ANALYSES OF TWO ASPECTS OF STUDY DESIGN FOR BIOASSESSMENT WITH BENTHIC MACROINVERTEBRATES: SINGLE VERSUS MULTIPLE HABITAT SAMPLING AND TAXONOMIC IDENTIFICATION LEVEL

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(Abstract)

Bioassessment is the concept of evaluating the ecological condition of habitats by surveying the resident assemblages of living organisms. Conducting bioassessment with benthic macroinvertebrates is still evolving and continues to be refined. There are strongly divided opinions about study design, sampling methods, laboratory analyses, and data analysis. Two issues that are currently being debated about study design for bioassessment in streams were examined here: 1) what habitats within streams should be sampled; 2) and is it necessary to identify organisms to the species level? The influence of habitat sampling design and level of taxonomic identification on the interpretation of ecological conditions of ten small streams in western Virginia was examined. Cattle watering and grazing heavily affected five of these streams (impaired sites). The other five streams, with no recent cattle activity or other impact by man, were considered to be reference sites because they were minimally impaired and represented best attainable conditions. Inferential and non-inferential statistical analyses concluded that multiple habitat sampling design was more effective than a single habitat design (riffle only) at distinguishing impaired conditions, regardless of taxonomic level. It appeared that sampling design (riffle habitat versus multiple habitats) is more important than taxonomic identification level for distinguishing reference and impaired ecological conditions in this

bioassessment study. All levels of taxonomic resolution, which were studied, showed that the macroinvertebrate assemblages at the reference and impaired sites were very different and the assemblages at the impaired sites were adversely affected by perturbation. This study supported the sampling of multiple habitats and identification to the family level as a design for best determining the ecological condition of streams in bioassessment.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Bioassessment is the concept of evaluating the ecological condition of water bodies and other habitats by surveying the resident assemblages of living organisms. It is a valuable tool to help achieve the primary goal of the Clean Water Act of 1972 and its amendments of 1987. The primary objective of the Clean Water Act is "to restore and maintain the chemical, physical, and biological integrity of the Nation's waters." Measuring biological assemblages through bioassessment directly reflects the overall integrity of water bodies.

There are several advantages to using benthic macroinvertebrates in bioassessment which have made them the biological assemblage of choice for environmental monitoring programs (Voshell et al. 1997). They inhabit the bottom substrates of streams, rivers, and lakes, where they have limited movement. Benthic macroinvertebrates are abundant in most waters and can be sampled without a great deal of effort or specialized equipment. Aquatic biologists can easily identify them to the family level, and genus-level identification requires only slightly more training and experience. The many taxa exhibit range of sensitivity to all types of pollution and environmental stress. Many benthic macroinvertebrates have life cycles that last about one year, which is long enough to ensure that they will be exposed to any type of perturbation that occurs, and to demonstrate responses to those stressors.

Benthic macroinvertebrates have been used for bioassessment for more than 100 years, during which time there have been many changes in how the studies are conducted (Voshell et al. 1997). The practice began with very simple sampling and interpretation based on the presence or absence of individual taxa, or perhaps their relative abundance, to place water bodies into pre-established classifications of environmental quality. Over time the practice became much more complicated, emphasizing sampling methods that provide replication, estimates of density, and inferential statistical testing. By the 1980s, those evaluating the waters of the United States were on the verge of eliminating benthic macroinvertebrates from assessment practice (Voshell et al. 1997). The emphasis on rigorous quantitative ecology was not feasible for the extensive biomonitoring that needed to be done to be in compliance with environmental regulations. In the 1980s, this led to the development of cost-effective, yet scientifically defensible, methods that have come to be known as the "rapid bioassessment approach" (Karr 1981, Hilsenhoff 1988, Plafkin et al. 1989). This approach reduces the need for continuously comparing sites by replicated samples and inferential statistical tests in favor of establishing prior knowledge of expected biological conditions over broad geographic areas known as ecoregions. The rapid bioassessment protocols developed by the United States Environmental Protection Agency (USEPA) (Plafkin et al. 1989, Barbour et al. 1999) are now used by a majority of the state regulatory agencies in the United States. However, in spite of some signs of standardization, bioassessment is still evolving and continues to be refined. There are strongly divided opinions about study design, sampling methods, laboratory analyses, and data analysis. Two issues that are currently being debated about study design for bioassessment in streams were the subject of the research presented here: 1) what habitats within streams should be sampled; 2) and is it necessary to identify organisms to the species level? Cost effectiveness is the issue for both questions. Does one reach the same conclusion about the ecological condition of a stream by sampling just the single most productive habitat, usually a riffle, as by sampling all available habitats, which may take more time and effort? Does one reach the same conclusion about the ecological condition of a stream by identifying organisms only to the family or genus level, as by identifying as many organisms as possible to the species level, which takes more time and effort?

Sampling single or multiple habitats related to the rapid bioassessment approach was addressed in a study conducted on two North Carolina rivers. This was a pilot study for the original USEPA rapid bioassessment protocols (RBPs) (Plafkin et al. 1989). This pilot study supported sampling only the riffles/run habitat, by combining a sample from slow current and a sample from fast current into one single composite sample to represent the study site. This provided a standardized method of sampling and comparing streams, because it has been well documented that the greatest abundance and diversity of macroinvertebrates occurs in the riffle/run habitat. When the USEPA RBP manual was revised (Barbour et al. 1999), the single habitat design (riffle/run) was still emphasized, but it was recommended that the available habitat be analyzed to make sure that riffle/run habitat accounts for at least 30% of what is available. Sampling in alternate or multiple habitats was recommended where riffle/run represents less than 30% of the available habitat. Parsons and Norris (1996) also recommended single-habitat sampling based upon predictive modeling studies of biological assessment of water quality. For broad scale biological monitoring and stream classification, multiple habitat sampling was deemed unnecessary because sampling riffle, edge, and combined habitats resulted in similar characterizations (Hewlett 2000). Conversely, Lenat (1988) reported that sampling in multiple habitats provided a more complete inventory of stream assemblages than just sampling in the riffle/run habitat. The data, especially taxa richness, generated from the multiple habitat design were necessary to precisely rank water quality conditions. Some state agencies (e.g., North Carolina Division of Environmental Management) have incorporated the multiple habitat design into their regular biomonitoring protocol. Another bioassessment issue currently being debated is whether benthic macroinvertebrates need to be identified to the species level, or will investigators reach the same conclusions about ecological condition by identifying them only to the genus or even just the family level? Like the question about sampling single or multiple habitats, the level of taxonomy necessary for bioassessment was also evaluated as part of the original development of RBPs by the USEPA (Plafkin et al. 1989). In the same pilot study that investigated sampling design, an analysis of seven metrics based on family-level identifications resulted in similar conclusions about ecological condition as when the metrics were calculated on species-level information. Family-level data were sufficient to distinguish between severely impaired, moderately impaired, and non-impaired conditions (Plafkin et al. 1989). Other studies have shown that family-level data were sufficient to detect moderate and severe disturbances (Wright et al. 1995), and that family, genus, and species levels of taxonomic identification resulted in similar interpretations of ecological condition (Hewlett 2000). In a thorough statistical analysis of the taxonomy issue, Bailey et al. (2001) concluded that multivariate characterizations of macroinvertebrate assemblages do not seem to be sensitive to taxonomic resolution and that additional information attained from genus or species-level identification may represent undesirable noise in a bioassessment. These authors also made a very interesting observation about why they think so many aquatic biologists insist upon identifying organisms to the species level in bioassessment studies. It satisfies the soul of the naturalist that lives in most that study macroinvertebrates to be able to name the species of an organism and gain a morsel of insight into the species unique attributes. Most benthologists yearn to place a species level name on the fauna they collect from lakes and streams (Bailey et al. 2001).

Other investigators have provided evidence, or at least expressed opinions, that species-level identifications are necessary for accurate bioassessments. Some have argued that any increase in the cost for species-level identifications is more than compensated for by an improvement in the quality of data (Lenat and Resh 2001). Species-level identifications are supported by several studies (Resh and Unzicker 1975, Hawkins and Norris 2000, Lenat and Resh 2001). Faunal surveys of the genus *Ceraclea*, a caddisfly, showed that responses to pollution effects were completely different when analyzed at the species level rather than at the genus level (Resh and Unzicker 1975). Family-level data did not perform well enough to allow reasonable detection of biological impairment for mountain streams in California (Hawkins and Norris 2000). Lenat and Resh (2001) suggested that rare species might constitute most of the species in stream macroinvertebrate assemblages, and argued that an important component of biological monitoring is lost when family-level identifications are used. In contrast to the original USEPA RBP manual, the second edition advocated identifying most organisms to the lowest possible taxonomic level, usually to the genus or species level (Barbour et al. 1999). Mounting the Chironomidae (midges) on slides and identifying below the family level was also suggested.

Others have warned of the liability of species identifications in bioassessment (Voshell et al. 1997). Because most specimens collected in benthic assemblage studies are immatures, it is impossible to accurately identify all of them to the species level, especially the early instars. Often, so-called species-level identifications are based on expected distributions, rather than actually observing morphological features, which would appear to be a violation of the principles of science (Voshell et al. 1997).

OBJECTIVES

My research had three objectives, all related to the use of benthic macroinvertebrates for bioassessment of the ecological condition of streams:

1. to determine if sampling in riffle habitat only and sampling in multiple habitats produce the same conclusion about ecological condition;
2. to determine if identification to the family, genus, and species level produce the same conclusion about ecological condition;

3. to determine which combination of habitat sampling and taxonomic identification level best determines ecological condition.

METHODS

Study Design

The overall design of this study was to compare streams that were known to be in good ecological condition to streams that were known to have impaired ecological condition. Samples would be taken according to different spatial designs (riffle habitat versus multiple habitats), and the organisms in the samples would be identified to different taxonomic levels (family, genus, species). The purpose was to determine which approaches produced the conclusions that agreed most strongly with the prior knowledge of impaired ecological conditions in some streams.

Benthic macroinvertebrate assemblages from ten small streams in western Virginia were sampled once during the months of April and May 1997. Cattle watering and grazing heavily affected five of these streams (impaired sites). The other five streams, with no recent cattle activity, were considered to be reference sites because they were minimally impaired and represented best attainable conditions.

The criteria for designating study sites as impaired versus reference were:

1. cattle observed using the stream,
2. evidence of cattle, such as feces or hoof prints, in the stream and on the banks,
3. sparse shrubs or trees in the riparian zone,
4. obvious bare soil on the banks of the stream near the channel,
5. grass on flood plain grazed close to the ground,
6. eroded gullies leading into stream or slumped stream banks.

For reference sites, these conditions did not exist in the immediate vicinity of the site or upstream. Samples were taken in the single most productive habitat (riffle) and at multiple habitats at all ten study sites. The benthic macroinvertebrates in both types of samples were identified to the family and genus levels at all ten study sites. Because of the effort required for identifying benthic macroinvertebrates to the species level, this was only done at one impaired site and one reference site (Mill Creek tributary and Sidney Creek, respectively). Most of the organisms in small streams are immature stages of aquatic insects that can only be identified to species by collecting adults in the nearby terrestrial environment or rearing the immatures to the adult stage in the laboratory and then associating the adults with the immatures. Collections of immatures and adults were made once a week or once every two weeks at these sites from May through September 1996.

Habitat and environmental parameters were also measured at each site.

Study Sites

The area of study was confined to western Virginia, in the Blue Ridge and Ridge and Valley ecoregions. The study sites with descriptive information are listed in Table 1. All the sites were 1st order streams, ranging from 1 to 3 m wide. Watershed area ranged from 0.12 to 2.92 km^2, with the elevation of the sites varying from 387 to 787 meters. The study sites were located within two ecoregions and four subecoregions.

Table 1. Study site information. Ecoregion and subecoregion numbers: 66c = Blue Ridge Interior Plateau, 66e = Blue Ridge Sedimentary Ridges, 67a = Ridge and Valley Northern Limestrone/Dolomite Valleys, 67g = Ridge and Valley Southern Shale Valley

	Reference					Impaired				
	Sidney Creek	Piney Creek	Crawford's Spring	Moffatts Tributary	Marl Creek	Mill Creek Tributary	Piney Creek	Cox's Spring	Wilson's Site	Ogden Site
Stream Order	1	1	1	1	1	1	1	1	1	1
Width (m)	2	2	1	2	2	1	3	1	1	2
Watershed Area (km^2)	2.43	0.95	0.14	0.47	2.80	1.73	2.82	0.12	0.39	2.92
Elevation (m)	646	774	750	463	387	634	747	787	585	418
Ecoregion, subecoregion	66e and 67g	66c	66c	67a	67a	67g	66c	66c	67a	67a
UTM	47209630	11465104	8845156	48420412	44848606	49909986	10025030	10985106	53210046	47348789
County	Montgomery	Carroll	Grayson	Rockbridge	Rockbridge	Montgomery	Grayson	Carroll	Rockbridge	Rockbridge

Sampling Methods

Benthic Macroinvertebrate Assemblages

Benthic macroinvertebrate assemblages were sampled in accordance with methods recommended in the USEPA RBP protocols (Plafkin et al. 1989, Barbour et al. 1999). A 100-m tape was stretched longitudinally in the stream so that it followed the channel at the study site. For the single habitat procedure, riffle areas within the 100-m reach were surveyed and two riffles with different velocities (one slow and one fast) were selected as sampling locations. A 1-m kick net (500 µm mesh) was used to collect a sample in each of the two riffle areas. The kick net was spread out and held in place in each riffle area, while a 1-m^2 area upstream of the net was thoroughly disturbed by moving and rubbing all individual stones, thereby causing the macroinvertebrates to be dislodged and swept into the net. The two 1-m kick net samples in riffle areas were combined to form one composite sample for the study site.

For the multiple habitat sampling, 21 D-frame dip net samples were taken randomly within the 100-m reach. A random number table was used to choose 21 sample locations out of 153 possible sample areas. Possible sample areas were at the left and right bank and in the center of the stream channel at each 2-m interval along the 100-m longitudinal reach. At each of the sample locations that had current, the D-frame dip net (0.305 m wide, 500 µm mesh catch net) was placed on the bottom, and the substrate immediately upstream of the net was disturbed for approximately 30 seconds, allowing the macroinvertebrates to flow into the net. If the sample location had no current, the D-frame dip net was moved along the bottom approximately 0.30 m in an upstream direction. The contents of the 21 D-frame dip net samples were combined into one composite sample for the study site. The area of the 21 D-frame dip net samples that were combined for the multiple habitat design was equal to the area of the 2 kick net samples that were combined for the single habitat design.

The benthic macroinvertebrate samples were placed in plastic bags, preserved in the field with 95% ethanol, stored in plastic buckets, and returned to the laboratory for processing. An identification label, made of plastic embossing tape and stating the site, date, and sample type, was placed in each sample.

Supporting Data For Species Identification

In order to accomplish the identification of specimens to the species level at Mill Creek tributary and Sidney Creek, it was necessary to collect immatures and adults of the aquatic insects and to associate the two stages. Late instars of immatures were collected at the two sites, placed in plastic buckets with stream water and aeration, and returned alive to the laboratory in coolers to maintain ambient temperatures. Adults were also collected in the field with an aerial net and placed in individual vials with the collection information.

Habitat And Other Environmental Characteristics

Habitat parameters were measured at each study site using the recommendations of Plafkin et al. (1989), resulting in a habitat score with 135 possible points. The habitat assessment was performed on the same 100-m reach from which the benthic macroinvertebrate samples were collected. The dominant substrate at each benthic sampling area was categorized as bedrock, boulder, cobble, pebble, gravel, sand, or silt, and the dominant substrate type for the study site was assigned.

Water temperature and conductivity were measured with a YSI Model 33 meter. Stream discharge was measured by the USGS .6 method with a Marsh-McBirney, Inc. model 2000 portable flow meter as outlined by Buchanan and Somers (1969).

Laboratory Analyses

In the laboratory, each sample was washed through a series of nested sieves proceeding from coarse to fine. The larger debris captured by the coarser sieves was examined visually for any clinging macroinvertebrates. Materials from the finer sieves were examined under light, and all macroinvertebrates were removed and placed in a 2- oz. jar containing 70% ethanol and the original sample label.

All specimens collected from the ten study sites in April and May 1997 were identified to family and Lowest Identification Level Attainable (LILA) with the most recent taxonomic literature (Brigham et al. 1982, Merritt and Cummins 1996, Stewart and Stark 1988, Wiggins 1996, Pennak 1991) and enumerated. LILA is the taxonomic resolution that is readily achieved under the normal constraints of time and effort to complete benthic macroinvertebrate studies. Under LILA, most specimens were identified to genus. Early instars of some insect taxa and all non-biting midges (Diptera: Chironomidae) were identified only to family, and other non-insect invertebrates were often identified to higher taxonomic levels.

Live specimens collected at Mill Creek tributary and Sidney Creek in May through September 1996 were placed in a Living Stream® in an environmental chamber and allowed to molt to the adult stage. Freshly emerged adults were allowed to harden before placing them in individual vials with pertinent collection and emergence information.

Field collected and reared specimens from Mill Creek tributary and Sidney Creek were identified to family, genus, and species, using the most recent taxonomic literature (Brigham et al. 1982, Merritt and Cummins 1996, Stewart and Stark 1988, Wiggins 1996, Pennak 1991), supplemented with specialized literature for each specific taxonomic group. Most specimens were successfully identified to the genus and species level, however in some cases, specific names could not be assigned. Chironomidae (Order:Diptera) immatures collected at these two sites in May of 1996 and 1997 were permanently mounted on slides with CMC-10 mounting media and identified to genus using the latest literature (Epler 1995, Epler 1999, Merritt and Cummins 1996, Wiederholm 1983). Species recognition of larvae in this group is not possible. After associating all possible adults and larvae from Mill Creek tributary and Sidney Creek, the benthic samples taken in those two streams in 1997 were identified to species for as many specimens as possible and enumerated.

Data Analysis

Eighteen biological metrics were calculated for each sample (Table 2). These particular metrics were selected because they had been previously shown to exhibit low variance and to be effective at discriminating impaired and reference sites (Smith and Voshell 1997), and because they provided a variety of ecological information. The 18 metrics included: 5 that measured richness, 4 that measured balance, 3 that measured tolerance, 2 that measured habit, and 4 that measured trophic relations.

TABLE 2. List and explanation of metrics used to characterize the benthic macroinvertebrate assemblage.

Metric	Category	Description	Expected response to perturbation
Total taxa richness	Richness	Total number of taxa in the macroinvertebrate assemblage	Decrease
EPT Index	Richness	Total number of taxa in the orders Ephemeroptera (E), Plecoptera (P), and Trichoptera (T)	Decrease
No. of Ephemeroptera taxa	Richness	Number of taxa in the order Ephemeroptera	Decrease
No. of Plecoptera taxa	Richness	Number of taxa in the order Plecoptera	Decrease
No. of Trichoptera taxa	Richness	Number of taxa in the order Trichoptera	Decrease
Simpson's Diversity Index	Balance	Integrates richness and evenness into a measure of general diversity	Decrease
% Top 1 dominant taxa	Balance	Relative abundance of most abundant taxon	Increase
% Top 3 dominant taxa	Balance	Relative abundance of the 3 most abundant taxa	Increase
% Top 5 dominant taxa	Balance	Relative abundance of the 5 most abundant taxa	Increase
HBI	Tolerance	Modified Hilsenhoff Biotic Index – weighted sum of total taxa by pollution tolerance values	Increase
% Tolerant	Tolerance	Percent abundance of macroinvertebrates with tolerance values of 7, 8, 9, or 10	Increase
% Intolerant	Tolerance	Percent abundance of macroinvertebrates with tolerance values of 0, 1, 2, or 3	Decrease
% Haptobenthos	Habit	Relative abundance of macroinvertebrates requiring clean, firm, coarse substrates (crawlers and clingers)	Decrease
% Herpobenthos	Habit	Relative abundance of macroinvertebrates requiring soft, fine substrates (sprawlers and burrowers)	Increase
% Collector-gatherers	Trophic	Relative abundance of macroinvertebrates in the collector-gatherer functional feeding group	Increase
% Scrapers	Trophic	Relative abundance of macroinvertebrates in the scraper functional feeding group	Decrease
% Shredders	Trophic	Relative abundance of macroinvertebrates in the shredder functional feeding group	Decrease
% Collector-filterers	Trophic	Relative abundance of macroinvertebrates in the collector-filterer functional feeding group	Increase

A two-sampled t-test was performed on individual metrics from the 10 sites sampled in April and May 1997. This test compared the means from the 5 reference sites and 5 impaired sites for each individual metric. Minitab 13 for Windows-95/98/NT was used for this statistical analysis. An alpha level of 0.05 was set to indicate significance for the null hypothesis ($\mu_1 = \mu_2$).

A multivariate cluster analysis was performed on the metric data (Minitab 13 for Windows-95/98/NT). Using a hierarchical method with Euclidean distance, a hierarchical tree diagram or dendrogram was produced to classify the 40 observations from the 5 reference and 5 impaired sites sampled in April and May 1997 (10 study sites, two sampling protocols, and two taxonomic levels attained at each study site).

Since there were only two study sites that yielded species data, inferential statistics could not be performed. Measures of similarity were used with non-metric data and metric data to compare these two sites. Non-metric data included the individual abundance of each taxon. The metric data incorporated the individual values for each metric.

The Coefficient of Jaccard (Krebs 1998) was used with the non-metric family, genus, and species-level data to compare Mill Creek tributary and Sidney Creek. The formula is

$$S_j = a / (a+b+c)$$

where S_j = Jaccard's similarity coefficient

a = Number of joint species in both sample A and sample B

b = Number of species in sample B but not in sample A

c = Number of species in sample A but not in sample B

This binary coefficient measured the degree of similarity of Mill Creek tributary to Sidney Creek in terms of taxa presence or absence. The values can range from 0.0 to 1.0 and increase with degree of similarity.

The Bray-Curtis percent similarity measure (Krebs 1998) was used with both the non-metric and metric data to compare the family, genus, and species-level data from Mill Creek tributary and Sidney Creek. The formula is

$$B = 1 - \frac{\sum_{i=1}^{n} \left| Xij - Xik \right|}{\sum_{i=1}^{n} \left(Xij + Xik \right)} \text{x100}$$

where B = Bray-Curtis measure of percent similarity

Xij, Xik = Number of individuals in taxa i in each sample (j,k)

n = Number of taxa in samples

In the Bray-Curtis measure, the occurrence of rare taxa add little to the similarity value, so the measure is dominated by the abundant taxa. The Bray-Curtis can range from 0-100%; with the greater the similarity between the two samples resulting in a higher Bray-Curtis value.

The Manhattan index (Krebs 1998) was also used on the metric data from Mill Creek tributary and Sidney Creek . The formula is

$$M = \sum_{i=1}^{n} \left| Xij - Xik \right|$$

where M = Manhattan index

Xij, Xik = Value of observation i in each sample (j,k)

n = Number of observations in samples

This function measures the summation of absolute differences in the observation values of two samples. In this case, the function was a measure of differences in the metric values of the two study sites. The greater the difference between the two samples, the greater the value of the Manhattan index.

RESULTS

Habitat and Other Environmental Characteristics of Study Sites

Habitat quality scores and measures of some basic environmental characteristics supported my classification of the 5 reference and 5 impaired sites (Table 3). Habitat scores ranged from a high of 114 at one of the reference sites (Piney Creek) to a low of 22 at one of the impaired sites (Cox's Spring). The mean habitat score was twice as high at the reference sites than the impaired sites (92 versus 46, respectively) . Habitat scores at Moffats tributary (75) and Marl Creek 72) were noticeably lower than the other reference sites, but this was not related to cattle activity. At both of these sites, the bottom substrate, available instream cover, and diversity of flow regimes were noticeably different than at the other reference sites. The lower habitat scores at the impaired sites were caused by parameters related to the presence of cattle, especially bank stability and vegetation, streamside riparian cover, and substrate embeddedness. There was a considerable range in discharge among the sites, but this was approximately the same within the reference and impaired sites. Conductivity ranged from 20 to 385 μmhos and water temperature ranged from 9 to 16 ^{o}C among all sites. Conductivity was slightly higher at the impaired sites (mean = 206 μmhos) than at the reference sites (mean = 148 μmhos). Water temperature was also slightly higher at the impaired sites (means = 13.9 ^{o}C versus 12.6 ^{o}C). Higher conductivity and water temperature at the impaired sites were probably related to the impaired ecological conditions brought about by cattle activity, such as higher concentrations of nutrients and greater turbidity. The substrate was composed predominantly of larger particles (cobble and bedrock) at the reference sites, which provided firmer, more stable, and more complex stream bottom. The substrate at the impaired sites had higher proportions of silt, sand, and gravel, which made the stream bottom softer, less stable, and less complex. The different substrate conditions at the impaired sites were the result of erosion caused by cattle activity.

Table 3. Habitat scores and other environmental characteristics (April-May 1997).

	Reference					Impaired				
	Sidney Creek	Piney Creek	Crawford's Spring	Moffatts Tributary	Marl Creek	Mill Creek Tributary	Piney Creek	Cox's Spring	Wilson's Site	Ogden Site
Habitat Score	93	114	106	75	72	65	74	22	39	29
Discharge (cms)	0.012	0.026	0.004	0.018	0.007	0.027	0.083	*	0.007	0.017
Water Temperature (^{o}C)	15	9	11	12	16	13	12.5	15	13	16
Conductivity (umhos)	50	20	30	280	360	230	20	55	385	340
Dominant Substrate	cobble	cobble	cobble	bedrock	bedrock	silt	cobble	silt	gravel	silt

Benthic Macroinvertebrates

Raw data on the benthic macroinvertebrate assemblages from the 10 study sites sampled in April and May 1997 are presented in Appendices A-D. Counts of individual taxa collected from the riffle habitat and identified to the family level are presented in Appendix A, while Appendix B contains counts of individual taxa and collected from the riffle habitat and identified to LILA. Counts of individual taxa and collected by multiple habitat sampling and identified to the family level and LILA are presented in Appendices C and D, respectively. Values for all 18 biological metrics that were used to compare impaired and reference sites are reported for each of the 10 sites in Appendices E-H. Appendices E and F contain the values for the 18 metrics from the riffle habitat identified to the family level and LILA, respectively. Appendices G and H contain the metric values from multiple habitat sampling identified to the family level and LILA, respectively.

The means of the metrics that were used to characterize the benthic macroinvertebrate assemblages at different taxonomic levels and different sampling designs, along with the results of two sample t-tests comparing reference and impaired sites, are presented in Tables 4 and 5.

Table 4. Means (±SE) of metrics for benthic macroinvertebrate assemblages collected in the riffle habitat at reference and impaired sites and results of two sample t-test (α = 0.05, n = 5). Metrics were calculated for two taxonomic levels: family and lowest identification level attainable (LILA), which was usually genus.

	Family			LILA		
Metric	Reference	Impaired	*p*-values	Reference	Impaired	*p*-values
Total taxa richness	19.6 ± 1.5	12.4 ± 2.5	0.048	23.2 ± 2.3	15.6 ± 3.7	0.130
EPT Index	11.8 ± 0.86	5.8 ± 2.10	0.048	13.4 ± 1.2	7.4 ± 2.9	0.120
No. of Ephemeroptera taxa	4.2 ± 0.58	2.8 ± 1.00	0.278	5.0 ± 0.77	3.4 ± 1.50	0.395
No. of Plecoptera taxa	4.4 ± 0.68	1.4 ± 0.51	0.010	4.6 ± 0.81	1.6 ± 0.51	0.020
No. of Trichopetera taxa	3.2 ± 0.66	1.6 ± 0.68	0.136	3.8 ± 0.37	2.4 ± 1.00	0.257
Simpson's Diversity Index	0.85 ± 0.02	0.67 ± 0.10	0.160	0.87 ± 0.02	0.69 ± 0.10	0.170
% Top 1 dominant taxa	30.1 ± 4.1	47.7 ± 9.7	0.150	28.5 ± 3.6	46.8 ± 9.8	0.140
% Top 3 dominant taxa	53.3 ± 4.6	76.5 ± 6.4	0.022	51.2 ± 3.7	74.4 ± 3.6	0.019
% Top 5 dominant taxa	69.3 ± 4.0	89.3 ± 3.9	0.009	66.7 ± 3.7	86.2 ± 4.6	0.013
HBI	3.6 ± 0.25	5.0 ± 0.34	0.013	3.0 ± 0.19	5.1 ± 0.34	0.002
% Tolerant	0.2 ± 0.12	1.2 ± 0.75	0.260	0.2 ± 0.12	1.2 ± 0.75	0.260
% Intolerant	55.3 ± 6.9	18.6 ± 7.2	0.014	64.4 ± 3.9	17.6 ± 7.8	0.003
% Haptobenthos	80.9 ± 2.3	49.8 ± 11.0	0.046	82.3 ± 2.6	52.0 ± 11.0	0.049
% Herpobenthos	15.8 ± 2.5	49.1 ± 11.0	0.041	14.5 ± 2.7	46.9 ± 11.0	0.043
% Collector-gatherers	35.5 ± 8.3	54.3 ± 8.6	0.160	35.7 ± 8.2	54.6 ± 8.6	0.150
% Scrapers	13.9 ± 4.8	11.8 ± 3.4	0.730	13.9 ± 4.7	12.1 ± 3.6	0.780
% Shredders	16.4 ± 4.3	2.9 ± 1.0	0.037	15.1 ± 3.9	0.6 ± 0.4	0.020
% Collector-filterers	20.3 ± 5.3	19.9 ± 7.1	0.960	20.1 ± 5.3	19.9 ± 7.1	0.980

Table 5. Means (±SE) of metrics for benthic macroinvertebrate assemblages collected in multiple habitats at reference and impaired sites and results of two sample t-test (α = 0.05, n = 5). Metrics were calculated for two taxonomic levels: family and lowest identification level attainable (LILA), which was usually genus.

	Family			LILA		
Metric	Reference	Impaired	*p*-values	Reference	Impaired	*p*-values
Total taxa richness	25.0 ± 1.3	20.4 ± 2.3	0.130	30.2 ± 2.2	25.0 ± 7.8	0.260
EPT Index	15.4 ± 1.0	8.4 ± 1.9	0.017	17.6 ± 1.4	10.8 ± 2.7	0.068
No. of Ephemeroptera taxa	5.0 ± 0.32	3.6 ± 0.93	0.226	6.2 ± 0.49	5.0 ± 1.40	0.458
No. of Plecoptera taxa	4.2 ± 0.37	2.6 ± 0.68	0.084	4.6 ± 0.40	2.8 ± 0.66	0.059
No. of Trichopetera taxa	6.2 ± 0.80	2.2 ± 0.58	0.005	6.8 ± 0.97	3.0 ± 0.89	0.024
Simpson's Diversity Index	0.86 ± 0.03	0.54 ± 0.07	0.011	0.86 ± 0.03	0.54 ± 0.08	0.012
% Top 1 dominant taxa	26.3 ± 6.4	64.7 ± 6.2	0.004	26.3 ± 6.4	64.7 ± 6.2	0.004
% Top 3 dominant taxa	52.0 ± 5.5	84.5 ± 2.6	0.003	49.9 ± 5.4	82.2 ± 3.3	0.002
% Top 5 dominant taxa	69.1 ± 4.4	90.0 ± 1.8	0.007	65.7 ± 4.3	87.5 ± 2.6	0.005
HBI	4.0 ± 0.27	5.4 ± 0.17	0.007	3.6 ± 0.40	5.4 ± .016	0.009
% Tolerant	1.4 ± 0.34	3.8 ± 0.87	0.047	1.4 ± 0.34	3.9 ± 0.90	0.048
% Intolerant	42.4 ± 7.2	15.4 ± 3.8	0.016	51.5 ± 8.9	14.8 ± 3.5	0.012
% Haptobenthos	64.3 ± 6.8	27.5 ± 7.7	0.009	67.8 ± 6.5	28.5 ± 7.6	0.006
% Herpobenthos	33.8 ± 6.5	71.0 ± 7.9	0.008	30.3 ± 6.2	70.0 ± 7.9	0.005
% Collector-gatherers	42.2 ± 9.2	73.0 ± 6.7	0.030	42.5 ± 9.1	73.2 ± 6.7	0.030
% Scrapers	13.9 ± 3.5	4.2 ± 1.2	0.059	14.1 ± 3.2	4.3 ± 1.2	0.035
% Shredders	21.4 ± 5.4	2.3 ± 0.9	0.025	18.0 ± 5.4	1.3 ± 0.4	0.037
% Collector-filterers	12.9 ± 2.2	11.4 ± 6.2	0.840	12.7 ± 2.2	11.3 ± 6.2	0.840

The effectiveness of the two sampling designs and two taxonomic identification levels for distinguishing impaired ecological conditions, was compared by counting the number of metrics that showed a significant difference between the reference and impaired sites (Table 6). Having the most metrics significantly different between known reference and impaired sites can be interpreted as providing greater weight of evidence for drawing conclusions from bioassessment studies. The multiple habitat sampling design was more effective at distinguishing impaired conditions, regardless of taxonomic level. At both taxonomic levels, almost three-fourths of the metrics were significantly different between reference and impaired sites with the multiple habitat sampling design. When samples were collected only in riffle habitat, only about one-half of the metrics were significantly different between known reference and impaired sites.

Table 6. Summary of results from two sample t-test presented in Tables 4 and 5. Values in the table are the number and percent of 18 metrics that indicated the ecological conditions at the impaired sites were significantly worse than those at the reference sites ($\alpha = 0.05$, n = 5).

		Sampling Design	
		Riffle	Multihabitat
Taxonomic Identification Level	Family	10 (56%)	13 (72%)
	LILA	8 (44%)	13 (72%)

Multivariate cluster analysis provided a mechanism to integrate the data from all 18 metrics and examine the importance of sampling design versus taxonomic identification level for distinguishing impaired ecological conditions from reference conditions in bioassessment studies (Figure 1). The results of the multivariate cluster analysis on the metric values from the 10 study sites also indicated that sampling design had a stronger influence on distinguishing between reference and impaired conditions than taxonomic identification level. There were three major clusters and one smaller cluster on the dendrogram (i.e., greatest Euclidian distance): a fairly consistent group of impaired sites on the right (12 observations, distance = 52.4), a consistent group of reference sites towards the left (16 observations, distance = 49.8), another group of mostly impaired sites in the middle (8 observations, distance = 37.6), and a single impaired site on the left (4 observations, distance = 18.6). Within the cluster of mostly impaired sites on the right and cluster of reference sites on the left, samples from multiple habitats and samples from the riffle habitat formed very distinct clusters. Even within the mixed cluster of reference and impaired sites in the middle of the dendrogram and the smaller cluster on the left, samples from multiple habitats and samples from the riffle habitat formed fairly distinct clusters. There was no tendency for samples to cluster according to taxonomic identification level. These results suggest that sampling design (riffle habitat versus multiple habitats) is more important than taxonomic identification level for distinguishing reference and impaired ecological conditions in bioassessment studies.

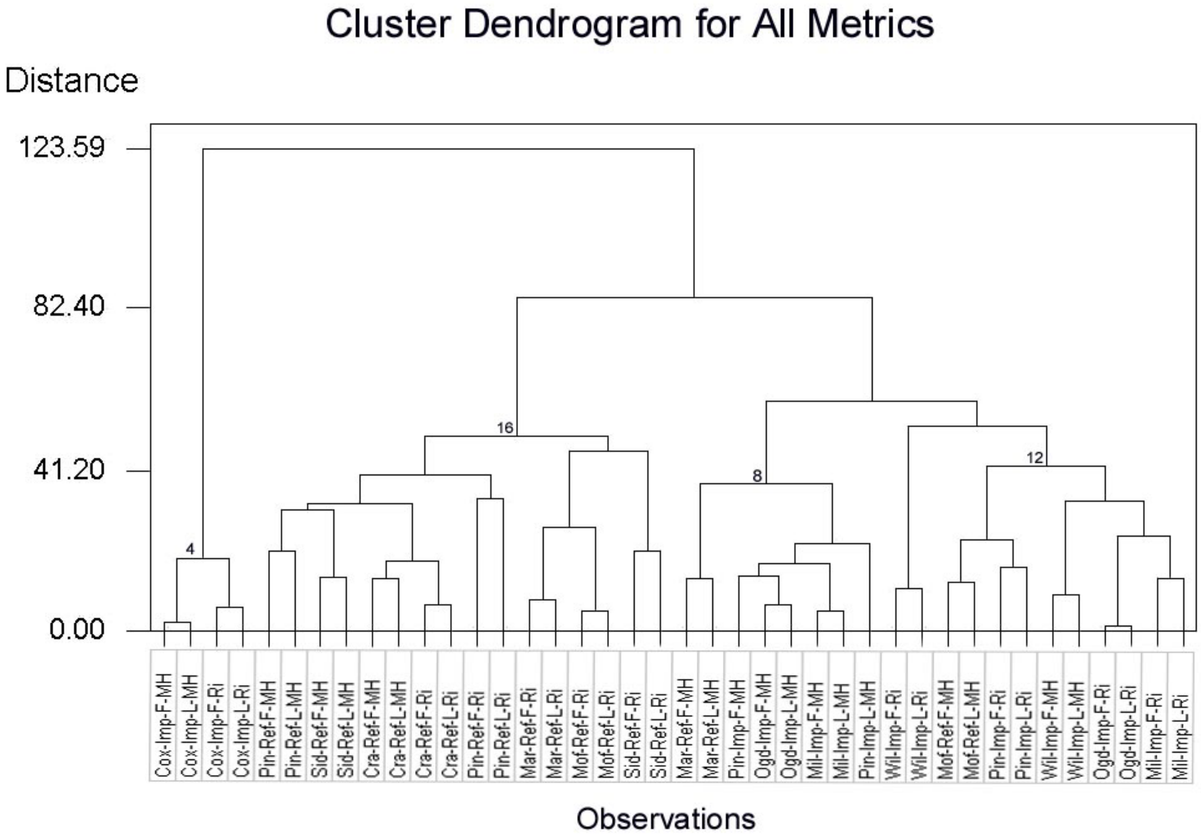

Figure 1. Dendrogram resulting from cluster analysis of metric data from 5 reference and 5 impaired sites, April-May 1997, with 40 observations (10 sites x 2 levels of identification x 2 habitats sampled). First 3 spaces in observation code = site (e.g. Pin = Piney Creek), next 3 spaces = reference (Ref) or impaired (Imp), next space = level of identification (F = family, L = LILA), and last spaces = habitats sampled (Ri = riffle, MH = multiple habitats).

Raw data from the two study sites (Sidney Creek and Mill Creek tributary) where species-level taxonomy was accomplished by rearing immatures and collecting adults in 1996 and 1997 are presented in Appendices I-N. Counts of individual taxa collected from the riffle habitat and multiple habitats at the two study sites in spring 1996 and identified to the family, genus, and species level are presented in Appendices I, J, and K, respectively. Appendices L-N contain the same data for collections made in spring 1997. Values for the 18 biological metrics calculated at family, genus, and species levels for samples from riffle habitat and multiple habitats in Sidney Creek and Mill Creek tributary are listed in Appendix O (spring 1996) and Appendix P (spring 1997). A complete list of all taxa determined from either immatures, reared adults, or adults collected in the field at Sidney Creek and Mill Creek tributary in 1996 and 1997 are listed in Appendix Q.

When species-level identifications were accomplished for most organisms by associating immatures and adults over a 2-year period, the reference site on Sidney Creek produced 66 taxa while the impaired site on Mill Creek tributary produced 44 taxa. (Table 7). These taxa belonged to 35 families and 58 genera in Sidney Creek and 24 families and 41 genera in Mill Creek tributary. The faunal composition at these two sites was analyzed at each taxonomic identification level by two similarity coefficients, the Jaccard coefficient (Table 8) and the Bray-Curtis percent similarity coefficient (Table 9). While the former coefficient only takes the presence or absence of taxa into consideration and the latter uses the abundance of taxa, both coefficients produced the same results. Regardless of sampling design or taxonomic level, the values for both of these similarity coefficients were less than half of their maximum possible values. There was practically no difference in the values for samples from riffle habitat and multiple habitats at any taxonomic level. In both riffle and multiple habitat samples the values for both similarity coefficients dropped considerably from the family to genus taxonomic level but hardly changed from the genus to species taxonomic level. However, the values for both similarity coefficients were low enough at the family taxonomic level that one could confidently state that the faunal composition was different at the reference and impaired site.

Table 7. Taxa list of immatures, reared adults, and adults collected from Sidney Creek and Mill Creek tributary in 1996 and 1997. x denotes presence

Taxa	Sidney	Mill
OTHER INVERTEBRATES		
Cambaridae		
Cambarus sp.1	*x*	*x*
Ancylidae		
Ferrissia sp.1		x
Hirudinea (Genus 1, Species 1)		x
Oligochaeta (Genus 1, Species 1)	x	x
Sphaeridae (Genus 1, Species 1)		x
EPHEMEROPTERA		
Baetidae		
Acentrella turbida	x	
Baetis sp.1		x
Baetis sp.2		x
Baetiscidae		
Baetisca sp.1	x	
Ephemeridae		
Ephemera guttulata	x	x
Ephemerellidae		
Ephemerella dorothea	x	x
Ephemerella rotunda	x	
Eurylophella cf. funeralis	x	x
Leptophlebiidae		
Habrophlebiodes sp.1	x	x
Paraleptophlebia adoptiva	x	
Heptageniidae		
Epeorus cf. vitreus	x	
Stenacron carolina		x
Stenonema sp.2	x	x
Stenonema vicarium	x	
ODONATA		
Aeshnidae		
Boyeria vinosa	x	
Gomphidae		
Lanthus vernalis	x	
Stylogomphus albistylus	x	
Cordulegastridae		
Cordulegaster maculata	x	
Calopterygidae		
Calopteryx sp.1	x	x
PLECOPTERA		
Nemouridae		
Amphinemura nigritta	x	x

Table 7 *(continued)*

Taxa	Sidney	Mill
Perlidae		
Eccoptura xanthenes	x	
Perlesta placida (group)		x
Perlesta teysia		x
Perloidae		
Remenus bilobatus	x	
Isoperla sp.1	x	
Isoperla sp.2		x
Chloroperlidae		
Haploperla brevis	x	
Sweltsa sp.1	x	
Leuctridae		
Leuctra cf. ferruginea	x	
Peltoperlidae		
Tallaperla maria	x	
MEGALOPTERA		
Corydalidae		
Nigronia fasciatus	x	
Nigronia serricornis	x	
Sialidae		
Sialis sp.1	x	
TRICHOPTERA		
Hydropsychidae		
Cheumatopsyche cf. campayla	x	x
Diplectrona modesta	x	
Hydropsyche morosa group	x	x
Hydropsyche 'non' morosa		x
Philopotamidae		
Chimarra cf. aterrima		x
Dolophilodes distinctus	x	
Wormaldia cf. moesta	x	
Hydroptilidae		
Hydroptila sp.1		x
Uenoidae		
Neophylax cf. ornatus	x	
Polycentropodidae		
Polycentropus sp.1	x	
Limnephilidae		
Pycnopsyche cf.guttifer	x	
Rhyacophilidae		
Rhyacophila cf. carolina/fuscula	x	
COLEOPTERA		
Ptilodactylidae		
Anchytarsus bicolor	x	

Table 7 *(continued)*

Taxa	Sidney	Mill
Elmidae		
Dubiraphia sp.1		x
Optioservus sp.1	x	x
Oulimnius latiusculus	x	
Stenelmis sp.1	x	x
Psephenidae		
Ectopria sp.1	x	
Psephenus herricki	x	
Dryopidae		
Helichus sp.1	x	
DIPTERA		
Chironomidae		
Ablabesmyia sp.	x	
Clinotanypus sp.		x
Cryptochironomus sp.	x	x
Cryptotendipes sp.		x
Labrundinia sp.	x	
Orthocladius sp.	x	x
Polypedilum sp.	x	x
Procladius sp.	x	
Rheotanytarsus sp.	x	x
Tanytarsus sp.	x	x
Thienemanniella sp.		x
Thienemannimya (group)	x	x
Ceratopogonidae		
Bezzia sp.1	x	x
Tipulidae		
Antocha sp.1	x	x
Erioptera sp.1		x
Hexatoma sp.1	x	x
Pilaria sp.1	x	x
Tipula sp.1	x	x
Dixidae		
Dixa sp.1		x
Empididae		
Hemerodromia sp.1		x
Simuliidae		
Simulium cf. vittatum	x	x
Tabanidae (Genus 1, Species 1)	x	

Total number of families	35	24
Total number of genera	58	41
Total number of species (or assumed species)	66	44

Table 8. Analysis of similarity between fauna at reference site on Sidney Creek and impaired site on Mill Creek tributary, according to different taxonomic levels, using the Jaccard coefficient. This coefficient considers the presence or absence of individual taxa. Values can range from 0.0 to 1.0 and increase with degree of similarity.

	1996		1997	
Taxonomic Identification Level	Riffle	Multiple Habitats	Riffle	Multiple Habitats
Family	0.42	0.46	0.40	0.30
Genus	0.28	0.28	0.21	0.18
Species	.025	0.30	0.19	0.19

Table 9. Analysis of similarity between fauna at reference site on Sidney Creek and impaired site on Mill Creek tributary, according to different taxonomic levels, using the Bray-Curtis percent similarity coefficient. This coefficient considers the numerical abundance of individual taxa. Values can range form 0-100% and increase with degree of similarity.

	1996		1997	
Taxonomic Identification Level	Riffle	Multiple Habitats	Riffle	Multiple Habitats
Family	16.35	11.74	40.00	38.74
Genus	7.88	7.59	26.97	28.80
Species	7.65	7.35	26.97	28.80

The Manhattan index was used to compare the values for all 18 biological metrics collectively between the reference site on Sidney Creek and the impaired site on Mill Creek tributary, according to sampling design and taxonomic identification level (Table 10). The metrics calculated from the multiple habitat samples distinguished the reference and impaired sites much more decisively with family-level identifications than with genus or species-level identifications. In 1996, genus and species-level identifications from riffle samples separated reference and impaired conditions slightly better than the same levels of identification from multiple habitat samples, but the opposite occurred in 1997.

Table 10. Analysis of similarity between metric data at reference site on Sidney Creek and impaired site on Mill Creek tributary, according to different taxonomic levels, using the Manhattan measure. This measure considers the similarity by the absolute difference in two sets of values.

	1996		1997	
Taxonomic Identification Level	Riffle	Multiple Habitats	Riffle	Multiple Habitats
Family	262.9	365.0	179.0	331.2
Genus	276.9	198.2	233.0	283.3
Species	281.4	202.3	234.3	287.3

DISCUSSION

Does sampling only in riffle habitat and sampling in multiple habitats produce the same conclusion about the ecological condition of streams? The majority of previous research on sampling design for bioassessment studies in streams has recommended sampling in the single most productive habitat, which is usually the riffle/run habitat (Plafkin et al. 1989, Chessman 1995, Parsons and Norris 1996, Barbour et al. 1999, Hewlett 2000). The primary reason that is given for this recommendation is that sampling in multiple habitats produces redundant results and only serves to increase time and costs appreciably. Chessman (1995) went farther and suggested that sampling in multiple habitats would interfere with interpreting bioassessment data and reaching conclusions because the same habitats may not be present at all of the sites being investigated. My results indicate that sampling only in the riffle habitat will usually work satisfactorily for stream bioassessment, but I think that sampling in multiple habitats offers some advantages. Most contemporary bioassessment involves analyzing the benthic macroinvertebrate assemblage by means of metrics, either individually or combined into a multimetric index (Barbour et al. 1999). My analyses showed that only about one-half of the metrics calculated from riffle samples distinguished impaired sites as being significantly different from reference sites. In contrast, about three-fourths of the metrics calculated from multiple habitat samples distinguished impaired sites as being significantly different from reference sites. Thus, a multiple habitat sampling design would probably be better for detecting intermediate levels of perturbation.

The likely explanation for why multiple habitat sampling is more effective than single habitat sampling is that more taxa with a greater range of ecological requirements are encountered. For example, the multiple habitat sampling design that I used in this study, collected macroinvertebrates from microhabitats such as cobble, pebble, gravel, sand, silt, aquatic plants, terrestrial vegetation drooping in the water, leaf packs, woody debris, fast current, slow current, and nearly still water in pools. The occurrence of different habitats at different sites is a primary effect of certain types of pollution or other environmental stress, especially from non-point sources that cause eutrophication, increased turbidity, and sedimentation. The best way to include the effects of habitat alteration in bioassessment studies is to use a multiple habitat sampling design.

I disagree that a multiple habitat sampling design takes more time and effort. To stay within reasonable cost constraints, it is only necessary to standardize the level of effort for the individual subsamples from the various microhabitats, as well as the overall effort for the entire reach, as suggested by Lenat (1988). My protocol is especially effective because I adhere strictly to a standard sampling design for taking the subsamples within the reach. In this study I used a random sampling design to choose transects and positions along each transect, but a systematic sampling design would work equally well.

Does taxonomic identification to the family, genus, and species level produce the same conclusion about the ecological condition of streams? The majority of previous research on the effects of taxonomic level on the resolution of bioassessment studies indicates that analyses based on genus and species-level identifications do not distinguish impaired ecological conditions any better than analyses based on family-level identifications. My results are in complete agreement with previous studies. There was no difference in the ability to discriminate impaired conditions with macroinvertebrate data at the family level, lowest practical level, genus level, or species level. There are several probable explanations for my results. There were not many species within genera, even at the reference sites, so genus-level and species-level analyses tend to be the same. In spite of intensive efforts to obtain adult specimens for species identification, I was only able to distinguish about two-thirds of the fauna to the species level. However, other investigations have reported that only about one-half of the fauna could be identified to species (Lenat 1988, Hewlett 2000). Some metrics are based on a measure of the organisms' ability to withstand pollution (tolerance values), and it has been shown that there is not much difference in these values within the different taxonomic levels (Bailey et al. 2001). Many other metrics have also been shown to not change their values a great deal in relation to the number of species within genera, genera within families, and families within orders (Bailey et al. 2001).

The time and costs for species-level identifications are definitely much higher than identification to higher taxonomic levels, but identification to genus would not require more effort for an experienced aquatic biologist. Given the ever-increasing body of evidence that trying to identify benthic macroinvetebrates to species in bioassessment studies does not improve the ability to distinguish impaired ecological conditions, why do aquatic biologists continue to recommend doing this (Barbour et al. 1999, Lenat and Resh 2001)? Bailey et al. (2001) explained that most aquatic biologists are naturalists at heart, and that assigning species names to organisms they find in their samples is done to satisfy their "soul of the naturalist." In many cases it would be preferable to identify everything correctly to family rather than attempt to lower identification levels that produce errors and inconsistency (Voshell et al. 1997). There will always be a place for species-level identification, such as studies emphasizing conservation biology, production, distribution ranges, and population dynamics, but this is not necessary for studies aimed at broad-scale bioassessment (Bailey et al. 2001).

Which combination of habitat sampling design and taxonomic identification level best determines the ecological condition of streams? The results of my study clearly indicated that sampling design according to habitats is more important than taxonomic identification level for distinguishing impaired ecological conditions. The probable explanation for these results is that adding different microhabitats to the sampling design adds appreciable ecological diversity, such as substratum to live on or in, food sources, current velocities. Metrics based on benthic macroinvertebrates adapted for this wide range of ecological conditions are likely to change noticeably when pollution or environmental stress occurs. Identification to lower taxonomic levels only results in the recognition of various taxa that are closely related phylogenetically and tend to have similar ecological requirements. Thus, metrics based on lower taxonomic levels, especially species, are not likely to exhibit any greater magnitude of change when pollution or environmental stress occurs than metrics based on higher taxonomic levels, such as genus or family. My results were opposite from Hewlett (2000), who found that the influence of taxonomic level was greater than habitat sampling design for bioassessment studies, but she only had two habitats in her sampling design whereas I had many different habitats in my design.

In summary, I recommend identifying benthic macroinvertebrates to family, possibly LILA, in a multiple habitat sampling design for bioassessment studies that are intended to determine if the ecological conditions in streams are acceptable. The multiple habitat sampling design must be carefully planned to include many different microhabitats in a composite sample but to avoid large samples that require extensive effort for sorting and identifying the organisms. It should be noted that this study only involved small streams and impairment caused by cattle grazing and watering. However, small streams comprise the greatest lengths of running waters that must be monitored, and the effects of cattle activity are similar to the effects of non-point source pollution from other common activities such as crop production, forestry, and urban development.

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Appendix

Contents:

Appendix Tables A-D:	Counts for individual taxa collected in April and May 1997 from 5 reference and 5 impaired sites
Appendix Tables E-H:	Metric data collected in April and May 1997 from 5 reference and 5 impaired sites
Appendix Tables I-N:	Counts for individual taxa collected in 1996 and 1997 from Sidney Creek and Mill Creek tributary
Appendix Tables O-P:	Metric data collected in 1996 and 1997 from Sidney Creek and Mill Creek tributary
Appendix Table Q:	Taxa list of macroinvertebrates collected form Sidney Creek and Mill Creek tributary

Appendix A. Benthic macroinvertebrates collected by riffle-only sampling protocol and identified to family level, Spring 1997

	Site									
	Reference	Reference	Reference	Reference	Reference	Impacted	Impacted	Impacted	Impacted	Impacted
Taxa	Piney	Marl	Moffatts	Crawford	Sidney	Piney	Wilson	Ogden	Cox's	Mill
OTHER INVERTEBRATES										
Cambaridae	14	6	6	1	9	2	4			5
Oligochaeta					1		1			
Pleuroceridae	6									
EPHEMEROPTERA										
Ameletidae				2						
Baetidae	3	11	18	2		5		14		
Baetiscidae						2				
Ephemerellidae	14	53	82	17	20	48		11		12
Ephemeridae			2							
Heptegeniidae	14	4	2	35	33	13	3			
Isonychiidae		13				2	3	1		
Leptophlebiidae	7	4	3	23				2		3
Neoephemeridae						1				
ODONATA										
Aeshnidae							2	2		
Calopterygidae							1	1		
Cordulegastridae				4						
Gomphidae	1			9	9	2				
PLECOPTERA										
Chloroperlidae	1				1	1				
Leuctridae	10	6	4		10					
Nemouridae	11	7		28	10			1		
Peltoperlidae	19			28	2					
Perlidae	3	1	9		2	7				26
Perlodidae	15	19	13	1	1	32	7	20		
MEGALOPTERA										
Corydalidae		2	8							
TRICHOPTERA										
Hydropsychidae	65	21	17	19	89	30	130	30		20
Limnephilidae				1		2				
Odontoceridae				4						
Philopotamidae	1					3	8			
Polycentropodidae	1		1							
Rhyacophilidae	1		3		5	2				
Uenoidae	1			1	2					
COLEOPTERA										
Elmidae	22		4	1	4	3	29	18		39
Psephenidae	18		1	1	1	16	16			2
DIPTERA										
Blephariceridae	2									
Ceratopogonidae	1									
Chironomidae	11	17	16	14	44	78	92	62	84	89
Dixidae		1		1						1
Empididae				1						
Muscidae									3	
Simuliidae	1	1				2		3	8	
Tabanidae		1						1	1	
Tipulidae	7	17	9	4	1	16	3	2	3	4

Appendix B. Benthic macroinvertebrates collected by riffle-only sampling protocol and identified to LILA , Spring 1997

	Reference	Reference	Reference	Reference	Reference	Impacted	Impacted	Impacted	Impacted	Impacted
Taxa	Piney	Marl	Moffatts	Crawford	Sidney	Piney	Wilson	Ogden	Cox's	Mill
OTHER INVERTEBRATES										
Cambarus	14	6	6	1	9	2	4			5
Oligochaeta					1		1			
Pleuroceridae	6									
EPHEMEROPTERA										
Ameletus				2						
Baetis (complex)	3	11	18	2		5		14		
Baetisca						2				
Cinygmula subaequalis	1									
Drunella						1				
Epeorus	1					3				
Ephemerella	14	53	78	17	20	46		11		12
Eurylophella			4			1				
Habrophlebiodes										3
Isonychia		13				2	3	1		
Leucrocuta				1						
Litobrancha recurvata			2							
Neoephemera						1				
Paraleptophlebia	7	4	3	23				2		
Stenonema	12	4	2	34	33	10	3			
ODONATA										
Boyeria							2	2		
Calopteryx							1	1		
Cordulegaster				4						
Lanthus	1			9	8	2				
Stylogomphus					1					
PLECOPTERA										
Acroneuria	3					7				
Agnetina		1	9							
Amphinemura	11	7		28	10			1		
Cultus		19	13				1			
Eccoptura xanthenes					2					
Isoperla	14			1	1	32	6	20		
Leuctra	10	6	4		10					
Perlesta placida (group)										26
Sweltsa	1				1	1				
Tallaperla	19			28	2					
Yugus	1									
MEGALOPTERA										
Nigronia fasciatus		2	5							
Nigronia serricornis			3							
TRICHOPTERA										
Cheumatopsyche		2			14	11	14			15
Chimarra							8			

Appendix B. Continued. Site

Taxa	Reference Piney	Reference Marl	Reference Moffatts	Reference Crawford	Reference Sidney	Impacted Piney	Impacted Wilson	Impacted Ogden	Impacted Cox's	Impacted Mill
Diplectrona modesta	65	3		19	75					
Dolophilodes distinctus	1					2				
Goera						2				
Hydropsyche		16	17			19	116	30		5
Neophylax	1			1	2					
Polycentropus	1		1							
Psilotreta				4						
Pycnopsyche				1						
Rhyacophila	1		3		5	2				
Wormaldia						1				
COLEOPTERA										
Dubiraphia							1			
Ectopria	16		1		1	1				
Optioservus			3		1		22	1		34
Oulimnius latiusculus	22			1	3	3				
Promoresia			1							
Psephenus herricki	2			1		15	16			2
Stenelmis							6	17		5
DIPTERA										
Antocha	1					3				3
Blephariceria	2									
Ceratopogonidae	1									
Chelifera				1						
Chironomidae	11	17	16	14	44	78	92	62	84	89
Dicranota	4	4		1		2				
Dixa		1		1						1
Hexatoma	1			3		11			3	1
Muscidae									3	
Simulium	1	1				2		3	8	
Tabanidae		1						1	1	
Tipula	1	13	9		1		3	2		

Appendix C. Benthic macroinvertebrates collected by multihabitat sampling protocol and identified to family level, Spring 1997

te

	ference	ference	ference	ference	ference	pacted	pacted	pacted	pacted	pacted
Taxa	ey	rl	ffatts	awford	ney	ey	son	den	x's	l
MISC. INVERTEBRATES										
Ancylidae										1
Cambaridae	5	7	3	3	1	1	4	1	1	1
Oligochaeta	2		1			1		1		
Physidae							7			
Planariidae								1		
Pleuroceridae	15						1	1		
Sphaeriidae				2				1	5	6
EPHEMEROPTERA										
Ameletidae		2		6						
Baetidae	1	34	16	1		2	2	5		3
Baetiscidae					1	1				
Ephemerellidae	36	26	51	33	22	51	2	8		15
Ephemeridae	2		8							3
Heptegeniidae	8	3	5	39	17	8	12	1		
Isonychiidae		3								
Leptohyphidae								1		
Leptophlebiidae	6	4	1	9	2	2	1	1		1
ODONATA										
Aeshnidae		1			1		2			
Calopterygidae	2		6		1	2	2	2		
Coenagrionidae								1		
Cordulegastridae	2			4	1				2	
Gomphidae	13			13	5	4	1			
Libellulidae								2	1	
PLECOPTERA										
Chloroperlidae				1	2					
Leuctridae	31	12	1	2		3			1	
Nemouridae	25	12	5	46	25	2		2		
Peltoperlidae	12			55	1	2				
Perlidae	3		5		1	1	1	1		2
Perlodidae	11	11	5	3		7	12	42		11
HEMIPTERA										
Corixidae		1						6		
MEGALOPTERA										
Corydalidae		1	1		3	1		1		
Sialidae	1	3								
TRICHOPTERA										
Hydropsychidae	30	32	21	54	38	39	93	6		7
Hydroptilidae		1								
Lepidostomidae	1		3							
Limnephilidae	10	2		9	9	2			1	

Appendix C. *Continued.* Site

	Reference	Reference	Reference	Reference	Reference	Impacted	Impacted	Impacted	Impacted	Impacted
Taxa	Piney	Marl	Moffatts	Crawford	Sidney	Piney	Wilson	Ogden	Cox's	Mill
Molannidae	1		2							
Odontoceridae				4						
Philopotamidae	1	2	1				42	1		
Phryganeidae							1			
Polycentropodidae	2	1				2				
Psychomyiidae		1								
Rhyacophilidae	1	2	7	1	3					
Uenoidae	2	1	1	2	9		2			
COLEOPTERA										
Dytiscidae									1	
Elmidae	37		24		8	4	11	8		7
Hydrophilidae							1	1		
Psephenidae	14		1		2	8	2			1
Ptilodactylidae						1				
DIPTERA										
Ceratopogonidae		2							1	1
Chironomidae	49	172	91	32	38	239	182	181	332	121
Dixidae				2						
Empididae	3			4		2		1		
Ephydridae									21	
Ptychopteridae									1	
Simuliidae					1		2		1	
Stratiomyidae									1	
Tabanidae					1	2			15	
Tipulidae	11	23	10	21	7	13	4	2	4	3

Appendix D. Benthic macroinvertebrates collected by multihabitat sampling protocol and identified to LILA , Spring 1997

	Reference	Reference	Reference	Reference	Reference	Impacted	Impacted	Impacted	Impacted	Impacted
Taxa	Piney	Marl	Moffatts	Crawford	Sidney	Piney	Wilson	Ogden	Cox's	Mill
OTHER INVERTEBRATES										
Cambarus	5	7	3	3	1	1	4	1	1	1
Ferrissia										1
Oligochaeta	2		1			1		1		
Physa							7			
Planariidae								1		
Pleuroceridae	15						1	1		
Sphaeriidae				2				1	5	6
EPHEMEROPTERA										
Ameletus		2		6						
Baetis (complex)	1	34	16	1		2	2	4		3
Baetisca					1	1				
Centroptilum								1		
Cinygmula subaequalis	1									
Drunella						1				
Epeorus	3					1				
Ephemera	2									3
Ephemerella	26	26	39	33	20	45	2	6		14
Eurylophella	10		12		2	5		2		1
Habrophlebiodes		4			1		1			1
Isonychia		3								
Litobrancha recurvata			8							
Paraleptophlebia	6		1	9	1	2		1		
Stenacron							1			
Stenonema	4	3	5	39	17	7	11	1		
Tricorythodes								1		
ODONATA										
Boyeria		1			1		2			
Calopteryx	2		6		1	2	2	2		
Coenagrionidae								1		
Cordulegaster	2			4	1				2	
Gomphus						2	1			
Lanthus	13			13	2	2				
Libellulidae										
Plathemis								2	1	
Stylogomphus					3					
PLECOPTERA										
Acroneuria	3					1				
Agnetina			5							
Amphinemura	25	12	5	46	25	2		2		
Cultus		4					5			
Eccoptura xanthenes					1					
Isoperla	9	7	5	3		7	7	42		11
Leuctra	31	12	1	2		3			1	
Perlesta placida (group)							1	1		2
Sweltsa				1	2					
Tallaperla	12			55	1	2				
Yugus	2									

Appendix D. *Continued.* Site

Taxa	Piney	Marl	Moffatts	Crawford	Sidney	Piney	Wilson	Ogden	Cox's	Mill
	Reference	Reference	Reference	Reference	Reference	Impacted	Impacted	Impacted	Impacted	Impacted
HEMIPTERA										
Corixidae		1						6		
MEGALOPTERA										
Corydalus cornutus								1		
Nigronia fasciatus		1	1		1					
Nigronia serricornis					2	1				
Sialis	1	3								
TRICHOPTERA										
Cheumatopsyche		1			3	14	18			7
Chimarra							42	1		
Diplectrona modesta	30	1		54	35	3		1		
Dolophilodes distinctus			1							
Hydropsyche		30	21			22	75	5		
Hydroptila		1								
Lepidostoma	1		3							
Lype diversa		1								
Molanna	1		2							
Neophylax	2	1	1	2	9		2			
Polycentropus	2	1				2				
Psilotreta				4						
Ptilostomis							1			
Pycnopsyche	10	2		9	9	2			1	
Rhyacophila	1	2	7	1	3					
Wormaldia	1	2								
COLEOPTERA										
Anchytarsus						1				
Berosus							1	1		
Dubiraphia							1			3
Dytiscidae									1	
Ectopria	12		1		2					
Optioservus	3		14		2		8	6		3
Oulimnius latiusculus	34				6	4				
Promoresia			10							
Psephenus herricki	2					8	2			1
Stenelmis							2	2		1
DIPTERA										
Antocha		5	1		2	7				
Ceratopogonidae		2							1	1
Chelifera	3			4						
Chironomidae	49	172	91	32	38	239	182	181	332	121
Dicranota	1	16	7	3		2				
Dixa				2						
Ephydridae									21	
Erioptera										
Hemerodromia						2		1		
Hexatoma	6			15		3		1	4	1
Ptychopteridae									1	
Simulium					1		2		1	
Stratiomyidae									1	
Tabanidae					1	2			15	
Tipula	4	2	2	3	5	1	4	1		2

Appendix E. Metric data from all sites collected by riffle-only sampling protocol and identified to family level, Spring 1997

	Ref	Ref	Ref	Ref	Ref	Imp	Imp	Imp	Imp	Imp
	Piney	Marl	Moffatts	Crawford	Sidney	Piney	Wilson	Ogden	Cox's	Mill
Total Taxa Richness	25	17	17	21	18	20	13	14	5	10
EPT	15	10	11	12	11	13	5	7	0	4
No. of Ephemeroptera Taxa	4	5	5	5	2	6	2	4	0	2
No. of Plecoptera Taxa	6	4	3	3	6	3	1	2	0	1
No. of Trichoptera Taxa	5	1	3	4	3	4	2	1	0	1
Simpson's D	0.90	0.87	0.80	0.89	0.81	0.85	0.70	0.80	0.27	0.74
% Top Dominant	26.10	28.80	41.41	17.77	36.48	29.21	43.48	36.90	84.85	44.28
% Top 3 Dominant	42.57	50.54	59.09	46.19	68.03	59.18	83.95	66.67	95.96	76.62
% Top 5 Dominant	55.82	69.02	73.74	67.51	80.33	76.40	91.97	85.71	100.00	92.54
HBI	3.76	3.43	3.24	3.01	4.45	4.04	5.39	4.83	6.01	4.57
% Tolerant	0.00	0.54	0.00	0.00	0.41	0.00	0.67	1.19	4.04	0.00
% Intolerant	42.57	58.70	58.59	77.66	38.93	42.32	7.69	22.02	0.00	20.90
% Haptobenthos	86.35	77.72	83.33	83.76	73.77	62.55	66.22	60.71	8.08	51.24
% Herpobenthos	8.03	19.02	13.64	15.74	22.54	36.70	32.11	38.69	91.92	46.27
% CG	14.06	46.74	61.11	28.93	26.64	50.19	31.10	52.98	84.85	52.24
% SC	25.30	2.17	3.54	22.34	16.39	11.99	16.05	10.71	0.00	20.40
% SH	18.88	16.30	6.57	30.96	9.43	6.74	1.00	1.79	3.03	1.99
% CF	27.31	19.02	9.09	9.64	36.48	13.86	47.16	20.24	8.08	9.95

Appendix F. Metric data from all sites collected by riffle-only sampling protocol and identified to LILA, Spring 1997

	Ref	Ref	Ref	Ref	Ref	Imp	Imp	Imp	Imp	Imp
	Piney	Marl	Moffatts	Crawford	Sidney	Piney	Wilson	Ogden	Cox's	Mill
Total Taxa Richness	32	20	20	23	21	28	17	15	5	13
EPT	18	12	12	13	12	18	7	7	0	5
No. of Ephemeroptera Taxa	6	5	6	6	2	9	2	4	0	2
No. of Plecoptera Taxa	7	4	3	3	6	3	2	2	0	1
No. of Trichoptera Taxa	5	3	3	4	4	6	3	1	0	2
Simpson's D	0.90	0.88	0.81	0.90	0.84	0.86	0.74	0.80	0.27	0.75
% Top Dominant	26.10	28.80	39.39	17.26	30.74	29.21	38.80	36.90	84.85	44.28
% Top 3 Dominant	42.57	48.37	57.07	45.69	62.30	58.43	76.92	66.67	95.96	74.13
% Top 5 Dominant	54.62	64.13	71.72	67.01	76.23	71.16	86.96	85.12	100.00	87.56
HBI	2.51	3.40	3.20	2.55	3.30	3.94	5.54	4.95	5.98	5.24
% Tolerant	0.00	0.54	0.00	0.00	0.41	0.00	0.67	1.19	4.04	0.00
% Intolerant	75.10	60.33	57.07	72.59	56.97	46.07	6.69	22.02	3.03	9.95
% Haptobenthos	88.76	79.89	83.33	85.79	73.77	68.54	66.22	60.71	11.11	53.23
% Herpobenthos	5.62	16.85	13.64	13.71	22.54	30.71	32.11	38.69	88.89	44.28
% CG	14.86	46.74	61.11	28.93	26.64	52.06	31.10	52.98	84.85	52.24
% SC	24.90	2.17	3.54	22.34	16.39	11.99	16.05	10.71	0.00	21.89
% SH	16.47	14.13	6.57	28.93	9.43	0.00	1.00	1.79	0.00	0.00
% CF	26.91	19.02	8.59	9.64	36.48	13.86	47.16	20.24	8.08	9.95

Appendix G. Metric data from all sites collected by multihabitat sampling protocol and identified to family level, Spring 1997

	Ref	Ref	Ref	Ref	Ref	Imp	Imp	Imp	Imp	Imp
	Piney	Marl	Moffatts	Crawford	Sidney	Piney	Wilson	Ogden	Cox's	Mill
Total Taxa Richness	30	25	23	23	24	25	22	25	15	15
EPT	18	17	15	15	12	13	10	10	2	7
No. of Ephemeroptera Taxa	5	6	5	5	4	5	4	5	0	4
No. of Plecoptera Taxa	5	3	4	5	4	5	2	3	1	2
No. of Trichoptera Taxa	8	8	6	5	4	3	4	2	1	1
Simpson's D	0.93	0.74	0.83	0.90	0.89	0.62	0.71	0.55	0.26	0.55
% Top Dominant	14.54	47.91	33.83	15.90	19.10	59.75	47.03	65.11	85.57	66.12
% Top 3 Dominant	36.20	66.30	61.71	44.80	50.75	82.25	81.91	83.09	94.85	80.33
% Top 5 Dominant	54.30	79.94	75.46	65.61	70.35	87.50	88.11	88.13	97.16	89.07
HBI	3.48	4.84	4.35	3.37	4.14	5.07	5.19	5.22	6.03	5.25
% Tolerant	1.48	1.11	2.60	0.58	1.01	1.25	2.58	5.40	5.93	3.83
% Intolerant	49.55	22.28	31.60	63.58	45.23	20.25	19.64	20.50	0.77	15.85
% Haptobenthos	73.89	42.06	55.02	78.32	72.36	34.25	47.80	28.42	0.77	26.23
% Herpobenthos	24.04	55.71	41.64	20.81	26.63	65.00	50.13	67.99	98.71	73.22
% CG	28.49	66.02	62.45	22.25	31.66	74.00	50.13	71.22	91.49	78.14
% SC	22.85	1.67	12.27	14.74	18.09	5.00	7.24	3.60	0.00	4.92
% SH	26.71	13.65	7.06	38.44	21.11	5.75	1.29	1.44	1.55	1.64
% CF	9.79	10.58	8.18	16.18	19.60	10.25	35.40	2.88	1.55	7.10

Appendix H. Metric data from all sites collected by multihabitat sampling protocol and identified to LILA, Spring 1997

	Ref	Ref	Ref	Ref	Ref	Imp	Imp	Imp	Imp	Imp
	Piney	Marl	Moffatts	Crawford	Sidney	Piney	Wilson	Ogden	Cox's	Mill
Total Taxa Richness	38	30	27	25	31	34	27	30	15	19
EPT	22	20	16	15	15	18	13	13	2	8
No. of Ephemeroptera Taxa	8	6	6	5	6	8	5	7	0	5
No. of Plecoptera Taxa	6	4	4	5	4	5	3	3	1	2
No. of Trichoptera Taxa	8	10	6	5	5	5	5	3	1	1
Simpson's D	0.93	0.75	0.85	0.90	0.90	0.63	0.73	0.55	0.26	0.55
% Top Dominant	14.54	47.91	33.83	15.90	19.10	59.75	47.03	65.11	85.57	66.12
% Top 3 Dominant	33.83	65.74	56.13	44.80	49.25	76.50	77.26	82.37	94.85	79.78
% Top 5 Dominant	50.45	77.44	67.29	65.61	67.84	82.00	84.75	86.69	97.16	86.89
HBI	2.87	4.77	4.31	2.71	3.48	5.02	5.39	5.28	6.02	5.34
% Tolerant	1.48	1.11	2.60	0.58	1.01	1.25	2.58	5.76	5.93	3.83
% Intolerant	66.47	27.30	34.94	73.12	55.78	21.50	16.02	19.78	1.80	14.75
% Haptobenthos	75.96	47.91	57.99	83.53	73.37	37.25	47.80	28.78	1.80	26.78
% Herpobenthos	21.96	49.86	38.66	15.61	25.63	62.00	50.13	67.63	97.68	72.68
% CG	29.38	66.02	62.83	22.25	32.16	75.75	50.13	71.22	91.49	77.60
% SC	21.96	3.06	12.27	14.74	18.59	5.00	7.24	3.60	0.00	5.46
% SH	24.63	7.80	4.09	33.24	20.10	2.75	1.29	1.08	0.52	1.09
% CF	9.20	10.31	8.18	16.18	19.60	9.75	35.40	2.88	1.55	7.10

Appendix I. Benthic macroinvertebrates collected at Sidney Creek and Mill Creek tributary by riffle-only and multihabitat sampling protocols and identified to family level, Spring 1996

	Riffle-Only	Riffle-Only	Multihabitat	Multihabit
	Reference	Impacted	Reference	Impacted
Taxa	Sidney	Mill	Sidney	Mill
MISC. INVERTEBRATES				
Ancylidae		3		
Cambaridae	3	2		3
Hirudinea		1		
Oligochaeta	1	5		6
Sphaeridae				1
EPHEMEROPTERA				
Baetidae	1	87		46
Ephemerellidae	44	8	20	10
Ephemeridae		1	2	2
Heptageniidae	26	10	19	1
Leptophlebiidae	7	183	10	66
ODONATA				
Calopterygidae				1
Cordulegastridae	1			
Gomphidae	18		7	
PLECOPTERA				
Chloroperlidae	1		3	
Leuctridae	10		9	
Nemouridae	126	2	108	1
Peltoperlidae	1			
Perlidae	2	149	3	147
Perlodidae	4	13	1	11
MEGALPOPTERA				
Corydalidae	2			
Sialidae	1			
TRICHOPTERA				
Hydropsychidae	94	42	24	39
Hydroptilidae				1
Limnephilidae	1			
Philopotomidae	3	3		
Polycentropodidae	1			
Rhyacophilidae	1		1	
Uenoidae	10		16	
COLEOPTERA				
Dryopidae	1			
Elmidae	18	167	13	111
Psephenidae		2		3
Ptilodactylidae	2			
DIPTERA				
Ceratopogonidae			2	2
Chironomidae	32	603	22	941
Empididae		4		2
Simuliidae		6		11
Tipulidae	9	14	10	12

Appendix J. Benthic macroinvertebrates collected at Sidney Creek and Mill Creek Tributary by riffle-only and multihabitat sampling protocols and identified to genus level, Spring 1996

	Riffle-Only	Riffle-Only	Multihabitat	Multihabit
	Reference	Impacted	Reference	Impacted
Taxa	Sidney	Mill	Sidney	Mill
MISC. INVERTEBRATES				
Cambarus	3	2		3
Ferrissia		3		
Hirudinea (Genus 1)		1		
Oligochaeta (Genus 1)	1	5		6
Sphaeridae (Genus 1)				1
EPHEMEROPTERA				
Acentrella	1			
Baetis (complex)		87		46
Epeorus	2		2	
Ephemera		1	2	2
Ephemerella	39	8	17	9
Eurylophella	5		3	1
Habrophlebiodes	5	183	8	66
Paraleptophlebia	2		2	
Stenacron		10		
Stenonema	24		17	1
ODONATA				
Calopteryx				1
Cordulegaster	1			
Lanthus	18		7	
PLECOPTERA				
Amphinemura	126	2	108	1
Eccoptura xanthenes	2		3	
Haploperla brevis			1	
Isoperla	3	13	1	11
Leuctra	10		9	
Perlesta placida (group)		149		147
Remenus	1			
Sweltsa	1		2	
Tallaperla	1			
MEGALOPTERA				
Nigronia	2			
Sialis	1			
TRICHOPTERA				
Cheumatopsyche	1	34	1	35
Chimarra		3		
Diplectrona modesta	93		22	
Dolophilodes distinctus	1			
Hydropsyche		8	1	4
Hydroptila				1
Neophylax	10		16	

Appendix J. Continued.

	Riffle-Only	Riffle-Only	Multihabitat	Multihabit
	Reference	Impacted	Reference	Impacted
Taxa	Sidney	Mill	Sidney	Mill
Polycentropus	1			
Pycnopsyche	1			
Rhyacophila	1		1	
Wormaldia	2			
COLEOPTERA				
Anchytarsus	2			
Dubiraphia		36		43
Helichus	1			
Optioservus	7	92	12	36
Oulimnius latiusculus	7		1	
Psephenus herricki		2		3
Stenelmis	4	39		32
DIPTERA				
Antocha	2	5	2	5
Bezzia			2	2
Erioptera		2		2
Hemerodromia		4		2
Hexatoma		4	1	5
Orthocladius .	23	531	13	358
Pilaria	5	2	3	
Polypedilum .	4	42	7	508
Rheotanytarsus	2	6		
Simulium		6		11
Tanytarsus	1			
Thienemannimya (group)	2	24	2	75
Tipula	2	1	4	

Appendix K. Benthic macroinvertebrates collected at Sidney Creek and Mill Creek Tributary by riffle-only and multihabitat sampling protocols and identified to species level, Spring 1996

	Riffle-Only	Riffle-Only	Multihabitat	Multihabit
	Reference	Impacted	Reference	Impacted
Taxa	Sidney	Mill	Sidney	Mill
MISC. INVERTEBRATES				
Cambarus sp.1	3	2		3
Ferrissia sp.1		3		
Hirudinea (Genus 1, Species 1)		1		
Oligochaeta (Genus 1, Species 1)	1	5		6
Sphaeridae (Genus 1, Species 1)				1
EPHEMEROPTERA				
Acentrella turbida	1			
Baetis sp.1		80		43
Baetis sp.2		7		3
Epeorus cf. vitreus	2		2	
Ephemera guttulata		1	2	2
Ephemerella dorothea	30	8	16	9
Ephemerella rotunda	9		1	
Eurylophella cf. funeralis	5		3	1
Habrophlebiodes sp.1	5	183	8	66
Paraleptophlebia adoptiva	2		2	
Stenacron carolina		10		
Stenonema sp.2	3		1	1
Stenonema vicarium	21		16	
ODONATA				
Calopteryx sp.1				1
Cordulegaster maculata	1			
Lanthus vernalis	18		7	
PLECOPTERA				
Amphinemura nigritta	126	2	108	1
Eccoptura xanthenes	2		3	
Haploperla brevis			1	
Isoperla sp.1	3		1	
Isoperla sp.2		13		11
Leuctra cf. ferruginea	10		9	
Perlesta placida (group)		149		147
Remenus bilobatus	1			
Sweltsa sp.1	1		2	
Tallaperla maria	1			
MEGALOPTERA				
Nigronia serricornis	2			
Sialis sp.1.	1			
TRICHOPTERA				
Cheumatopsyche cf. campayla	1	34	1	35
Chimarra cf. aterrima		3		

Appendix K. Continued.

	Riffle-Only	Riffle-Only	Multihabitat	Multihabit
	Reference	Impacted	Reference	Impacted
Taxa	Sidney	Mill	Sidney	Mill
Diplectrona modesta	93		22	
Dolophilodes distinctus	1			
Hydropsyche morosa group		1	1	
Hydropsyche 'non' morosa		7		4
Hydroptila sp.1				1
Neophylax cf. ornatus	10		16	
Polycentropus sp.1	1			
Pycnopsyche cf. guttifer	1			
Rhyacophila cf. carolina/fuscula	1		1	
Wormaldia cf. moesta	2			
COLEOPTERA				
Anchytarsus bicolor	2			
Dubiraphia sp.1		36		43
Helichus sp.1	1			
Optioservus sp.1	7	92	12	36
Oulimnius latiusculus	7		1	
Psephenus herricki		2		3
Stenelmis sp.1	4	39		32
DIPTERA				
Antocha sp.1	2	5	2	5
Bezzia sp.1			2	2
Erioptera sp.1		2		2
Hemerodromia sp.1		4		2
Hexatoma sp.1		4	1	5
Orthocladius sp.	23	531	13	358
Pilaria sp.1	5	2	3	
Polypedilum sp.	4	42	7	508
Rheotanytarsus sp.	2	6		
Simulium cf.vittatum		6		11
Tanytarsus sp.	1			
Thienemannimya (group)	2	24	2	75
Tipula sp.1	2	1	4	

Appendix L. Benthic macroinvertebrates collected at Sidney Creek and Mill Creek Tributary by riffle-only and multihabitat sampling protocols and identified to family level, Spring 1997

	Riffle-Only	Riffle-Only	Multihabitat	Multihabit
	Reference	Impacted	Reference	Impacted
Taxa	Sidney	Mill	Sidney	Mill
OTHER INVERTEBRATES				
Ancylidae				1
Cambaridae	9	5	1	1
Oligochaeta	1			
Spheariidae				6
EPHEMEROPTERA				
Baetidae				3
Baetiscidae			1	
Ephemerellidae	20	12	22	15
Ephemeridae				3
Heptageniidae	33		17	
Leptophlebiidae		3	2	1
ODONATA				
Aeshnidae			1	
Calopterygidae			1	
Cordulegastridae			1	
Gomphidae	9		5	
PLECOPTERA				
Chloroperlidae	1		2	
Leuctridae	10			
Nemouridae	10		25	
Peltoperlidae	2		1	
Perlidae	2	26	1	2
Perlodidae	1			11
MEGALOPTERA				
Corydalidae			3	
TRICHOPTERA				
Hydropsychidae	89	20	38	7
Limnephilidae			9	
Rhyacophilidae	5		3	
Uenoidae	2		9	
COLEOPTERA				
Elmidae	4	39	8	7
Psephenidae	1	2	2	1
DIPTERA				
Ceratopogonidae				1
Chironomidae	44	89	38	121
Dixidae		1		
Simuliidae			1	
Tabanidae			1	
Tipulidae	1	4	7	3

Appendix M. Benthic macroinvertebrates collected at Sidney Creek and Mill Creek Tributary by riffle-only and multihabitat sampling protocols and identified to genus level, Spring 1997

	Riffle-Only	Riffle-Only	Multihabitat	Multihabit
	Reference	Impacted	Reference	Impacted
Taxa	Sidney	Mill	Sidney	Mill
OTHER INVERTEBRATES				
Cambarus	9	5	1	1
Ferrissia				1
Oligochaeta (Genus 1)	1			
Spheariidae (Genus 1)				6
EPHEMEROPTERA				
Baetis (complex)				3
Baetisca			1	
Ephemera				3
Ephemerella	20	12	20	14
Eurylophella			2	1
Habrophlebiodes		3	1	1
Paraleptophlebia			1	
Stenonema	33		17	
ODONATA				
Boyeria			1	
Calopteryx			1	
Cordulegaster			1	
Lanthus	8		2	
Stylogomphus	1		3	
PLECOPTERA				
Amphinemura	10		25	
Eccoptura xanthenes	2		1	
Isoperla	1			11
Leuctra	10			
Perlesta placida (group)		26		2
Sweltsa	1		2	
Tallaperla	2		1	
MEGALOPTERA				
Nigronia fasciatus			1	
Nigronia serricornis			2	
TRICHOPTERA				
Cheumatopsyche	14	15	3	7
Diplectrona modesta	75		35	
Hydropsyche		5		
Neophylax	2		9	
Pycnopsyche			9	
Rhyacophila	5		3	
COLEOPTERA				
Dubiraphia				3
Ectopria	1		2	
Optioservus	1	34	2	3
Oulimnius latiusculus	3		6	
Psephenus herricki		2		1
Stenelmis		5		1
DIPTERA				
Ablabesmyia			2	
Antocha		3	2	

Appendix M. Continued.

Taxa	Riffle-Only Reference Sidney	Riffle-Only Impacted Mill	Multihabitat Reference Sidney	Multihabit Impacted Mill
Bezzia				1
Clinotanypus				1
Cryptochironomus			3	8
Cryptotendipes				1
Dixa		1		
Hexatoma		1		1
Labrundinia	1		1	
Orthocladius	18	74	11	68
Polypedilum	19	5	17	18
Procladius			4	
Rheotanytarsus	1			2
Simulium			1	
Tabanidae (Genus 1)			1	
Tanytarsus		1		
Thienemanniella				1
Thienemannimyia (group)	5	9		22
Tipula	1		5	2

Appendix N. Benthic macroinvertebrates collected at Sidney Creek and Mill Creek Tributary by riffle-only and multihabitat sampling protocols and identified to species level, Spring 1997

	Riffle-Only	Riffle-Only	Multihabitat	Multihabit
	Reference	Impacted	Reference	Impacted
Taxa	Sidney	Mill	Sidney	Mill
OTHER INVERTEBRATES				
Cambarus sp.	9	5	1	1
Ferrissia sp.				1
Oligochaeta (Genus 1, Species 1)	1			
Sphaeriidae (Genus 1, Species 1)				6
EPHEMEROPTERA				
Baetis sp.2				3
Baetisca sp.1			1	
Ephemera guttulata				3
Ephemerella dorothea	18	12	19	14
Ephemerella rotunda	2		1	
Eurylophella cf. funeralis			2	1
Habrophlebiodes sp.1		3	1	1
Paraleptophlebia adoptiva			1	
Stenonema vicarium	33		17	
ODONATA				
Boyeria vinosa			1	
Calopteryx sp.1			1	
Cordulegaster maculata			1	
Lanthus vernalis	8		2	
Stylogomphus albistylus	1		3	
PLECOPTERA				
Amphinemura nigritta	10		25	
Eccoptura xanthenes	2		1	
Isoperla sp.1	1			
Isoperla sp.2				11
Leuctra cf. ferruginea	10			
Perlesta placida (group)		26		2
Sweltsa sp.1	1		2	
Tallaperla maria	2		1	
MEGALOPTERA				
Nigronia fasciatus			1	
Nigronia serricornis			2	
TRICHOPTERA				
Cheumatopsyche cf. campayla	14	15	3	7
Diplectrona modesta	75		35	
Hydropsyche morosa group		1		
Hydropsyche 'non' morosa		4		
Neophylax cf. ornatus	2		9	
Pycnopsyche cf. guttifer			9	
Rhyacophila cf. carolina/fuscula	5		3	

Appendix N. Continued.

Taxa	Riffle-Only Reference Sidney	Riffle-Only Impacted Mill	Multihabitat Reference Sidney	Multihabit Impacted Mill
COLEOPTERA				
Dubiraphia sp.1				3
Ectopria sp.1	1		2	
Optioservus sp.1	1	34	2	3
Oulimnius latiusculus	3		6	
Psephenus herricki		2		1
Stenelmis sp.1		5		1
DIPTERA				
Ablabesmyia sp.			2	
Antocha sp.1		3	2	
Bezzia sp.1				1
Clinotanypus sp.				1
Cryptochironomus sp.			3	8
Cryptotendipes sp.				1
Dixa sp.1		1		
Hexatoma sp.1		1		1
Labrundinia sp.	1		1	
Orthocladius sp.	18	74	11	68
Polypedilum sp.	19	5	17	18
Procladius sp.			4	
Rheotanytarsus sp.	1			2
Simulium cf. vittatum			1	
Tabanidae (Genus 1, Species 1)			1	
Tanytarsus sp.		1		
Thienemanniella sp.				1
Thienemannimyia (group)	5	9		22
Tipula sp.1	1		5	2

Appendix O. Metric data collected by riffle-only and multihabitat sampling protocols and identified to family, genus, and species at Sidney Creek and Mill Creek tributary, Spring 1996

	Family	Family	Family	Family	Genus	Genus	Genus	Genus	Species	Species	Species	Species
	Rif	Rif	MH	MH	Rif	Rif	MH	MH	Rif	Rif	MH	MH
	Ref	Imp	Ref	Imp	Ref	Imp	Ref	Imp	Ref	Imp	Ref	Imp
	Sidney1	Mill1	Sidney2	Mill2	Sidney1	Mill1	Sidney2	Mill2	Sidney1	Mill1	Sidney2	Mill2
Total Taxa Richness	27	20	17	21	41	30	29	29	43	32	31	30
EPT	16	10	12	10	22	11	18	12	24	13	20	13
No. of Ephemeroptera Taxa	4	5	4	5	7	5	7	6	9	6	9	7
No. of Plecoptera Taxa	6	3	5	3	7	3	6	3	7	3	6	3
No. of Trichoptera Taxa	6	2	3	2	8	3	5	3	8	4	5	3
Simpson's D	0.84	0.73	0.81	0.54	0.84	0.79	0.82	0.79	0.85	0.79	0.82	0.79
% Top Dominant	30.00	46.21	40.00	66.41	30.00	40.69	40.00	35.85	30.00	40.69	40.00	35.85
% Top 3 Dominant	62.86	73.03	57.04	84.62	61.43	66.13	54.44	71.49	59.29	66.13	54.07	71.49
% Top 5 Dominant	76.67	91.11	71.48	92.52	72.62	79.85	66.67	81.44	69.76	79.31	65.93	81.44
HBI	3.77	4.43	3.30	5.03	2.83	4.87	3.00	5.47	2.83	4.87	3.00	5.47
% Tolerant	0.48	0.69	0.00	0.56	0.71	0.69	0.00	0.56	0.71	0.69	0.00	0.56
% Intolerant	60.48	28.20	72.96	16.65	79.05	17.01	76.30	6.99	79.05	17.01	76.30	6.99
% Haptobenthos	84.52	52.03	84.07	31.69	86.67	56.40	87.78	68.24	86.67	56.40	87.78	68.24
% Herpobenthos	14.76	47.82	15.93	68.03	12.62	43.45	12.22	31.47	12.62	43.45	12.22	31.47
% CG	20.24	67.97	20.00	75.58	17.86	49.73	15.19	30.28	17.86	49.73	15.19	30.28
% SC	13.10	13.95	17.78	8.12	13.81	27.20	20.00	12.77	13.81	27.20	20.00	12.77
% SH	35.48	1.23	47.04	0.92	34.76	3.45	47.41	35.92	34.76	3.45	47.41	35.92
% CF	23.33	3.91	8.89	3.60	23.81	4.37	8.89	3.60	23.81	4.37	8.89	3.60

Appendix P. Metric data collected by riffle-only and multihabitat sampling protocols and identified to family, genus, and species at Sidney Creek and Mill Creek tributary, Spring 1997

	Family	Family	Family	Family	Genus	Genus	Genus	Genus	Species	Species	Species	Species
	Rif	Rif	MH	MH	Rif	Rif	MH	MH	Rif	Rif	MH	MH
	Ref	Imp	Ref	Imp	Ref	Imp	Ref	Imp	Ref	Imp	Ref	Imp
	Sidney1	Mill1	Sidney2	Mill2	Sidney1	Mill1	Sidney2	Mill2	Sidney1	Mill1	Sidney2	Mill2
Total Taxa Richness	18	10	24	15	25	16	36	26	26	17	37	26
EPT	11	4	12	7	12	5	15	8	13	6	16	8
No. of Ephemeroptera Taxa	2	2	4	4	2	2	6	5	3	2	7	5
No. of Plecoptera Taxa	6	1	4	2	6	1	4	2	6	1	4	2
No. of Trichoptera Taxa	3	1	4	1	4	2	5	1	4	3	5	1
Simpson's D	0.81	0.74	0.89	0.55	0.86	0.81	0.92	0.83	0.86	0.81	0.92	0.83
% Top Dominant	36.48	44.28	19.10	66.12	30.74	36.82	17.59	37.16	30.74	36.82	17.59	37.16
% Top 3 Dominant	68.03	76.62	50.75	80.33	52.46	66.67	40.20	59.02	52.05	66.67	39.70	59.02
% Top 5 Dominant	80.33	92.54	70.35	87.98	67.62	80.10	57.29	72.68	66.80	80.10	56.78	72.68
HBI	4.45	4.57	4.14	5.25	3.30	5.25	3.55	5.36	3.27	5.25	3.53	5.36
% Tolerant	0.41	0.00	1.01	3.83	0.41	0.50	4.02	4.37	0.41	0.50	4.02	4.37
% Intolerant	38.93	20.90	45.23	15.85	56.97	9.95	55.78	14.75	56.97	9.95	55.78	14.75
% Haptobenthos	73.77	51.24	72.36	26.23	81.97	56.22	81.91	37.70	81.97	56.22	81.91	37.70
% Herpobenthos	22.54	46.27	26.63	73.22	14.34	41.29	17.09	61.75	14.34	41.29	17.09	61.75
% CG	26.64	52.24	31.66	78.14	15.98	44.78	18.59	49.73	15.98	44.78	18.59	49.73
% SC	16.39	20.40	18.09	4.92	16.39	21.89	18.59	5.46	16.39	21.89	18.59	5.46
% SH	9.43	1.99	21.11	1.64	17.21	2.49	28.64	10.93	17.21	2.49	28.64	10.93
% CF	36.48	9.95	19.60	7.10	36.89	10.45	19.60	8.20	36.89	10.45	19.60	8.20

Appendix Q. Taxa list of immatures, reared adults, and adults collected from Sidney Creek and Mill Creek tributary in 1996 and 1997. x denotes presence

Taxa	Sidney	Mill
OTHER INVERTEBRATES		
Cambarus sp.1	x	x
Ferrissia sp.1		x
Hirudinea (Genus 1, Species 1)		x
Oligochaeta (Genus 1, Species 1)	x	x
Sphaeridae (Genus 1, Species 1)		x
EPHEMEROPTERA		
Acentrella turbida	x	
Baetis sp.1		x
Baetis sp.2		x
Baetisca sp.1	x	
Epeorus cf. vitreus	x	
Ephemera guttulata	x	x
Ephemerella dorothea	x	x
Ephemerella rotunda	x	
Eurylophella cf. funeralis	x	x
Habrophlebiodes sp.1	x	x
Paraleptophlebia adoptiva	x	
Stenacron carolina		x
Stenonema sp.2	x	x
Stenonema vicarium	x	
ODONATA		
Boyeria vinosa	x	
Calopteryx sp.1	x	x
Cordulegaster maculata	x	
Lanthus vernalis	x	
Stylogomphus albistylus	x	
PLECOPTERA		
Amphinemura nigritta	x	x
Eccoptura1anthenes	x	
Haploperla brevis	x	
Isoperla sp.1	x	
Isoperla sp.2		x
Leuctra cf. ferruginea	x	
Perlesta placida (group)		x
Perlesta teysia		x
Remenus bilobatus	x	
Sweltsa sp.1	x	
Tallaperla maria	x	
MEGALOPTERA		
Nigronia fasciatus	x	
Nigronia serricornis	x	
Sialis sp.1	x	

Appendix Q. Continued.

Taxa	Sidney	Mill
TRICHOPTERA		
Cheumatopsyche cf. campayla	x	x
Chimarra cf. aterrima		x
Diplectrona modesta	x	
Dolophilodes distinctus	x	
Hydropsyche morosa group	x	x
Hydropsyche 'non' morosa		x
Hydroptila sp.1		x
Neophylax cf. ornatus	x	
Polycentropus sp.1	x	
Pycnopsyche cf.guttifer	x	
Rhyacophila cf. carolina/fuscula	x	
Wormaldia cf. moesta	x	
COLEOPTERA		
Anchytarsus bicolor	x	
Dubiraphia sp.1		x
Ectopria sp.1	x	
Helichus sp.1	x	
Optioservus sp.1	x	x
Oulimnius latiusculus	x	
Psephenus herricki	x	
Stenelmis sp.1	x	x
DIPTERA		
Ablabesmyia sp.	x	
Antocha sp.1	x	x
Bezzia sp.1	x	x
Clinotanypus sp.		x
Cryptochironomus sp.	x	x
Cryptotendipes sp.		x
Dixa sp.1		x
Erioptera sp.1		x
Hemerodromia sp.1		x
Hexatoma sp.1	x	x
Labrundinia sp.	x	
Orthocladius sp.	x	x
Pilaria sp.1	x	x
Polypedilum sp.	x	x
Procladius sp.	x	
Rheotanytarsus sp.	x	x
Simulium cf. vittatum	x	x
Tabanidae (Genus 1, Species 1)	x	
Tanytarsus sp.	x	x
Thienemanniella sp.		x
Thienemannimya (group)	x	x
Tipula sp.1	x	x

Stephen W. Hiner

VITA

I was born in Marlington, WV in 1956 and grew up in nearby Bath County, VA. In 1981, I graduated from Virginia Tech with a bachelor's degree in Fisheries Science. I began working in the Department of Entomology at Virginia Tech shortly there after, and have been employed by the department and university ever since. During my employment at Virginia Tech I began studies towards a M.S. degree. My research addressed taxonomic resolution and habitat sampling in biomonitoring studies using benthic macroinvertebrates. In 1999, I received the Virginia Tech President's Award for Excellence for service to the university.