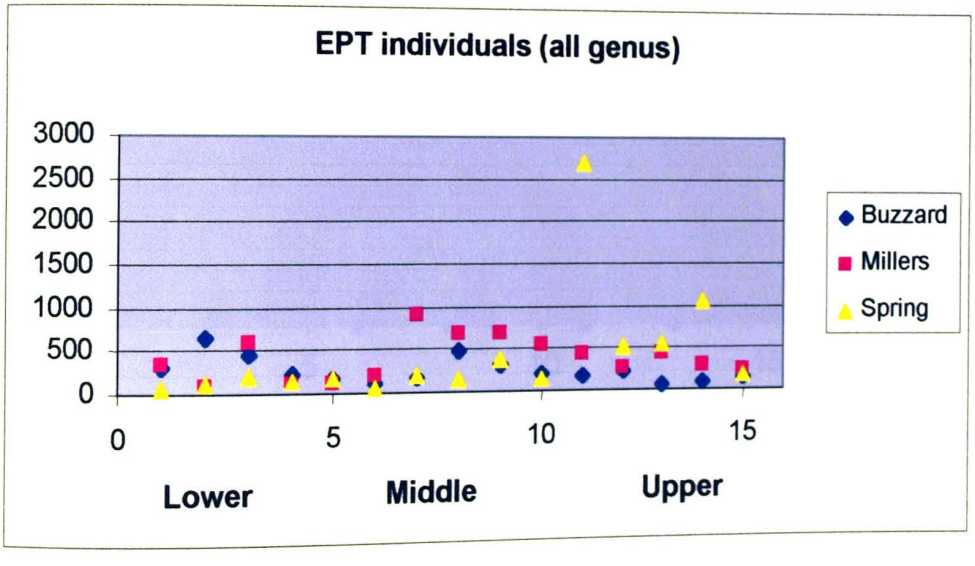


Figure 3.17. The metric EPT individuals using the all genus data set.

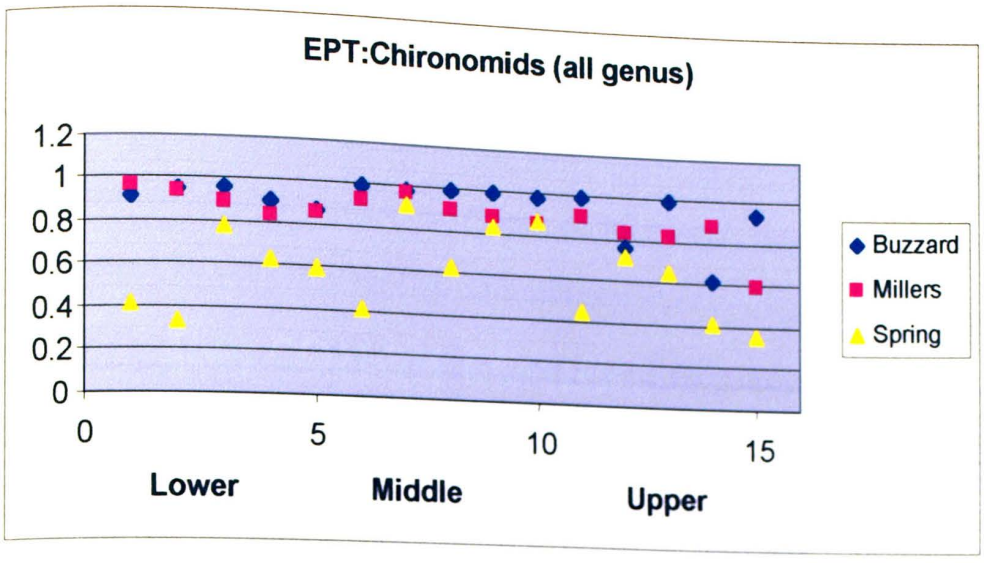


Figure 3.18. The metric EPT:Chironomid ratio using the all genus data set.

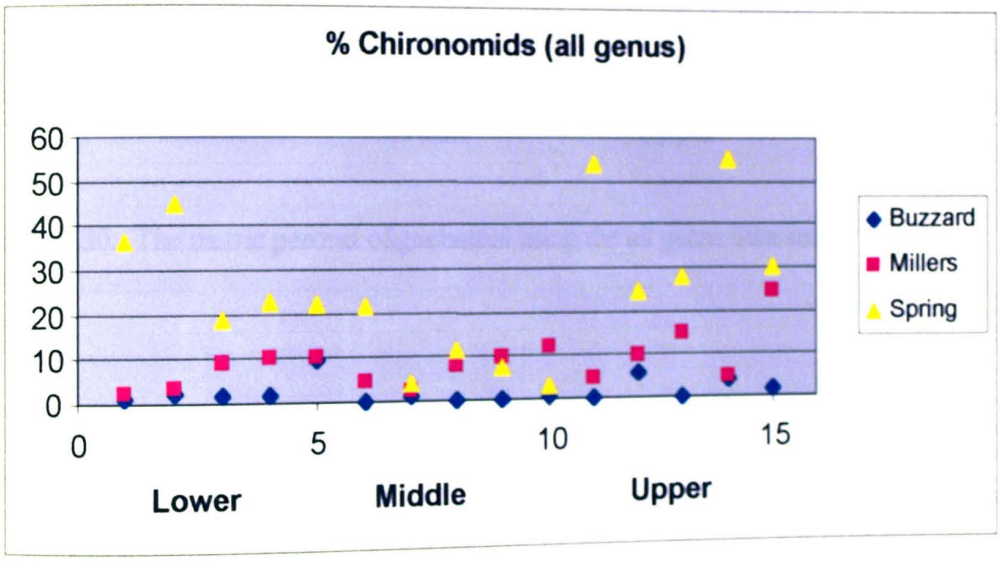


Figure 3.19. The metric percent chironomids using the all genus data set.

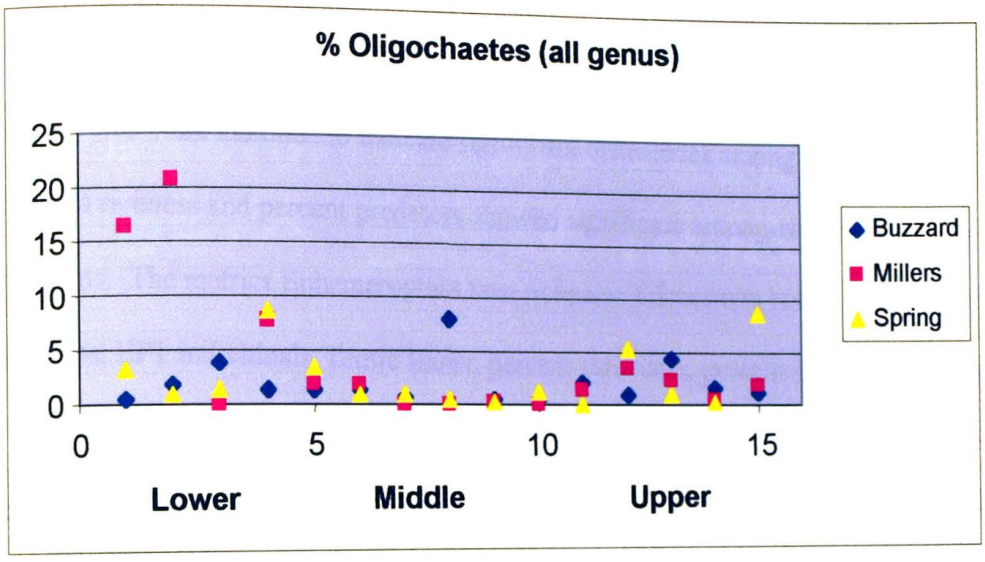


Figure 3.20. The metric percent oligochaetes using the all genus data set.

An analysis of variance (ANOVA) was performed on the metric scores calculated from the data set identified to genus-level to evaluate among-reach variance within streams (Table 3.1). Only 14 of the 17 metrics evaluated could be transformed to satisfy the normality assumption of ANOVA. Thus, ANOVA was not performed on the following metrics: percent chironomids, percent omnivores, and percent oligochaetes. P-values of ≤ 0.05 were assumed to indicate significant differences among reaches. The metrics taxa richness and percent predators showed significant among-reach differences in all streams. The metrics Ephemeroptera taxa richness, Trichoptera taxa richness, EPT taxa richness, EPT individuals, Biotic Index, percent shredders, percent scrapers, percent tolerant, and intolerant taxa richness showed a significant among-reach difference in at least one stream. The metrics macroinvertebrate abundance, EPT:Chironomid ratio, and dominance did not show significant differences among reaches in any of the streams. These results suggest that when using 11 of these 14 metrics, more that one reach would need to be evaluated for a reliable bioassessment.

Summary of Spatial Variation

Field observations of stream conditions revealed differences among reaches within each stream. These results suggest "best professional judgment" was fairly reliable. However, this judgment required the advice of experienced aquatic ecologists.

Table 3.1 ANOVA of metric values by reach within streams. Values are the p values of the F test. Shaded cells indicate metrics that varied significantly among reaches in a stream ($p \leq 0.05$).

Metric	Buzzard Creek	Millers Creek	Spring Creek
Macroinvertebrate abd	0.2022	0.0530	0.0729
Taxa Richness	**0.0036**	**0.0214**	**0.0330**
Ephemeroptera Taxa Richness	**0.0237**	0.9971	0.3661
Caddisfly Taxa Richness	0.7403	**0.0059**	**0.0447**
EPT Taxa Richness	0.0833	0.2832	**0.0419**
EPT Individuals	0.0945	**0.0417**	0.0665
EPT / Chironomids + EPT	0.1594	0.1361	0.1985
Dominance	0.0644	0.9900	0.2508
Biotic Index	0.1109	**0.0294**	0.1641
% Shredders	0.7298	0.7863	**0.0412**
% Scrapers	0.3387	**0.0059**	**0.0083**
% Predators	0.0006	**0.0071**	**0.0067**
% Tolerant	0.0027	0.2703	**0.0039**
Intolerant Taxa Richness	0.0331	0.3293	**0.0739**

plots of metric scores supported the field observations. These results suggested that among-reach variation in streams was significant. These results also illustrated significant among-sample variation within each of the reaches. Thus, for reliable multimetric bioassessments, more than one reach should be sampled within streams of the RR/SFC watershed,.

Statistical analyses indicated that sampling from only one reach would likely yield inaccurate bioassessment results when using 11 of these metrics. These results explain and support the field observations and graphical analyses of metric scores.

The reference stream, Buzzard Creek, had less among-reach variation of metrics (36%) than Millers and Spring creeks. Spring Creek had greater among-reach variation of metrics (57%) than Millers Creek (43%). These results suggest that streams with greater habitat stress, may have greater among-reach variation within the RR/SFC watershed. These results all suggest that streams cannot be adequately scored by sampling from only one reach, as suggested by the EPA's RBP (Barbour et al 1999).

Selection of Metrics for use in Bioassessments

Graphically, the greatest difference among streams was in the lower reaches, riffle samples 1-5. This pattern was consistent in data sets at all three taxonomic resolutions. Thus, the ability of a metric to discriminate among streams, respond in a manner consistent with theory, and yield the best score in the reference stream, was greatest in

the lowest reach. These data points correspond to x-values 1-5, labeled "lowest" on the graphs.

Only the lowest reach was analyzed with ANOVA because the lowest-most reach of Buzzard Creek is the best reach to use as a reference. With some cattle access, the middle reach is more impacted, and the upper reach of Buzzard Creek differs from the other streams in that is it nearer the source (which is a cave for Buzzard Creek only). Graphical analyses confirmed that the lowest reach of Buzzard Creek was the most "reference-like" in character, and the deviations of the other streams from the reference condition seemed most pronounced in the lowest reach. Therefore, we decided to use only the lowest reach of each stream in an ANOVA because the lowest reach data responded more predictably

Graphical Analyses of the Lowest Reach Only

Graphical analyses of the metrics scores calculated from the all genus data set was performed with dot (Figures 3.1c – 3.12c and 3.16 – 3.20). Expected values are stated in Table 2.2, Chapter II, Methods. Criteria used to select metrics that could discriminate among streams included: 1) Buzzard Creek (reference) had the best value, 2) metric responded as predicted by theory, and 3) metric values in the lowest reach responded in the same way. The metrics taxa richness (Figure 3.1c), Trichoptera taxa richness (Figure 3.3c), EPT:Chironomid ratio (Figure 3.18), Biotic Index (Figure 3.6c), percent scrapers (Figure 3.8c), percent chironomids (Figure 3.19), percent predators (Figure 3.9c), percent

tolerant (Figure 3.10c), percent omnivores (Figure 3.11c), and intolerant taxa richness (Figure 3.12c) discriminated among streams using the stated criteria.

Statistical Analyses of the Lowest Reach Only

One criterion used to evaluate the importance of a metric was its statistical significance (a metric differed significantly among streams for P values ≤ 0.05). A second criterion was that a reasonably high percent of the variability among streams was explained by the metric (values of $R^2 \geq 50\%$ were accepted). . A one-way ANOVA of each metric by stream using the data from the lowest reach, samples 1-5 only, was performed. Table 3.2 lists P-values and adjusted R^2 resulting from ANOVA.[NOTE: the word significant should be reserved for statistical significance, and so not used for R^2 here.] Seven metrics met both criteria: macroinvertebrate abundance, EPT:Chironomid ratio, Biotic Index, percent scrapers, percent predators, percent tolerant, and percent omnivores.

The results of the Dunnett's Test are given in Table 3.3. Streams that were significantly different from Buzzard Creek are identified with and asterisk (*). Ideally, a robust metric would show a significant difference between the reference stream, Buzzard Creek, and the treatment streams, i.e. Millers and Spring creeks.

The metrics macroinvertebrate abundance, Biotic Index, percent scrapers, percent predators, percent tolerant, and percent omnivores in Buzzard Creek scored significantly different from both Millers and Spring creeks. The metrics, EPT:Chironomid ratio, and

Table 3.2. ANOVA results using the lowest reach of the three study streams. P-values were considered significant at the ≤ 0.05 level. Adjusted R^2 values are the percent of the variation explained by the model.

Metric	P-value	R^2
Macroinvertebrate Abundance	0.009	55%
Taxa Richness	0.49	40%
Ephemeroptera Taxa Richness	0.53	10%
Trichoptera Taxa Richness	0.084	34%
EPT Taxa Richness	0.43	13%
EPT Individuals	0.16	26%
EPT: Chironomid	0.0002	75%
Dominance	0.23	22%
Biotic Index	<0.0001	82%
% Shredders	0.037	42%
% Scrapers	0.0002	75%
% Chironomids	0.07	36%
% Predators	0.001	68%
% Oligochaetes	0.12	30%
% Tolerant	0.0004	72%
% Omnivores	0.0003	75%
Intolerant Taxa	0.27	20%

Table 3.3. Results of Dunnett's Test following significant results from one-way ANOVA. A metric in bold font indicates a metric that responded ideally, with the treatment streams significantly different than the control stream. NS indicates no significant difference from the control stream. An asterisk (*) indicates a significant difference from the control stream.

Metric	Millers Creek Treatment	Spring Creek Treatment
Macroinvertebrate Abundance	*	*
EPT: Chironomid	NS	*
Biotic Index	*	*
% Shredders	NS	*
% Scrapers	*	*
% Predators	*	*
% Tolerant	*	*
% Omnivores	*	*

percent shredders scored significantly different from Buzzard in Spring Creek, but not in Millers Creek.

Summary of Metric Selection

Metrics were selected for inclusion in the multimetric index for bioassessment based on four criteria: 1) the metric responded as predicted by theory, 2) Buzzard Creek had the best metric scores, 3) ANOVA of the metric explained over half of the variation among streams ($R^2 \geq 50\%$) and was significant ($P \leq 0.05$), and 4) Dunnett's test revealed Buzzard Creek to be significantly different from the treatment streams.

The metric macroinvertebrate abundance was not selected for inclusion, although it met the four criteria. Macroinvertebrate abundance can only be used if all macroinvertebrates are removed and identified (whole-count) from quantitative benthic samples. If fixed-count (non-proportional) subsampling is being employed for a bioassessment, as is typical with rapid bioassessment protocols, the abundance would always approximate the set minimum value established *a priori* (typical 100, 200, or 300). In this study, the average number of macroinvertebrates in each Hess sample was approximately 900. Also, a sample in which 900 out of 1000 organisms were chironomids or oligochaetes would rate similar to a sample of 1000 organisms representing 40 taxa even though the former assemblage would be considered much impaired compared to the latter. Thus, abundance was not selected as a metric for use in the multimetric bioassessment to discriminate among streams.

The metrics Biotic Index (Figure 3.6c) percent scrapers (Figure 3.8c), percent predators (Figure 3.9c), percent tolerant (Figure 3.10c), and percent omnivores (Figure 3.11c) met the four criteria set forth by this study and were included in the multimetric index. Thus, these metrics are recommended for reliable multimetric bioassessments in the RR/SFC watershed.

Multimetric Bioassessment

Table 3.4 represents mean metric values obtained from the five riffle samples from the lower reach of Buzzard Creek (reference condition) and 15 samples (upper, middle, and lower reaches) of Millers and Spring creeks . Only five of the 17 metrics evaluated met the four criteria for inclusion in the multimetric index. Shaded cells in Table 3.3 did not meet these criteria.

Table 3.5 represents the metrics selected for inclusion (non-shaded cells) where metric scores (Table 3.4) have been normalized to a 100-point scale for equal weight. Table 3.5 also has a row entitled "RANK," which is the mean metric score, i.e., the multimetric index score. These values were used in the final bioassessment in Table 3.6. Bioassessment ranks were calculated as the percent similarity of the study stream multimetric score to that of the reference condition, lowest reach of Buzzard Creek, multimetric score. The ranks used were "relatively nonimpaired" (>83% similar), "slightly impaired" (54 - 83 % similar), "moderately impaired" (21 - 53 % similar), and "severely impaired" (<21% similar) (modified from Plafkin et al. 1989). By default, Buzzard Creek, the reference stream, ranked "relatively nonimpaired" with a value of

Table 3.4. Mean metric scores of five riffle samples in the lowest reach only of Buzzard Creek (reference condition) and 15 samples from Millers and Spring creeks using the genus-level data set. Shaded columns are metrics not included in the multimetric index for bioassessment.

Metrics	Buzzard	Millers	Spring
macroinvertebrate abundance	1419	653	1145
Taxa Richness	33	32	28
Ephemeroptera Taxa Richness	6	6	5
Trichoptera Taxa Richness	4	4	3
EPT Taxa Richness	10	11	8
EPT abundance	362	391	435
EPT:Chironomids	0.92	0.87	0.60
Dominance	0.33	0.28	0.29
Biotic Index	4	5	5
% Shredders	1	1	12
% Scrapers	56	22	14
% Chironomids	3	9	25
% Predators	1	5	7
% Oligochaetes	2	4	2
% Tolerant	4	27	19
% Omnivores	2	6	8
Intolerant Taxa Richness	6	5	5

Table 3.5. Similarity of treatment stream metric scores to those of Buzzard Creek. The mean metric scores were normalized to a percentage score by dividing each by the best value and multiplying by 100 [(metric/best metric value) x 100]. Metric values were calculated using generic level of taxonomic resolution, including chironomids, except select macroinvertebrates. The last row, RANK, thus represents average score for each stream's metric and represents the streams multimetric score.

Metrics	Buzzard	Millers	Spring
Biotic Index	100	76	74
% Scrapers	100	39	24
% Predators	100	30	21
% Tolerant	100	14	21
% Omnivores	100	33	26
RANK	**100**	**38**	**33**

Table 3.6. Multimetric bioassessment using multimetric index derived from table 3.4. The similarity to reference score synonymous with "RANK" in Table 3.5. Bioassessment ranks were calculated as the percent similarity of the study stream multimetric score to that of the reference stream, Buzzard Creek. The ranks used were "relatively nonimpaired" (>83% similar), "slightly impaired" (54 - 83 % similar), "moderately impaired" (21 - 53 % similar), and "severely impaired" (<21% similar) (modified from Plafkin et al. 1989). By default, Buzzard Creek, the reference stream, ranked as relatively nonimpaired with a value of 100%.

Stream	Similarity to Reference see note above	Multimetric Bioassessment Score
Buzzard	100	relatively nonimpaired
Millers	38	moderately impaired
Spring	33	moderately impaired

100%. Millers Creek ranked "moderately impaired" with a similarity to the reference of 38%. Spring Creek ranked "moderately impaired" with a 33% similarity to the reference stream.

Further Study

Further studies on multimetric bioassessments in the RR/SFC watershed may include analyzing the behavior of metrics on macroinvertebrates with identification of all macroinvertebrates to lowest practical taxonomic level. Lowest practical taxon may include species-level taxonomy. Given the increased ability of some metrics to detect significant differences among streams when increasing the level of taxonomic resolution for chironomid identification from family to genus, it is likely that some metrics would exhibit increased ability to detect differences among streams if identification to the lowest practical taxon for all groups was applied.

Further study may also include analyses of samples from reaches 2 and 4 of this study. Recall that only reaches 1, 3, and 5 were evaluated. All five reaches were sampled on the same two days. Evaluation of these reaches may further understanding of how many reaches should be evaluated in multimetric bioassessments while allowing for spatial variability within streams in the RR/SFC watershed. These reaches have been sorted from detritus and are in Dr. Hamilton's laboratory at Austin Peay State University, Clarksville, Tennessee.

Analyses of temporal effect of times of year. Sampling during seasons other fall may describe a need for a specific set of metrics to be used for bioassessments.

Spring Creek samples from the lowest reach in this study may be used as the baseline aquatic macroinvertebrate survey for post-restoration analyses. Five riffle samples were collected from this reach in October 2000 following a restoration of that reach. These samples are in the laboratory of Dr. Hamilton.

Analysis of temporal variation may be performed by studying the samples collected in October 2000. These samples include five Hess samples collected randomly from five reaches in Buzzard, Millers and Spring creeks (five samples x five reaches) totaling 25 samples per stream.

Additional studies could test the multimetric index derived in this study in other streams in the RR/SFC watershed or in other watersheds in the same ecoregion. This study only evaluated three streams, so the general application of the multimetric index developed here remains to be tested.

The need for reliable bioassessments for the Red River watershed is a concern for all who live within it.

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Appendix

Genus-level data set for Buzzard Creek. TV = Tolerance Value, a value of 0 indicates no TV assigned; TRPH = Trophic Group, 1= herbivores, 2= detrivores, 3= filter feeder, 4= collector gatherer, 5= scraper, 6= predator, 7= omnivore, 1+2= shredder ; B1:1 = Buzzard Creek, reach one, sample 1; B1:2 = Buzzard Creek, reach one, sample 2; and so on.

Genus	TV	TRPH	B1:1	B1:2	B1:3	B1:4	B1:5	B3:1	B3:2	B3:3	B3:4	B3:5	B5:1	B5:2	B5:3	B5:4	B5:5
Turbellaria	0	6	21	30	24	11	11		6		4		5	5		82	
Nematoda	4.8	0	5		5	2	1	2						1	1		
Prostoma	0	0												2	2	18	
Corbiculidae	6.12	3				3			1	15		1	3				
Ancylidae	0	5	1									1	4				2
Physidae	0	5	1							1							
Planorbidae	5.23	5									1					2	
Pleuroceridae	2.46	5	1461	359	452	73	2	1	10	124	74	154	871	328	147	149	99
Sphaeriidae	7.58	3	20	18	12	41		1		2	1	2		1	2		
Oligochaeta	7.11	7	14	27	60	16	4	2	3	110	4	3	31	12	38	22	7
Crangonx sp.	7.87	4													1		
Gammarus	9.1	4	12	5	11	115		11	68	267	125	103	144	176	243	721	177
Hydracarina	5.53	6	24	10	24	14	1		1	1	4	2	9	3	7	9	3
Orconectes	7.5	7							2			2					
Cambarus sp.	7.62	7		1	1	1						1					
Lirceus sp.	7.85	4			1	16	1									20	
Cambaridae	9.46	7		1		1											
Baetisca sp.	3.4	4															
Caenis sp.	7.41	4	3	1			2					1		1			
Serratella sp.	1.7	4	1	2	2												
Isonychia sp.	3.45	3	29	41	11	4	23	1	2	12	11				1		
Diphetor	4	5										1					
Baetidae	4	5		6		1					3	2					
Acentrella sp.	3.61	1			1			6					2	18		7	5
Baetis sp.	5.4	4	30	176	81	26	42	26	21	6	16	4			3		
Leptophlebiidae	2	4													1		
Heptagenia sp.	2.57	5		5		1			1	1	5	4				1	
Stenacron sp.	3.57	5	16	47	11	14			14	89	27	32		1	3	3	
Stenonema sp.	7.2	4	147	284	147	45		23	46	174	143	113	14	1		4	
Heptageniidae ?	0	6		1						1							
Rhagovelia	6	6					1										
Argia sp.	8.17	6	3		3												
Calopteryx sp.	7.78	6	1														
Gomphidae ?	6	6	1														
Boyeria sp.	5.97	6	1														
Stylurus sp.	5.8	6	1														
Nemouridae	0	2							3					1			
Corydalus	5.16	6		1	3	1	1				1						
Nigronia sp.	5.25	6	1	2					3	9		10		2	2	6	1
Sialis sp.	7.17	6						1									

Genus level data set for Buzzard Creek

Genus	TV	TRPH	B1:1	B1:2	B1:3	B1:4	B1:5	B3:1	B3:2	B3:3	B3:4	B3:5	B5:1	B5:2	B5:3	B5:4	B5:5
Pyralidae ?		7	4														
Optioservus sp.	2.36	5	269	192	239	340		16	109	292	196	340	220	365	391	253	206
Stenelmis sp.	5.1	5	97	50	115	227		1	22	49	26	22				1	1
Promoresia sp.	2.35	5	3		1												
Microcylloepus	2.11	5			2					1							
Oulimnius sp.	1.8	5				1											
Dineutus sp.	5.54	6	1			2											
Helichus sp.	4.63	5				1											
Hydroptila sp.	6.22	1													2		
Dytiscidae ?	0	6				1											
Psephenus herricki	2.35	5	230	102	102	153	59	5	12	33	54	37	7		1		
Micrasema sp.	0.75	1	7	1	6	32						1			13		3
Glossosoma sp.	1.55	5						2		5	5		135	140	26	7	128
Helicopsychidae	3	4															
Ceratopsyche	2.18	3			9			7	10	5	1	2		1		2	
Cheumatopsyche sp.	6.22	3	68	82	189	72	82	30	74	163	106	33	23	50	7	44	10
Hydropsyche sp.	4.29	3		2	6	3		4	8	24	2				1	22	6
Hydroptila sp.	6.22	1											1				
Lype diversa	4.05	2													5		
Polycentropus sp.	3.53	6	1														
Chimarra sp.	2.76	3	2	3	3	24	21	5	3	4							
Trichoptera ?	4.46	1											8				
Simuliidae	6	4		1	2		1	5	3	2	4		1				5
Tabanus sp.	9.7	3								5	3			1	1		
Chironomidae pupae	0	0					10						1				
Tipulidae	3	4	3								1						
pupae	0	3	3	6	2	5	10						1			1	
Chironomidae ?	5.7	4	1			1	2									1	
Stempellinella sp.	4.62	1					4									2	
Thienemaniella sp.	5.86	4	4	7	2				1							1	
Thienemannimyia GR	0	6	4				7					2					
Tanypodinae unkn	6.66	0		1										1			1
Tanytarsus sp.	6.76	7	8	1	2	3	1							2		5	1
Corynoneura sp.	0	4				3	1										
Larsia sp.	9.3	6				2											
Parametriocnemus	3.65	4	2	5	6	9	2		1					2		3	2
Polypedilum sp.	6.7	4		1					2					9		30	1
Rheotanytarsus sp.	5.89	3	7	15	10	2						1		51		12	6

Genus-level data set for Millers Creek. TV = Tolerance Value, a value of 0 indicates no TV assigned; TRPH = Trophic Group, 1= herbivores, 2= detrivores, 3= filter feeder, 4= collector gatherer, 5= scraper, 6= predator, 7= omnivore, 1+2= shredder ; M1:1 = Miller Creek, reach one, sample 1; M1:2 = Miller Creek, reach one, sample 2; and so on.

Genus	TV	TRPH	M1:1	M1:2	M1:3	M1:4	M1:5	M3:1	M3:2	M3:3	M3:4	M3:5	M5:1	M5:2	M5:3	M5:4	M5:5
Turbellaria	0	6	26	6	3				4				4	3		21	3
Prostoma	0	0			25		4			4	2	14	1	6	4	9	3
Nematoda	4.8	0	3	2	6	8	1	1			4			3	8	5	3
Nematomorpha	5	0							4			4					
Corbicula fluminea	6.12	3	3	6				2	1								
Ancylidae	0	5			3	8		4	10	7	10						
Physidae	0	5	1	1									12		2	2	3
Pleuroceridae	2.46	5			13	6	7	11	16	2		6	74	6		251	4
Sphaeriidae	7.58	3		6	8								3	2		2	
Oligochaeta	7.11	7	92	38		16	3	6			2		15	20	14	3	12
Hydracarina	5.53	6	6	6	13	15	18		1	1	8	2	9	5	4	7	14
Orconectes	7.5	7			1								3		1		
Lirceus sp.	7.85	4	1	5	6	2	1		2	9	2	6	5	2	2	21	17
Gammarus	9.1	4											1	1		3	33
Caenis sp.	7.41	4	16	2	41	7	17	65	31	88	114	44	34	26	132	24	1
Isonychia sp.	3.45	3	62	4	225	20	20	7	223	118	120	66	23	19	34	14	9
Tricorythodes sp.	5.06	1	1	3	2			4									
Diphetor	5.4	4											6				
Heterocloeon sp.	3.48	5	3														
Baetidae	4	5		3				2	4		12		12				
Acentrella sp.	3.61	1	5	3				2	1		1		24				
Baetis sp.	5.4	4	1	2	7	17	1	6	102	91	72	21	9	13	2	20	23
Paraleptophlebia sp.	0.94	4											1			1	
Choroterpes sp.	2	4											2				
Heptagenia sp.	2.57	5	2	1	2				7		8		3	1			
Stenacron sp.	3.57	5	2	2				1		4			26	17		13	37
Stenonema sp.	7.5	4	212	57	261	31	41	82	234	327	212	276	82	179	200	135	58
Rhagovelia obesa	6	6												5		1	
Calopteryx sp.	7.78	6														1	
Boyeria sp.	5.97	6														1	
Argia sp.	8.17	6	12	7	3		3	3	3	4		16	10			1	
Gomphidae	6	6	53	1	2	1	1		1				1	1			4
Plecoptera unknown	2.52	6								3	2	4					2
Corydalus cornutus	5.16	6	1		5		1	1	11		6	24	4				
Nigronia sp.	5.25	6						2		5	2	4	2	5	6	1	2
Sialis sp.	7.17	6		1												1	
Optioservus sp.	2.36	5	11	3	22	9	2	8	24	23	12	36	21	42	34	83	80
Stenelmis sp.	5.1	5	6	4		2		7		5		4	20	18	28	20	1
Psephenus herricki	2.35	5	7	7	34	5	2	57	65	177	136	4	378	142	6	68	80
Ectopria sp.	4.16	5	1													1	
Micrasema sp.	0.75	1												2		1	3
Glossosoma sp.	1.55	5															4
Helicopsychidae	3	3			2			13	4	1	4		171			51	14

Genus-level data set for Millers Creek continued.

Genus	TV	TRPH	M1.1	M1.2	M1.3	M1.4	M1.5	M3.1	M3.2	M3.3	M3.4	M3.5	M5.1	M5.2	M5.3	M5.4	M5.5
Ceratopsyche	2.18	3								2	6	6					2
Cheumatopsyche sp	6.22	3	18	4	58	31	23	10	252	51	100	86	9	2	44	10	71
Hydropsyche sp	4.29	3		2					2	2	8	10					
Hydroptila sp	6.22	4															
Polycentropus sp	3.53	6					1	2	4		10	16		4	2	5	
Ptilostomis sp		4											9				
Chimarra sp	2.76	3			3	4			38	5	12	22	26	4	6	1	3
Oecetis sp	4.7	6	3	1													
Chimarra sp	2.76	3	3														
Diptera unknown	0	1					4	5									
Simuliidae	6	1				1						4	1			8	
Atrichopogon sp	6.49	5														1	
Culicidae sp	0	0					4										
Empididae	7.57	0				1											
Tipulidae	3	0						1	1	3		12	1	4			
Chironomidae pupae	0	3					2		8	7	12	18	9	9	18	5	20
Chironomidae	5.7	5						3			2						
Stempellinella sp	4.62	1		1						4	2	4	2	7		5	
Thienemaniella sp	5.86	4		3	10	5		1		3							8
Thienemannimyia GR	0	6	5		4		1	3		4	6	4	5	5	16	5	4
Tribelos sp	6.31	1												1			
Trissopelopia	0	6											1				
Tanypodinae unkn	6.66	0				1			1								8
Tanytarsus sp	6.76	7		2	46	3	10	4	8	4	24	36	25	9	10	5	14
Cladotanytarsus sp	4.09	4				1											
Corynoneura sp	0	4				3		2				2		15	6	2	16
Glyptotendipes	0	4										2					
Lopescladius sp	1.67	4															2
Labrundinia sp	5.9	6	1											1			
Larsia sp	9.3	6						1									
Parametriocnemus sp	3.65	4	6		3	1	4	1	1	2	12	6		3	8	1	28
Paratanytarsus sp	8.45	4								2	6	4				1	
Polypedilum sp	6.7	4			5	2			8	50	26	16	2	4	34	10	44
Rheotanytarsus sp	5.89	3			6	5			1	2	2	2	4	1	1	4	10

Genus level data set for Spring Creek. TV = Tolerance Value, a value of 0 indicates no TV assigned; TRPH = Trophic Group, 1= herbivores, 2= detrivores, 3= filter feeder, 4= collector gatherer, 5= scraper, 6= predator, 7= omnivore, 1+2= shredder ; S1:1 = Spring Creek, reach one, sample 1; S1:2 = Spring Creek, reach one, sample 2; and so on.

Genus	TV	TRPH	S1:1	S1:2	S1:3	S1:4	S1:5	S3:1	S3:2	S3:3	S3:4	S3:5	S5:1	S5:2	S5:3	S5:4	S5:5
Turbellaria	0	6				1			5		1	7	3	4	2	2	1
Prostoma	0								2	5	4	10					3
Nematoda	4.8	0	1				1	1				1	3		2	2	1
Ancylidae	0	5					5										
Lymnaea sp.	3	4															
Physidae	0	5								8			3		2	14	6
Planorbidae	5.23	5				1							3	1	2	2	
Pleuroceridae	2.46	5	2			1	1	48	1	34	3	42					
Sphaeriidae	7.58	3															41
Oligochaeta	7.11	7	6	5	4	30	19	3	5	4	2	8		42	10	6	88
Hirudinea	0	6															
Hydracarina	5.53	6	7		12	11	47	5	3	2	9	20	24	19	30	12	46
Orconectes	7.5	7										1					5
Lirceus sp.	7.85	4											30	3	70	16	203
Cambarus sp.	7.62	7								4							
Gammarus	9.1	4						5									
Caenis sp.	7.41	4	7	8	5	2	8			36	4	2					
Serratella sp.	1.7	4															
Hexagenia sp.	4.9	4															
Isonychia sp.	3.45	3	4	12		12	12	7	19	2	54	5	9	1	6	2	4
Tricorythodes sp.	5.06	1	3	18	146	70	2	7	2	13		7					
Baetidae	4	5						1						1			
Acentrella sp.	3.61	5	2			1								6			
Baetis sp.	5.4	1	8	41	35	25	47	10	26	44	131	42		74	88	12	42
Paraleptophlebia sp.	0.94	4						1		12			3				1
Heptagenia sp.	2.57	5				1	2	4		2		2				4	4
Stenacron sp.	3.57	5		2			2	9		8		1		1	4	14	11
Stenonema sp.	7.5	4	28	29	22	51	71	52	161	308	389	194	39	12	48	30	17
Hemiptera	6	6					2										7
Microvelia sp.	0	6															
Rhagovelia obesa	6	6					2										7
Argia sp.	8.17	6	7	40	2	11	3	10	1	16	9	9					
Calopteryx sp.	7.78	6														2	
Gomphidae	0	0										2					
Gomphidae	6	6						1									
Corydalus cornutus	5.16	6	9	1	1		7		1		15	3	3				
Nigronia sp.	5.25	6						5	3	7		6	3				
Sialis sp.	7.17	6						4		2							
Optioservus sp.	2.36	5	6	68	5	7	14	58	94	68	71	262	12	10	13	2	75
Stenelmis sp.	5.1	5		12	1	1	4	2	2	15	9	19		2	12	6	12

Genus-level data set for Spring Creek continued.

Genus	TV	TRPH	S1:1	S1:2	S1:3	S1:4	S1:5	S3:1	S3:2	S3:3	S3:4	S3:5	S5:1	S5:2	S5:3	S5:4	S5:5
Promoresia sp.	2.35	5										2					
Hydroptila sp.	6.22	1						3									
Dytiscidae	0	6															
Psephenus herricki	2.35	5		13	5	20	73	11	1	6	13	2		3			
Glossosoma sp.	1.55	5							1								
Ceratopsyche	2.18	3	1	5		2	6	1	7		29	6	576	41	8	36	17
Cheumatopsyche sp.	6.22	3	22	26	10	18	98	11	117	22	108	80	1554	342	352	764	52
Hydropsyche sp.	4.29	3					1	1	9		14		537	36	92	220	32
Hydroptila sp.	6.22	1							2								
Lype diversa	4.05	2								4		5					
Neotrichia sp.	0	1							1								
Polycentropus sp.	3.53	6									7						
Chimarra sp.	2.76	3					4		18		15						
Chimarra sp.	2.76	3				1				2		4					
Diptera unknown	0	0	1	2													
Tabanidae	6.73	6	1							3							
Simuliidae	6	4						8	1							4	1
Ceratopogonidae	6	0						8	1								
Chironomidae pupae	0	0	7						2	26	13	6	90	22	28	116	32
Tipulidae	3	4	1	3				1			1						1
chiro pupae	0	3	7	9	24	15	12						8	1		14	
Chironomidae	5.7	4			1					2			4				1
Stempellinella sp.	4.62	1		10	2			4		3					2		
Thienemaniella sp.	5.86	4	7	8	2		2	6		12	27	3	40			22	1
Thienemannimyia	0	6		13	5	9	1	5		11		2	120	20	28	134	37
Tribelos sp.	6.31	1								4							
Tanypodinae unkn	6.66	0	1													2	2
Tanytarsus sp.	6.76	7	15	48	11	2	3	18	4	15	2	1	2244	102	104	654	134
Chironomus sp.	9.63	4			1												
Corynoneura sp.	0	4								2	1		44			12	
Dicrotendipes sp.	8.1	4				1								1			
Paracladopelma sp.	5.51	6								1							
Labrundinia sp.	5.9	6								1			8		2		
Parametriocnemus sp.	3.65	4	37	7	1	26	27	15	3	9	17	9	316	24	20	86	18
Paratanytarsus sp.	8.45	4								1					2		
Rheotanytarsus sp.	5.89	3		62	1	5	25	11	3	13	21	6	328	38	118	384	47
Polypedilum sp.	6.7	4	1	68	10	21	52	15	10	10	5	2	240	14	16	222	60

VITA

Rebecca Houtman was born in Pasadena, California on April 13, 1969. She attended Jonas Salk Elementary School in Illinois and Salisbury High School in Red Bluff, California where she graduated in 1987. The following September she enrolled at Shasta Junior College in Redding, California. A year later she enrolled in Randy's Beauty College where she earned her certification in Cosmetology. In 1995 she moved to the Republic of Panama where she volunteered at the Smithsonian Tropical Research Institute until 1998. At that time she moved to Clarksville, Tennessee to complete her bachelor's degree in biology at Austin Peay State University. In December of 2000 she earned her undergraduate degree and is currently in their masters of science program in biology under Dr. Steven Hamilton.