

# JOURNAL OF ENVIRONMENTAL HYDROLOGY

*The Electronic Journal of the International Association for Environmental Hydrology*

*On the World Wide Web at <http://www.hydroweb.com>*

VOLUME 14

2006



## INFLUENCE OF WATER QUALITY ON MACROINVERTEBRATE POPULATION AND DIVERSITY

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*The environmental impact of urbanization is ubiquitous. Biodiversity within an ecosystem can be a simple means to determine the impact of urban development and overall environmental health of a region, and careful evaluation of waterways can provide insight into environmental health. Watersheds from three unique microenvironments in rural Tennessee were systematically compared. Water samples from rural and urban watersheds around Cookeville, TN were collected and analyzed. GIS was used to delineate watersheds, and land use and land cover data were computed to obtain urban areas in each watershed. Water samples were collected from three sites, all 3<sup>rd</sup> ordered streams. Macroinvertebrate identification, counting and indexes were developed. The North Carolina Biological Index was used to compute the Biotic Index Score. Habitat assessment and land use data were compared to measurements of water quality. Computation from percent dominance and percent clingers showed that watersheds exert their own characteristics. Percent urban area has negative impact on the diversity of macroinvertebrate community and dominance. Habitat assessment also supports such findings.*

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## INTRODUCTION

The 1987 Clean Water Act has been implemented for many years. Two of the most important policies are development of Total Maximum Daily Loading and nonpoint source pollution management (NCSE, 1998). Nonpoint source pollution has been a major concern in many United States watersheds (Sugiharto et al., 1994). Water quality of a local central Tennessee watershed has been a controversial topic at the local and state level. A local wastewater treatment plant has been discharging effluents into a small creek. Measurements from state officials and the local water department show contradictory results. Prior studies from the past two years focused on hydrology and simple indicators of water quality, but have not been able to provide valuable public information regarding water quality in the named watershed – Pigeon Roost Creek Watershed. Many researchers have used benthic macroinvertebrates as water quality indicators (Lenat and Barbour, 1993; Reynoldson et al., 1997). This study was aimed at providing a more thorough investigation of water quality in local watersheds using biological assessment tools.

Aquatic macroinvertebrates are found in lakes, streams, ponds, marshes, and puddles, and help maintain the health of the aquatic ecosystem by eating bacteria and dead, decaying plants and animals. Water quality affects the types of organisms that can survive in a body of water. To study the health and water quality of local watersheds, three watersheds were chosen to compare their biological index, physical parameters, and influence of land use patterns.

## LITERATURE REVIEW

Benthic macroinvertebrates have been used for studying water quality for many years. Many factors including stream orders, land use, drainage basin size, temperature, and sediment loads, have been found to have direct or indirect impacts on water quality. Parsons and Norris' (1996) study of effects due to different habitats showed that water quality from different streams can be determined from sampling macroinvertebrates from similar habitats. Richards and Host (1994) identified relationships between macroinvertebrates and stream physical habitat, and between habitat and land use patterns. Stewart et al. (2000) studied water quality in three different watersheds using macroinvertebrate communities as indicators. Quinn et al. (1997) found invertebrate densities were 3-fold higher in pasture than native streams, mainly because of more chironomids and snails, but mayflies, stoneflies, and caddisflies densities were 2- to 3-fold higher in forest streams compared to pasture. Other researchers, such as Klein (1979) characterized impairment of water quality with the levels of imperviousness. He found that when imperviousness reaches 30%, water quality becomes more degraded. Buckton (2000) and Benfield (2001) contributed their research on the water quality issue by using macroinvertebrate diversity as indicators. Bioassessment techniques have been developed and applied for water quality monitoring purposes (Metcalf, 1989). In this study, we used similar techniques to assess three local watersheds to provide insightful information for the research community and the public.

## STUDY AREA

Cookeville, TN is located at the western edge of the Cumberland Plateau. Situated at an altitude of 1,100 feet, Cookeville is in the center of a karst region. The population of Putnam County, in which Cookeville is the county seat, is around 64,000 (Census Bureau, 2005). Three watersheds, Pigeon Roost, Spring Creek, and Blackburn Fork, are selected for this study (Figure 1). Pigeon Roost watershed is an urban watershed. The creek receives water from urban runoff and a point

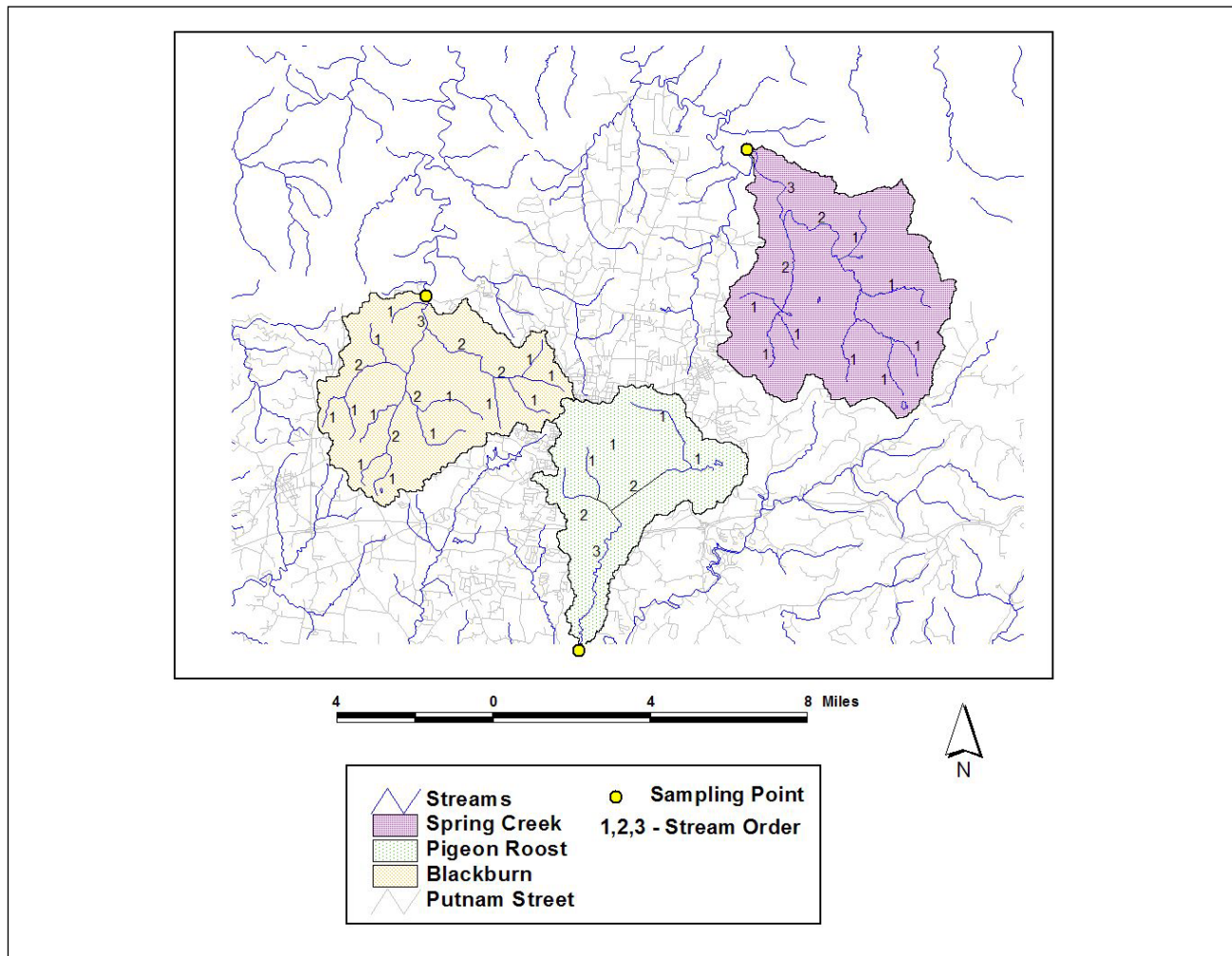


Figure 1. Watershed boundary, monitoring points and stream order.

source discharge from the city wastewater treatment plant. Spring Creek is a standard reference stream used for comparing water quality by the Tennessee Department of Environment and Conservation (TDEC, 2002). Blackburn Fork watershed is located at the north edge of the city, receiving water mainly from local small agricultural farms. Figure 1 shows the three watersheds with their stream orders (Marsh, 1998). To be consistent, all three sampling points were third-order streams for comparison. The numbers on the stream segments represent stream order. The yellow dots on the map represent the sampling site location.

### OBJECTIVES/PROCEDURES

1. To find watersheds containing urban and rural land use patterns in Cookeville, Tennessee
2. Collect macroinvertebrate samples from three selected points from the same stream order
3. Identify macroinvertebrates and classify to different categories based on biological index scores
4. Conduct habitat assessments in three watersheds
5. Process land use data to find percentage of land cover in three watersheds
6. Study correlation between land use pattern and biological index

## LANDUSE DATA ANALYSIS

Landuse information of an area can be effectively used for a comprehensive study of water quality (Stewart et al., 2000). Many researchers have investigated the relationships between land use and macroinvertebrate community (Lenat and Barbour, 1993; Sponseller et al. 2001). To obtain detailed land use data, GIS data were downloaded from <http://www.tngis.org> for spatial processing. Data include Digital Elevation Models (DEMs), showing elevation of the ground, stream files, and land use land cover grid file generated from compiling satellite images. Using ArcMap (ESRI, 2005), land use data were classified and computed to obtain percentage land cover in three watersheds. Detailed information about the watersheds (Table 1) and visual comparisons between them (Figure 2) were obtained. Pigeon Roost had a 33.5 percentage in urban/developed land usage. Compared to the other two streams, Blackburn Fork only had a 2.5 percentage while Spring Creek only had a 0.1 percentage in this category. One can also see, as a result of urbanization near Pigeon Roost, other land cover percentage has been reduced. The urbanized watershed will have more nutrients flushed into water, which in turn will affect macroinvertebrate communities. Water quality in an urban watershed is generally worse than in others.

Table 1. Land use and land cover percentage from all three watersheds.

Land Use Land Cover Name	Watershed (Land Use %)		
	Blackburn	Spring Creek	Pigeon Roost
Open Water	0.1%	0.1%	0.3%
Forested Wetland	0.1%	0.0%	0.1%
Nonforested Wetland	0.1%	0.2%	0.4%
Pasture/Grassland	80.7%	51.5%	45.4%
Row Crop	4.3%	3.3%	1.5%
Upland Deciduous Forest	10.3%	41.8%	11.4%
Upland Mixed Forest	1.5%	1.9%	3.9%
Upland Coniferous Forest	0.7%	0.2%	3.6%
Urban/Developed	2.5%	0.1%	33.5%
Non-vegetated	0.0%	0.9%	0.0%
Undefined	0.0%	0.0%	0.0%
Total Area (mi <sup>2</sup> )	21.99	26.95	16.91

Blackburn Fork has a higher percentage of pasture and grassland than the other two streams. Spring Creek has a higher percentage of forests than the other two streams. The grassland and forest serve as a good buffer for surface runoff which minimizes impacts on water quality. In addition to land cover difference, Pigeon Roost Creek has point source pollution from the Cookeville wastewater treatment plant, which discharges effluent into this stream. Urbanization also contributes heavily to nonpoint source pollution runoff. These two factors combine to create a unique pollution scenario in the Pigeon Roost watershed.

## METHODOLOGY

### Macroinvertebrate Sampling

Macroinvertebrates, animals that do not have backbones but are visible to the naked eye, are commonly referred to as insects or bugs. In streams, many macroinvertebrates live on stream bottoms. Macroinvertebrates play key roles as biological indicators of stream health because 1) they represent important links in the food chain as recyclers of nutrients and food for fish, 2) each insect acts as a mini, around-the-clock water quality monitoring device with its own value for pollution tolerance; the presence or absence of tolerant and intolerant types can indicate the

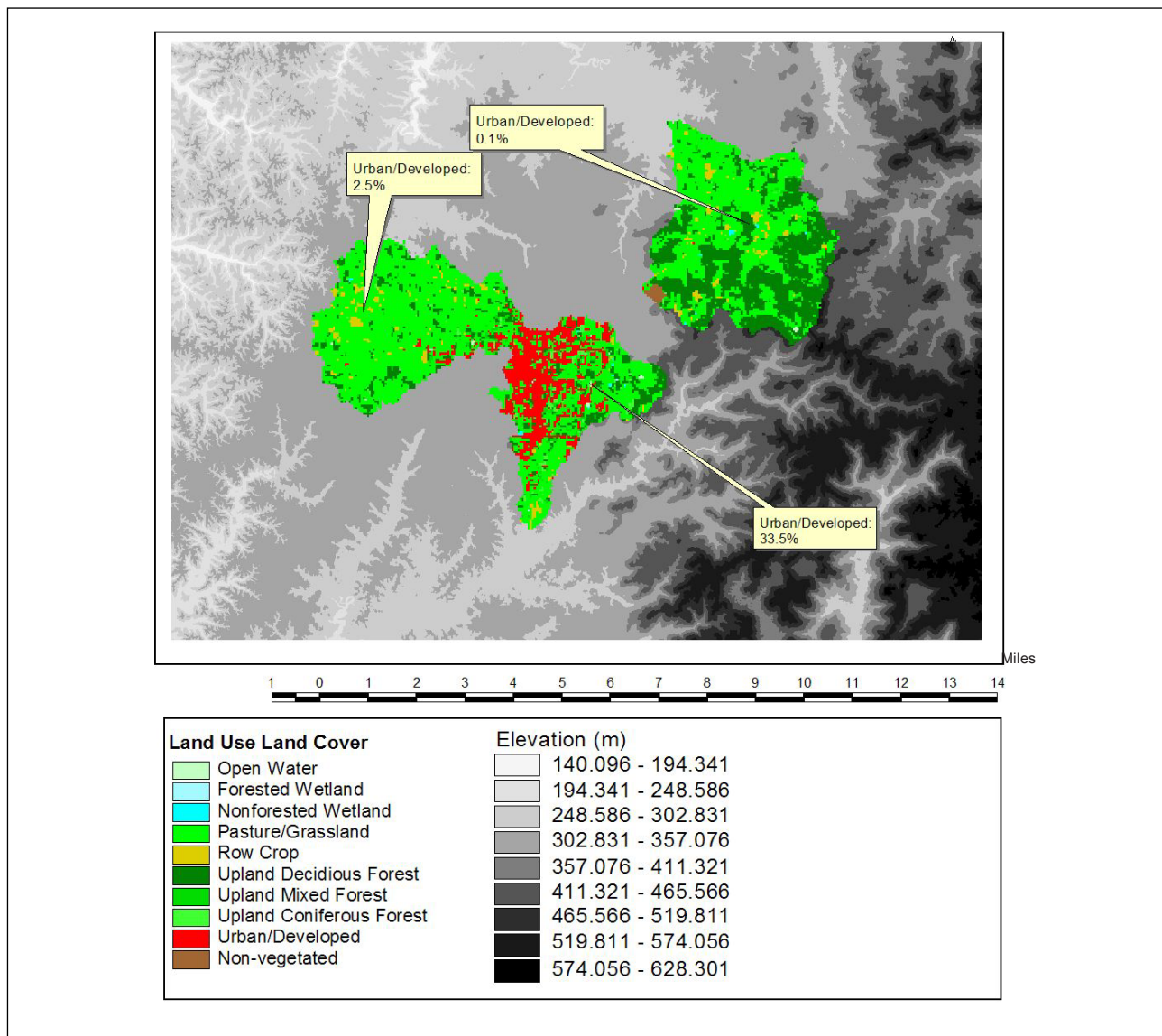


Figure 2. Land use land cover map with digital elevation models.

overall health of the stream, 3) many macroinvertebrates tend to have short life cycles, usually one season or less; therefore, becoming rather accurate indicators of the stream's quality and, 4) many macroinvertebrates are relatively sedentary residents of the stream's bottom, becoming a pollutant's captive.

Samples of macroinvertebrates were collected at three sites: Pigeon Roost downstream from the wastewater treatment plant, Blackburn Fork, and Spring Creek. All samples were collected at riffle habitats since this provides the best habitat for most stream-dwelling macroinvertebrates. According to a study by Parsons and Norris (1996), sampling from similar habitats is a required procedure when comparing water quality among different watersheds. Although riffles in a stream may be the result of anything from an uneven bedrock bottom to an aggregation of large boulders, the optimum habitat for macroinvertebrates is a riffle uniformly composed of moderately-sized particles. The constant flow of oxygenated water that the riffle areas provide offers a continuous and plentiful supply of food in the form of plant and animal matter. Riffle-dwelling macroinvertebrates generally require an environment that has a plentiful supply of oxygen and is free of toxic pollutants; therefore, making the riffle area the best place to sample .

### **Sampling Method: Semi-Quantitative Riffle Kick (SQKICK)**

SQKICKs are required for samples in several Tennessee ecological subregions (Arnwine and Denton, 2001). All of Cookeville's streams, located in subcoregion 71g, have riffles and do not require an upstream or off-site reference sample. The following describes the procedures used for field sampling:

Use a 500-micron mesh net to sample the riffle. Two kicks should be collected at each site: one from a slower current velocity and one from a faster current velocity. Always collect the downstream sample first to avoid organism drift.

Disturb the sampled site approximately one meter distant from and along the same width (one meter) as the net by kicking and shuffling the substrate. Rocks may be lifted and rubbed to remove clinging organisms. This allows organisms and debris to flow into the net.

After the kick is completed, allow time for the debris to finish floating into the net. A second biologist should carefully grab the bottom of the net from the water and carry the net horizontally to the bank for processing.

Thoroughly collect everything from the net and place in a plastic zip-lock bag and preserve with some field water and 70% alcohol. Organic material, such as whole leaves or twigs, should also be included in the sample. The sample should be returned to the laboratory for processing and identification.

### **Sub-sampling the SQKICK in lab**

All SQKICK samples are to be reduced to a 200+ organism subsample in the lab before beginning analysis. The following describes the procedures used for sub-sampling:

Thoroughly rinse the sample in a 25-micron mesh sieve to remove fine sediment. Whole leaves and twigs should be thoroughly rinsed, visually inspected, and then discarded.

Transfer the cleaned sample into a gridded subsampler pan with marked boxes 1 through 100.

Evenly disperse the insects throughout the pan. Using a random number generator and a counter, remove all organisms from the grids that are selected and place the material into a dish with a small amount of ethanol. Any organism that is in between two grids counts if its head is in the selected grid. If there is no head (like oligochaetes), then the grid containing most of the body will be the organism's grid.

Continue picking until there are 200+ organisms in the subsample. If 200 organisms is not yet reached, then keep choosing additional grids one at a time until the quota is reached or surpassed. All the organisms from the final grid that is randomly selected are removed, even if there are already 200+ organisms in the subsample.

Save the remaining original organisms not included in the subsample in a separate container.

## **MACROINVERTEBRATE IDENTIFICATION**

After a random subsample as been completed, the insects were ready for identification. Macroinvertebrate identification is complicated due to the existence of several forms during their life cycles. Some aquatic insect life cycles have nymphs while others have larvae. Nymphs hatch from eggs, molt, grow, and metamorphose into adults. After hatching from an egg, other insects are larvae, which then evolve into pupae, which later evolves into adults. Pupae, the stage between

the larva and the adult, were not identified in this study.

Insects are identified by a hierarchical system of Kingdom, Phylum, Class, Order, Family, and Genus. Taxa, a named group at any level, may be hard to remember but this type of identification eliminates all confusion. For example, the stonefly consists of over 460 species in North America (Cummins and Merritt, 1996; Smith, 2001).

All macroinvertebrates, except chironomids and oligochaetes, were identified using a stereoscopic microscope (7X - 45X magnification). Chironomids and oligochaetes were mounted on glass slides using CMCP-10 mounting media and were identified using a compound microscope (40X - 420X magnification). The macroinvertebrates samples collected from three watersheds were identified, recorded and compiled into tablet format. Several indicators are important in showing water quality in terms of characteristics of macroinvertebrate communities.

## RESULTS AND DISCUSSION

In this study, the North Carolina Biotic Index (NCBI) was used as a referencing index for pollution. It has been widely used for showing intolerance of macroinvertebrates in water. NCBI scores of 0.00 to 3.00 are regarded as intolerant to macroinvertebrate genera. For Tennessee taxa, the highest NCBI score is 9.86 and lowest is 0.00. Spring Creek has the lowest total NCBI value (Table 2). High values of NCBI are mostly attributed to two most dominant species, Trichoptera and Diptera. Low-valued NCBI species include Leuctra and Chimarra; both are lower than 3.00. Their presence in water proves that the water is still very clean. The total number of macroinvertebrates found in Blackburn Fork totals 219 with NCBI values of 905.93 (Table 3). One hundred and sixty-three Clingers were found. The more Clingers found shows, generally, the better the water quality. The high value of total NCBI may be due to the large number of individuals found in the subsamples. Two-hundred and nineteen individuals were identified in Blackburn Fork sample while there were only 172 identified in Spring Creek. Out of 183 individuals identified from Pigeon Roost Creek (Table 4), only one species has a NCBI value under 3. This explains why the high score of total NCBI was found in this creek.

To further study the water quality from different watersheds, Taxa Richness, EPT Richness, %EPT, %OC, NCBI, % Dominant and %Clingers were computed and their definitions are given below:

- Taxa Richness (TR) - This is the total number of distinct taxa (genera) found in the subsample.
- EPT Richness (Ephemeroptera Plecoptera Trichoptera) - This is the total number of genera found in the orders Ephemeroptera, Plecoptera, and Trichoptera.
- % EPT – (Number of Ephemeroptera + Plecoptera + Trichoptera X 100)/Total Number of individuals.
- % OC (percent of oligochaetes and chironomids) = (Total number of Oligochaeta + Chironomidae X 100) /Total number of individuals
- NCBI (North Carolina Biotic Index) -  $NCBI = \sum (X_i * T_i) / N$  where  $X_i$  =number of individuals in a taxon,  $T_i$  =tolerance value of a taxon,  $N$ =total number of individuals.
- % Dominant (percent contribution of the single most dominant taxon) = Total individuals in the most dominant taxon X 100 / Total number of individuals

Table 2. Results from Spring Creek macroinvertebrate identification.

Order	Family	Genus	Number	NCBI value	NCBI	Clingers
<i>Plecoptera</i>	<i>Perlidae</i>	<i>Perlesta</i>	16	4.7	75.2	16
<i>Coleoptera</i>	<i>Elmidae</i>	<i>Stenelmis</i>	2	5.1	10.2	2
<i>Ephemeroptera</i>	<i>Isonychiidae</i>	<i>Isonychia</i>	46	3.45	158.7	
<i>Ephemeroptera</i>	<i>Heptageniidae</i>	<i>Stenonema</i>	30	3.45	103.5	30
<i>Plecoptera</i>	<i>Leuctridae</i>	<i>Leuctra</i>	21	0.67	14.07	21
<i>Trichoptera</i>	<i>Hydropsychidae</i>	<i>Cheumatopsyche</i>	22	6.22	136.84	22
<i>Trichoptera</i>	<i>Hydropsychidae</i>	<i>Hydropsyche</i>	4	4.3	17.2	4
<i>Trichoptera</i>	<i>Philopotamidae</i>	<i>Chimarra</i>	7	2.76	19.32	7
<i>Diptera</i>	<i>Simuliidae</i>	<i>Simulium</i>	15	4	60	15
<i>Ephemeroptera</i>	<i>Caenidae</i>	<i>Caenis</i>	1	7.41	7.41	
<i>Diptera</i>	<i>Chironomidae</i>	<i>Polypedilum</i>	4	5.69	22.76	
<i>Diptera</i>	<i>Chironomidae</i>	<i>Tanytarsus</i>	1	6.76	6.76	
<i>Lumbriculida</i>	<i>Lumbriculida</i>	<i>Lumbriculus</i>	3	7.03	21.09	
<b>Total</b>			<b>172</b>		<b>653.05</b>	<b>117</b>

Table 3. Results from Blackburn Fork macroinvertebrate identification.

Order	Family	Genus	Number	NCBI value	NCBI	Clingers
<i>Decapoda</i>	<i>Cambaridae</i>	<i>Orconectes</i>	1	2.6	2.6	
<i>Ephemeroptera</i>	<i>Heptageniidae</i>	<i>Stenonema</i>	60	3.45	207	60
<i>Ephemeroptera</i>	<i>Caenidae</i>	<i>Caenis</i>	3	7.41	22.23	
<i>Plecoptera</i>	<i>Perlidae</i>	<i>Perlesta</i>	2	4.7	9.4	2
<i>Ephemeroptera</i>	<i>Isonychiidae</i>	<i>Isonychia</i>	32	3.45	110.4	
<i>Trichoptera</i>	<i>Philopotamidae</i>	<i>Chimarra</i>	13	2.76	35.88	13
<i>Trichoptera</i>	<i>Hydropsychidae</i>	<i>Cheumatopsyche</i>	51	6.22	317.22	51
<i>Megaloptera</i>	<i>Corydalidae</i>	<i>Nigronia</i>	1	5.25	5.25	1
<i>Trichoptera</i>	<i>Hydropsychidae</i>	<i>Hydropsyche</i>	2	4.3	8.6	2
<i>Diptera</i>	<i>Simuliidae</i>	<i>Simulium</i>	5	4	20	5
<i>Coleoptera</i>	<i>Psephenidae</i>	<i>Psephenus</i>	6	2.35	14.1	6
<i>Odonata</i>	<i>Gomphidae</i>	<i>Gomphus</i>	1	5.8	5.8	
<i>Coleoptera</i>	<i>Elmidae</i>	<i>Stenelmis</i>	13	5.1	66.3	13
<i>Diptera</i>	<i>Athericidae</i>	<i>Atherix</i>	13	2	26	
<i>Megaloptera</i>	<i>Corydalidae</i>	<i>Corydalis</i>	1	5.16	5.16	1
<i>coleoptera</i>	<i>Elmidae</i>	<i>Optioservus</i>	9	2.36	21.24	9
<i>Mesogastropoda</i>	<i>Pleuroceridae</i>	<i>Elimia</i>	2	2.46	4.92	
<i>Diptera</i>	<i>Chironomidae</i>	<i>Polypedilum</i>	3	5.69	17.07	
<i>Diptera</i>	<i>Chironomidae</i>	<i>Tanytarsus</i>	1	6.76	6.76	
<b>Total</b>			<b>219</b>		<b>905.93</b>	<b>163</b>

Table 4. Results from Pigeon Roost Creek macroinvertebrate identification.

Order	Family	Genus	Number	NCBI value	NCBI	Clingers
<i>Diptera</i>	<i>Chironomidae</i>	<i>Psetrocladius</i>	87	3.59	312.33	
<i>Diptera</i>	<i>Chironomidae</i>	<i>Pseudochironomus</i>	7	5.36	37.52	
<i>Diptera</i>	<i>Chironomidae</i>	<i>Rheotanytarsus</i>	5	5.89	29.45	5
<i>Diptera</i>	<i>Chironomidae</i>	<i>Endochironomus</i>	2	7.79	15.58	2
<i>Diptera</i>	<i>Chironomidae</i>	<i>Cardiocladius</i>	2	5.89	11.74	
<i>Diptera</i>	<i>Chironomidae</i>	<i>Chironomus</i>	2	9.63	19.26	
<i>Trichoptera</i>	<i>Hydropsychidae</i>	<i>Cheumatopsyche</i>	73	6.22	454.06	73
<i>Diptera</i>	<i>Simuliidae</i>	<i>Simulium</i>	2	4	8	2
<i>Lumbriculida</i>	<i>Lumbriculidae</i>	<i>Lumbriculus</i>	1	7.03	7.03	
<i>Diptera</i>	<i>Athericidae</i>	<i>Atherix</i>	1	2	2	
<i>Diptera</i>	<i>Tipulidae</i>	<i>Antocha</i>	1	4.25	4.25	1
<b>Total</b>			<b>183</b>		<b>901.22</b>	<b>83</b>



- % Clingers = Total number of clinger individuals X 100 / Total individuals

Clingers are the organisms that build fixed homes or have adaptations to attach to surfaces in flowing water. Clingers usually indicate good water quality.

After computing these values, they were equalized by assigning a score of 0, 2, 4, or 6 based on Tennessee’s ecoregion reference database for bioregion of 71g (Table 5). The values of each watershed then add up to a value and are then assigned an Bioregion Index Score (BIS) rating using the bioregion index scores chart. Table 6 shows calculated results of BIS scores for three watersheds, with their individual parameters. A detailed discussion of each parameter is provided in the following sections.

### Percent Clingers and Percent Dominance

Among the three watersheds, Pigeon Roost has the lowest percentage of Clingers. Clingers presence indicates a good quality of water. Spring Creek and Blackburn Fork both have high percentages (Figure 3). A high Percent Dominance is shown in Pigeon Roost Watershed while Blackburn and Spring have lower Percent Dominance values. A higher Percent Dominance indicates less diversity of species in the water. High percent of the dominant species indicates some disturbance has likely occurred to the invertebrate community.

### Number of Genera/Orders

Blackburn Fork contains the highest number of genera (Figure 4). While Pigeon Roost has the lowest number of genera, Blackburn Fork, again, has highest number of Orders found in the water compared to other two streams. Water samples from Pigeon Roost only found 11 genera from 3 orders. Such low diversity is an indication that taxa dominated the community. Therefore, the water quality in Pigeon Roost is obviously more impaired than that of the other two streams.

Table 5. Biocriteria Table (TDEC, 2002).

Bioregion 71fgh Target Index Score		Method = SQKICK Stream Order= 2,3,4,5		
Metric	6 (Non)	4 (Slight)	2 (Moderate)	0 (Severe)
Taxa Richness	> 27	19 – 27	10 -18	< 10
EPT Richness	> 9	7 – 9	4 -6	
% EPT	> 53.38	35.9 – 53.37	13- 35.8	< 18
% OC	< 27.5	27.5 – 51.6	51.7 – 75.8	> 75.8
NCBI	< 4.74	4.74 – 6.49	6.5 – 8.25	> 8.25
% Dominant	< 36.7	36.7 – 57.7	57.7 – 78.8	> 78.8
% Clingers	> 52.4	35.0 – 52.4	17.5 – 34.9	< 17.5

Table 6. BIS Scores in three streams.

	<b>Blackburn Fork</b>	<b>score</b>	<b>Spring Creek</b>	<b>score</b>	<b>Pigeon Roost</b>	<b>score</b>
<b>Taxa Richness (TR)</b>	19	4	13	2	11	2
<b>EPT Richness (EPT)</b>	7	4	8	4	1	0
<b>% EPT</b>	74.43	6	85.47	6	39.89	4
<b>% OC</b>	1.83	6	4.65	6	57.92	2
<b>NCBI</b>	4.14	6	3.8	6	4.93	4
<b>% Dominant</b>	27.4	6	26.74	6	47.54	4
<b>% Clingers</b>	74.43	6	68.02	6	45.36	4
<b>BIS Score</b>		38		36		20

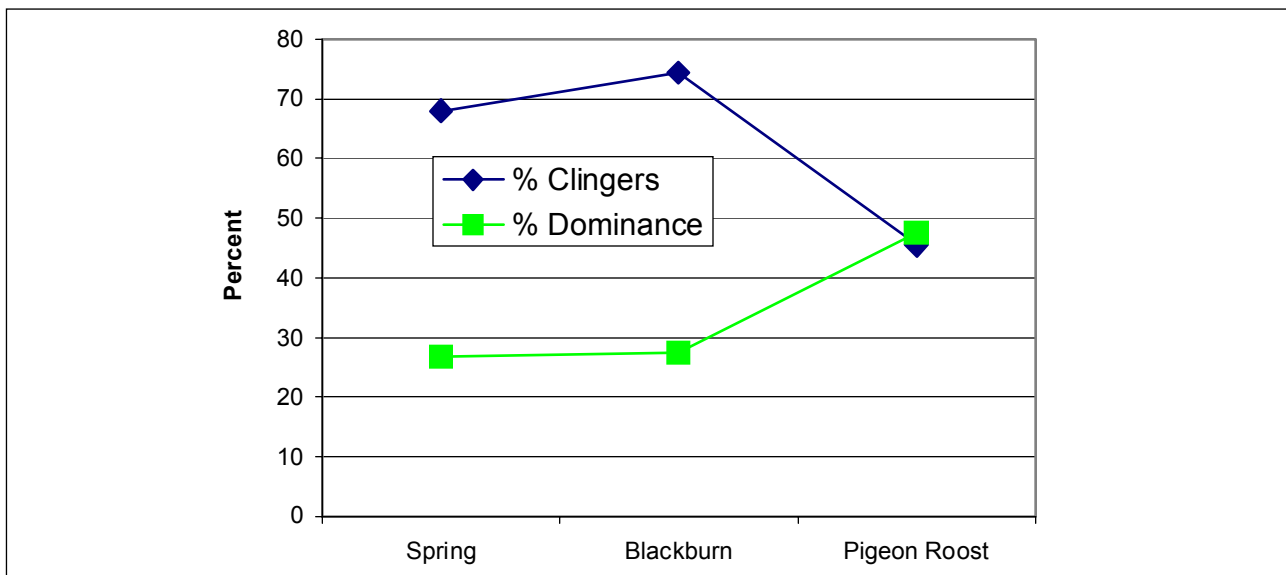


Figure 3. Percent clingers and percent dominance.

### Percent EPT/Percent OC

Percent EPT is the percentage of total number of genera found in the orders Ephemeroptera, Plecoptera, and Trichoptera. Species from these three orders are pollutant sensitive. Percent OC (Oligochaetes and Chironomids) shows the most pollution-tolerant macroinvertebrates in the water. High Percent EPT is always correlated with low Percent OC. Pigeon Roost has a high Percent OC and a low Percent EPT (Figure 5). Blackburn Fork and Spring Creek have two-fold Percent EPT of Pigeon Roost while the low Percent OCs are shown in Blackburn Fork and Spring Creek.

### Habitat Assessment

Habitat assessments are critical for monitoring water quality since it conveys the environment that the organisms live in. Habitat assessments should be conducted each time a biological sample is collected. The habitat will be visually computed by looking for gravel, cobble, bedrock, soft sediments, vegetation, boulders, and movable rocks in percentages. All sites were sampled on a

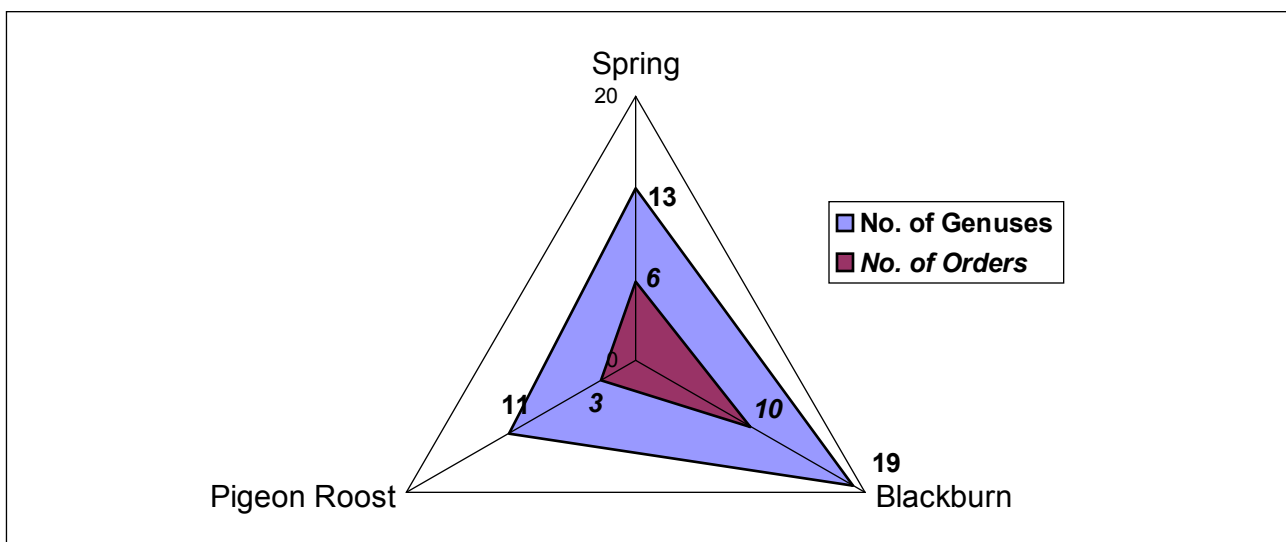


Figure 4. Number of orders/number of genus found in three streams.

February afternoon. The weather was good, temperature was in the 40s and sunny. Habitat assessment results varied by streams (Table 7).

From the habitat data presented of the three streams, Pigeon Roost clearly exhibits the worst habitat for intolerant organisms with 80% cobble and all cobble/boulder covered with algae. Along

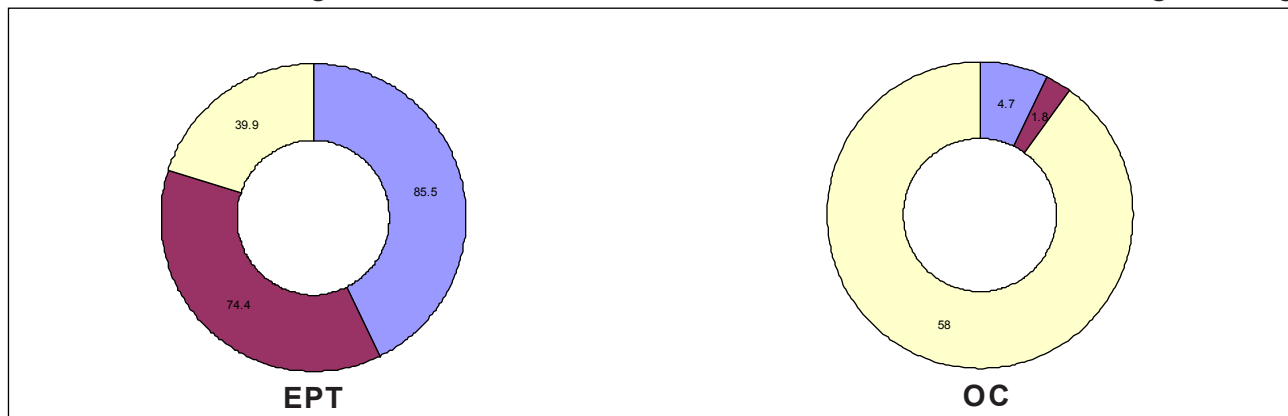


Figure 5. Percent EPT and OC.

with an index biotic score of 20, which is moderately impaired, one can see that Pigeon Roost has the worst habitat for supporting life. Blackburn Fork and Spring Creek show significant increases in percentages, only holding 40% and 60% cobble respectively. However, a stream which has uniform substrate will support fewer types of organisms than a stream that has a variety of substrates. Blackburn Fork has a greater variety of substrates than Spring Creek, and with a Biotic Index Score (BIS) of 38 as compared to 36, Blackburn Fork displays the healthiest habitat.

### Urbanization vs Biotic Index Score

When comparing urban/developed percentage vs. BIS, the higher the urbanization percentage, the lower the BIS becomes (Figure 8). The Pigeon Roost watershed has had many phosphorus and nitrogen nutrients as evidenced by the habitat assessment (100% algae over all cobble/boulders/rocks). As evident, urbanization has negative impact on water quality. The higher the urbanization, the lower the BIS score. The conclusion from this study supported findings from other researchers, such as Roy et al., (2003).

Table 7. Percent cobble/boulder/gravel/sand and presence of algae in three sampling sites.

Sampling Site	Habitat
<b>Pigeon Roost</b>	80% cobble 10% boulder/rip-rap 10% sand All cobble/boulder covered with filamentous green algae
<b>Blackburn Fork</b>	40% cobble 50% gravel 5% sand 5% boulder/rip-rap No algae on rocks
<b>Spring Creek</b>	60% cobble 30% gravel 10% sand No algae on rocks

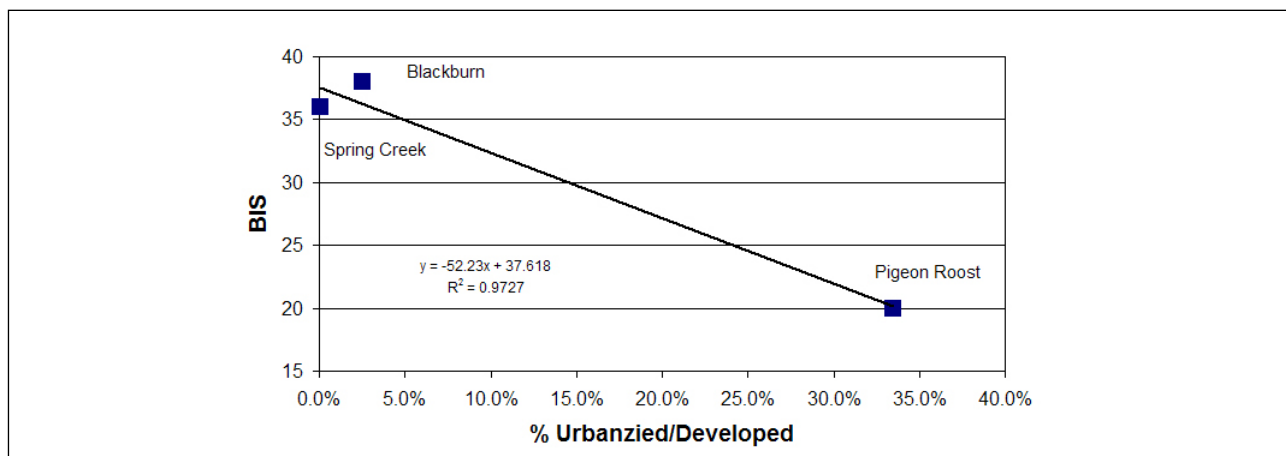


Figure 8. Percent urbanized vs bioregional index score (BIS).

### CONCLUSION

1. Three watersheds exhibit their unique characteristic properties in terms of macroinvertebrate population and diversity. Spring Creek, the state’s reference stream, demonstrates a non-impaired index score rating according to TDEC’s standards. Surprisingly, Blackburn Fork also holds a slightly higher non-impaired index score rating compared to Spring Creek. The finding from this study suggests that, in the future, TDEC may have two reference streams in this bioregion 71g. Pigeon Roost shows the lowest water quality out of the three due to various reasons as stated below.

2. The habitat assessment from this study shows Pigeon Roost has more dominant cobble (80%) and algae (covering 100% of the rocks) as opposed to the other two streams. Excessive algae generally degrades the environment for macroinvertebrates. The less diverse habitat found in Pigeon Roost may contribute to its lower grade water quality in terms of macroinvertebrate population and diversity. The other two streams, Spring Creek and Blackburn Fork, have no algae in their habitats. Blackburn Fork displays the best habitat according to this study, and this is verified by the high BIS value.

3. A higher percentage of urbanization contributes to a lower BIS value as evidenced by Pigeon Roost. The inverse relationship is evident from the chart produced from the data. In Pigeon Roost Creek, urbanization is one of the two factors contributing to its low water quality. The other factor is the point source pollution from the Cookeville Waste Water Treatment Plant. A smart urban growth policy is required to mitigate pollution.

4. Future research topics include devoting more time in the future to study additional watersheds and analyze more physical, chemical, and biological parameters, along with seasonal variations. These efforts can do much to protect the health of watersheds in middle Tennessee.

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