
MacroInvertebrate Survey of Wolf Creek: A Comparison of Agricultural and Wooded Areas

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1993

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Field Ecology
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December 15, 1993

Abstract
Macroinvertebrates were collected using artificial substrates at three different sites along Wolf Creek in Rice County, Minnesota for a period of six weeks. The three sites included a wooded area (site 1), an area with nearby agriculture (site 3), and a mixed site; both wooded and nearby agriculture (site 2). The purpose of the study was to examine any differences in macroinvertebrates found between the sites. Macroinvertebrates have been shown to be useful biomonitors in stream assessment studies, and the presence, or absence of certain species is a good indication of the relative "health" of the site, and the stream as a whole. This is assuming that the stream flow, bottom sub-stratum, and other physical characteristics are consistent between the sites, since these factors also play a role in what organisms are found. With that assumption in mind, the null hypothesis to be tested is that there will be no significant difference in the type and numbers of species found at each site.

The results indicated that there was a significant difference between the numbers of species found at each site ($p<.01$) using the Simpson diversity index. The mixed site had the highest number of species, as well as similarities to both the wooded ($C=.57$) and agricultural site ($C=.56$), which is to be expected because it was an intermediate of both sites. Many "clean water" species (of the family Chironomidae) were found at the wooded site such as, *Orthocladius* and *Thienemanniella* , whereas pollution tolerant species such as *Rheotanytarsus* and *Thienemannimyia* were more common at the agricultural site. Other species collected suggest the same general trends, but differences between sites can also be attributed to the changes in the flow of the stream over the six week period. At the end of the six weeks, site 3 (agricultural) had slowed down and ice was present on the surface, whereas the flow in the wooded area did not seem to change. Therefore, the differences between the sites may be due to a combination of environmental and physical characteristics.

Introduction:

Macroinvertebrates have been shown to be useful biomonitors in stream assessment studies. In stream communities most invertebrates are permanent fixtures, which monitor everything that goes by (such as pollutants). They are helpful because they continuously sample and tend to concentrate pollutants, allowing assessment of early environmental damage (Root, 1990). Organisms in streams are limited to specific ranges of physiological environment by their physiological tolerances to ranges of current velocities, water chemistry, etc. (Fiance, 1978; Vannote and Sweeny, 1980, as cited in Barnes and Minshall, 1983). Community structure of benthic macroinvertebrate populations has frequently been used to evaluate conditions in streams receiving organic enrichment. Bottom organisms are particularly suitable for such studies because their relatively low mobility does not enable them to escape deleterious substances that enter the environment (Wilhm and Dorris, 1968).

Comparisons of three different areas; agricultural, forested, and mixed sites should be a fair assessment of the variation found within a stream. Riparian vegetation has a large effect on the stream community. Forested areas act as a "buffer" to the stream, filtering out pollutants before they reach the stream. Sites in wooded areas should have "clean water" species whereas agricultural areas should have more pollution tolerant species, since run-off from herbicides and pesticides eventually end up in the stream. Faunal densities have been found to be highest in riffled areas of a stream (Hynes, 1970). Riffles are areas of faster-moving water and are normally characterized by larger substratum (such as rocks and boulders). Hester-Dendy type samplers are convenient invertebrate samplers, which are effective in studying riffled areas.

Some advantages of using Hester-Dendy samplers include: (1) they reduce variability of operator efficiency in taking samples, thus helping to standardize a sampling program; (2) are relatively inexpensive and simple to construct; and (3) permit nondestructive sampling of an environment. It should also be noted though that while artificial substrates increase sampling precision, they may also decrease sampling accuracy due to loss of information on spatial distribution of macroinvertebrates (Rosenberg and

Resh, 1982). Artificial substrate samplers (such as the Hester-Dendy) do not measure the condition of the natural substrate or the effect of pollution on the substrate itself. Hester-Dendy samplers also do not permit an estimation of actual densities, because they tend to favor species which cling to the substratum, but they are useful for comparative ecological studies, which is the aim of this study (Brower, Zar, and von Ende, 1989).

The object of this study is to find out to what extent surrounding terrestrial areas have an effect on the invertebrates of Wolf Creek. Wolf Creek is a tributary of the Cannon River (which in turn feeds into the Mississippi River) with its origin at Circle Lake. Although Wolf Creek is relatively small (only 5.6 miles long), it is a contributor to larger systems, such as the Cannon and Mississippi Rivers. Excluding various degrees of human perturbation (such as abnormally high light intensities, low oxygen levels, or inorganic and organic nutrient loads), the availability of food, nature of sediments, and current flow generally constitute the parameters of primary significance in determining microdistribution patterns (Whitton, 1975). Therefore, by choosing three sites that are relatively homologous in sediment type and current flow, any differences in species composition would likely be the result of different human interference. A major contributor of chemical contamination is the drainage, run-off, and leaching from agricultural or forested lands regularly treated with pesticide, or with a recent history of such treatment (Muirhead-Thomson, 1987). My null hypotheses for this study are as follows:

1. There will be no difference in the diversity of macroinvertebrates between agricultural and forested areas.

2. There will be no difference in the types of species found at each site (all sites should have the same species).

Materials and Methods:

Three different areas along the stream were identified (Fig. 1) which were fair representations of: 1. agricultural (dominated by farmland)

2. forested (wooded areas)

3. mixed (forested/agricultural) as an intermediate.

Samplers were placed in the stream in early October and recovered six weeks later in mid November. The Hester-Dendy samplers are composed of seven hardboard plates which are bolted together. To anchor the samplers in the stream, concrete bricks with three inverted "j" shaped rods are used. The samplers are screwed on to the ends of the rods (for a total of three samplers for each brick). Two bricks were then placed at each site (six samplers per cite), in riffled areas so that the top of j-shaped rod was at least six inches below the surface of the water. Maps of each area were drawn and a rough estimate was made as to the position of each brick in the stream to aide in relocating the samplers after the six weeks.

The samplers were collected after six weeks in the stream. To collect, a plastic bag is placed around one of the three samplers , and the sampler is carefully unscrewed to prevent invertebrate loss. The samples are then brought back to the lab , and the invertebrates are sorted taxonomically and stored in a 70% ethanol solution. Smaller invertebrates, such as midges, are difficult to identify. It is necessary to look at the jaw plate of each midge in order to key the midge down to genus. To do this, the head is first separated from the body under a microscope. The head is then washed with xylene, which makes it more transparent, and situated ventral side up on the slide. The head is then mounted using Cytoseal 280 mounting medium. A round coverslip was placed on top of the head, and gentle pressure was applied so that the mouth parts were visible. Three heads were mounted on each slide and later identified using taxonomic keys.

Statistical analysis included the Shannon-Weaver and Simpson diversity indices, species richness, equitability, community similarity, community loss, and the Macroinvertebrate Biotic Index. The Shannon and Simpson index was calculated using the Hyper Diversity disk on the Macintosh. Values for the Shannon index calculate the relative importance of each species collected. For unpolluted streams, the values are normally between 3 and 4 and for polluted streams usually less than one. Values for the Simpson index range between 0 and 1 with higher values indicating a healthier stream. The Simpson index also gives a p-value for the similarities between sites. If the p-value is less than .05, then there is a significant difference in the numbers of species found at each site.

Species richness is simply the number of different taxa found at each site. A greater amount of taxa is representative of a healthier community. Equitability is calculated by the equation $E=s'/s$, with s=the number of taxa and s'=the value provided by the U.S.E.P.A.. This value uses the Shannon-Weaver test value compared to MacArthur's broken stick model, which is a model based on the theory of the structures of communities in nature (Weber, 1973). These values range from 0 to 1, with higher values indicating a healthier (or less impacted) environment.

Community Similarity (C) and Community Loss (CL) are another way of measuring differences between sites. Community Similarity measures to what degree two sites are similar. The formula for this value is: $C=2w/(a+b)$, with a= the number of taxa from one site, b= the number of taxa from another site, and w= the number of taxa common to both sites (Brower et al., 1989). Community Loss is the measure of differences in taxa between sites. Values of dissimilarity range from 0 to infinity, with higher numbers showing greater dissimilarity. It is calculated by the value CL= (b-c)/a, with a=the number of taxa in common, b= the number of taxa in one site, and c= the number of taxa in the other site.

The Macroinvertebrate Biotic Index (MBI) is a measure that determines the average of tolerance ratings weighed by organism abundance. It is calculated by the formula MBI= (Summation) ni x ti/N, where ni= the number of individuals in the taxa, ti= tolerance value assigned to the specific taxa, and N= the total number of individuals at the site (Hilsenhoff, 1987). Pollution tolerance level values range from 0 to 11 with high numbers indicating higher levels of tolerance.

The final calculation was the percent frequency that each species occured at each site. This was done by dividing the number of individulas found in each taxa by the total number of individuals at each site. Frequency is simply a means of determining how common a species is at a particular site.

Results:

<u>Diversity between sites:</u>

Statistical analysis results can be found on Table 1. Diversity, MBI, Richness, and Equitability are all displayed, as well as what each number means on a scale of environmental impact, with slightly meaning low impact (healthy community), and severely meaning high impact (polluted area). Site 2 (mixed site) showed the highest level of richness. All the sites were in the moderately impacted range for richness. Non-impacted environments have a richness > 26. The Shannon-Weaver diversity index results showed site 2 as having the highest diversity followed by site 3, then site 1. These results put each site into a different category (slightly-severely), with significant differences between each site (p<.01).

Reasons for the discrepancies will be discussed later on, but keep in mind that some of these results are easily skewed by large numbers of individuals in a single species.

Community Similarity and Community Loss results showed that sites 1 & 2 and sites 2 & 3 were the most similar. Community Loss, which is a test of dissimilarity showed that sites 1 & 3 had the least in common. Species found at each of these sites followed expected trends. A breakdown of total numbers of individuals, % frequency, and tolerance values can be found in Tables 2-4.

<u>Differences in taxa found:</u>

A large number of *Simulium* were found in site 1. This value is responsible for some of the skewness in the diversity index. Basic "clean water" species include Tricoptera, Ephemoroptera, and Plecoptera, as well as the genus *Tanytarsus* of the Chironomidae. These organisms were all found in high numbers in sites one and two. Species in site 3, especially the Chironomidae, were all pollution tolerant. Species such as *Glyptotendipes* are especially tolerant to pollution. Notice also, that very few of the "clean water" species show up in great numbers in the third site. Site two shows a lot of the same species as the other two sites and varying degrees of tolerance levels, which was expected for this site.

Discussion:

The large number of *Simulium* in the first site can be attributed to a small population explosion and drift of these insects. Black fly larvae are found in the shallows of streams where the current is especially swift (riffles). "Sometimes they are so abundant that the substrate is almost obscured" (Pennak, 1978). Single-sample assessments taken only one time of the diel period are not representative of the cyclic patterns of a species (Waters, 1972).

The numbers of *Simulium* most likely decreased in the site and should not effect the overall diversity in that portion of the stream since these insects usually experience large population explosions in late fall and overwinter in the egg and larval forms (Pennak, 1978). Or possibly, it could have been that the large numbers of black flies out competed the other organisms for space on the Hester-Dendy plates, in which case other organisms would have found other places in the stream to colonize.

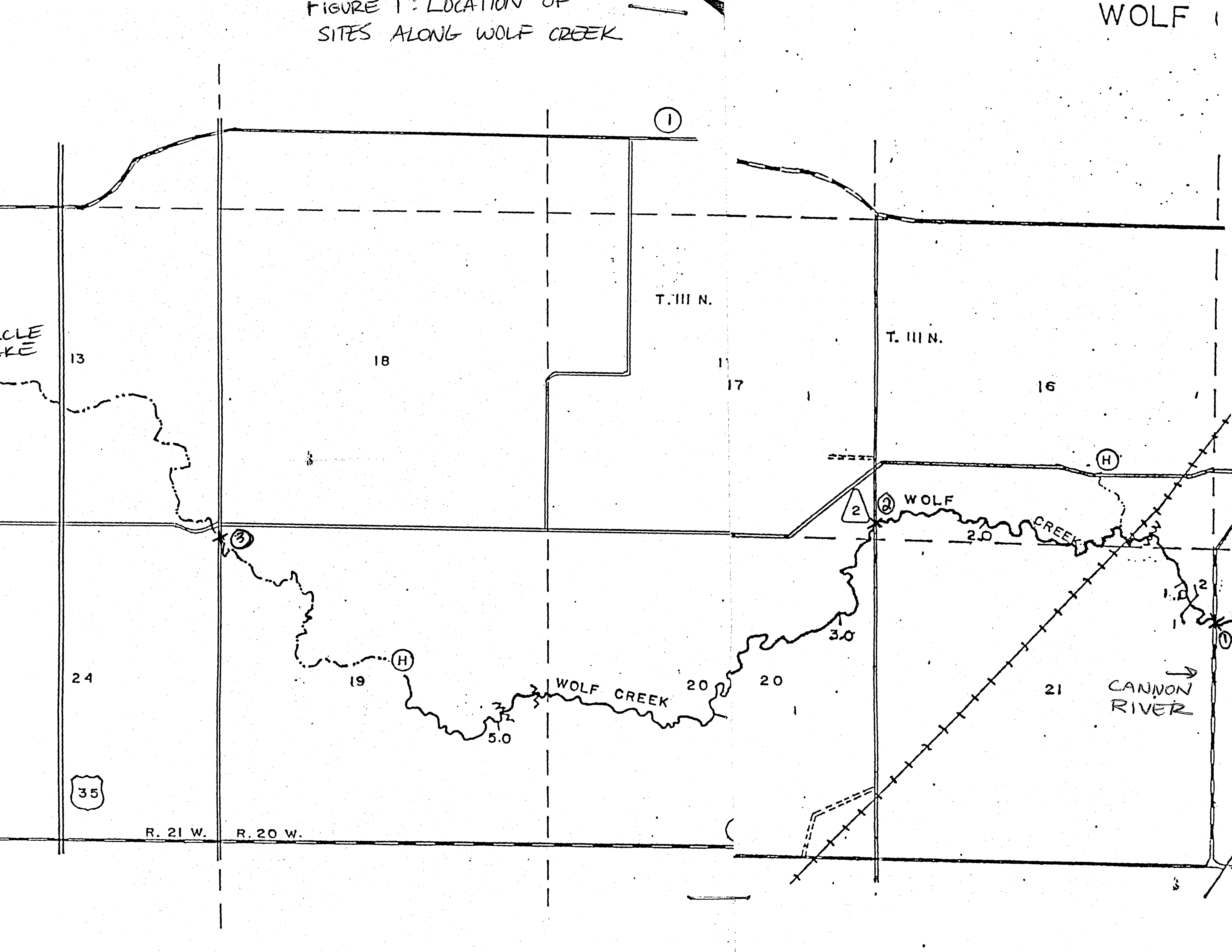
One of the sampling blocks in site 1 was also found on its side in the stream on the day of collection, this could have been due to either vandalism or the swift currents of the riffle. Effects of an overturned block change the flow of the water between the plates of the sampler, which may have an effect on colonization. Site 1 was also in a sense, the "guinea pig" of all the sites, and the site that was examined first in the lab. Poor lab technique on my part could have resulted in individuals being washed down the drain, or simply overlooked by my untrained eye. As time went on and I examined the plates at the other sites, my technique improved, and fewer individuals were lost to human error.

Still it is important that the types of species found at the first site were in some cases unique to this site, such as the *Baetis* mayfly. This species is especially common in swift moving waters. In the third site I found *Tricorythoded* mayflies which are more commonly found in slower moving areas. The third site also had a layer of ice on the surface at the end of the six weeks, leading me to believe that flow rate was probably significantly different at each of the sites, and had an effect on the species that were found. Careful determination of flow prior to sampling may have lead me to choose more homologous sites.

Chironimids can be used to enhance biomonitoring data. Basic life history information for species, species groups, or even genera can often reveal important traits about the water from which the organisms were collected (Simpson and Bode, 1979). Along with the "clean water" species of Tricoptera, Ephemoroptera, and Plecoptera, the Chironomids can be used to asses what areas in the stream are more polluted. If very few "clean water" species are found, then the entire stream could be severely impacted, which would have implications for the entire watershed. In the identification of the Chironimids, sites 2 and 3 showed the most variation. Site 2 had the largest number of midges, most of which were moderately tolerant. Site 3 had fewer numbers of midges, most of which were moderate to extremely tolerant (11 is the highest rating for tolerance). These highly tolerant midges, combined with the low numbers of "clean water" species at the third site, lead me to believe that the surrounding farmland, and lack of vegetation are having an effect on the stream environment. Further analysis of the macroinvertebrates in the spring should give this project more substantial data in which conclusions on the health of the stream can be made.

In conclusion, the null hypothesis that the diversity of each site would be the same was rejected. The Simpson diversity index showed prob-values<.01, which means that the differences were significant at the .05 level. Diversity trends did not fit the expected results, due to possible human error and large numbers of single species at site 1. Statistical analysis did show that overall Wolf Creek is a pollution impacted stream with vaules ranging from slightly to severely impacted, which is probably due to the nearby agriculture and pasture land. This assumption was further supported by the types of organisms found at each site. Types of organisms found varied from site to site, leading me to reject my second null hypothesis, which stated that they would be the same. Pollution tolerant species were more common to the agricultural site, "clean water" indicators were found in greater numbers and diversity at the wooded site, while the mixed site showed a combination of characteristics from both sites. Differences in the organisms is not only attributed to riparian vegetation, but also to inconsistent stream flow between sites. This study concludes that environmental as well as physical factors shape the community of the stream.

FIGURE 1: LOCATION OF
SITES ALONG WOLF CREEK



SITE	SHANNON	MBI	RICHNESS	EQUITABILITY	SIMPSON
1	0.074	6.696	11	0.091	0.022
RATING	SEVERELY	SEVERELY	MODERATE	SEVERELY	
2	2.078	4.95	17	0.294	0.825
RATING	SLIGHTLY	SEVERELY	MODERATE	SEVERELY	
3	1.574	5.12	15	5.12	0.658
RATING	MODERATE	SEVERELY	MODERATE	SLIGHTLY	
	COMMUNITY	COMMUNITY	SIMPSON		
SITES	SIMILARITY	LOSS			
1&2	0.571	0.75	p<.01		
2&3	0.563	0.22	p<.01		
1&3	0.308	1	p<.01		

Table 1: Statistical Analysis of Wolf Creek sites

SPECIES	site 1	site 2	site 3
AMPHIPODA			
Hyalella		8	137
GASTROPODA			
Stagnicola		1	
PLECOPTERA			
Perlesta	3		
EPHEMEROPTERA			
Baetis	38		
Heptagenia	46	113	7
Tricorythoded			21
TRICOPTERA			
Cheumatopsyche	8	28	3
Hydropsyche	245	8	
DIPTERA			
Simulium	3111	85	
Empididae		2	5
CHIRONOMIDAE			
Brilla		5	1
Chironomus			1
Dicrotendipes			1
Eukiefferiella	1	2	
Glyptotendipes			4
Microtendipes			10
Micropsectra			1
Orthocladius	7	20	1
Polypedilum		1	1
Rheotanytarsus	6	41	35
Tanytarsus		15	
Thienemanniella	3	2	
Thiennemannimyia		29	19
Trichocladius		8	
Paratendipes	1		
COLEOPTERA			
Stenelmis		2	

Table 2: Numbers of Individuals found at sites 1, 2, and 3

SPECIES	TOTAL	FREQUENCY	TOLERANCE
AMPHIPODA			
Hyalella			
GASTROPODA			
Stagnicola			
PLECOPTERA			
Perlesta	3	0.09	4
EPHEMEROPTERA			
Baetis	38	1.1	5
Heptagenia	46	1.3	3
Tricorythoded			
TRICOPTERA			
Cheumatopsyche	8	0.2	5
Hydropsyche	245	7.1	4
DIPTERA			
Simulium	3111	89.7	7
Empididae			
CHIRONOMIDAE			
Brilla			
Chironomus			
Dicrotendipes			
Eukiefferiella	1	0.03	?
Glyptotendipes			
Microtendipes			
Micropsectra			
Orthocladius	7	0.2	6
Polypedilum			
Rheotanytarsus	6	0.17	6
Tanytarsus			
Thienemanniella	3	0.09	2
Thiennemannimyia			
Trichocladius			
Paratendipes	1	0.03	8
COLEOPTERA			
Stenelmis			

Table 3: Taxa at site 1 (wooded), frequency, and tolerance

SPECIES	TOTAL	FREQUENCY	TOLERANCE
AMPHIPODA			
Hyalella	8	2.2	5
GASTROPODA			
Stagnicola	1	0.27	7
PLECOPTERA			
Perlesta			
EPHEMEROPTERA			
Baetis			
Heptagenia	113	30.5	3
Tricorythoded			
TRICOPTERA			
Cheumatopsyche	28	7.6	5
Hydropsyche	8	2.2	4
DIPTERA			
Simulium	85	23	7
Empididae	2	0.54	?
CHIRONOMIDAE			
Brilla	5	1.4	6
Chironomus			
Dicrotendipes			
Eukiefferiella	2	0.54	?
Glyptotendipes			
Microtendipes			
Micropsectra			
Orthocladius	20	5.4	6
Polypedilum	1	0.27	6
Rheotanytarsus	41	11.1	6
Tanytarsus	15	4.1	6
Thienemanniella	2	0.54	2
Thiennemannimyia	29	7.8	6
Trichocladius	8	2.2	?
Paratendipes			
COLEOPTERA			
Stenelmis	2	0.54	5

Table 4: Taxa at site 2 (mixed), frequency, and tolerance

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